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2 Induction of Defense Reactions in Chickpea (*Cicer arietinum* L.) by Pathogenic and Non-Pathogenic *Fusarium oxysporum*

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Abstract:

Chickpea or Bengal gram (*Cicer arietinum* L.) the oldest pulse crop has special significance in the diet of the predominantly vegetarian population of India. *Fusarium oxysporum* f.sp*. ciceri* is a very serious, destructive and wide spread disease on chickpea. It has threatened the cultivation of this crop at many places causing poor yield and caused a major economic loss in the affected areas. present investigation was taken up with the evaluation of the response of two distinct varieties of Chickpea (Wilt tolerant and wilt susceptible) to a non-pathogenic fungus, *Fusarium oxysporum* f.sp. *lycopersici*(FOL) and a pathogenic fungus *Fusarium oxysporum* f. sp *ciceris* (FOC) through Biochemical, Histochemical and Molecular parameters. *Fusarium oxysporum* f.sp. *ciceri*- The mycelia appeared initially white and later turned into pink and finally pigmentation was intensified throughout the plate. *Fusarium oxysporum* f.sp. *lycopersici*- The mycelia appeared white to pink initially which on later stages turned into violet. The present investigation was directed towards testing the possibility of using a non-pathogenic fungus induced resistance as a tool for building up Systemic Acquired Resistance (SAR) against pathogen attack in a crop plant. Chickpea was chosen as a model system along with two fungi viz: *Fusarium oxysporum* f.sp*. lycopersici* a non-pathogenic organism to chick pea and *Fusarium oxysporum* f. sp. *ciceri* the wilt pathogen of chickpea.

Keywords: Chickpea/ Biocontro/ *Fusarium oxysporum/ ciceris/ lycopersici*

Introduction:

India is the largest producer and consumer of pulses in the world accounting for 33 percent of the world area and 22 percent of the world production of pulses. The domestic demand and consumption, however, is much higher than production mainly because pulses are a major source of protein for a large section of the vegetarian population in the country. The cultivation of pulses also results in the production of large quantity of nutritious green fodder, a food for the livestock. Besides their high nutritional value, pulse crop has a unique characteristic of maintaining and restoring soil fertility through biological nitrogen fixation. Their cultivation improves the physical characteristics of the soil through their deep and well spread root system. The pulse crops add more nitrogen to the soil than the nitrogen provided by chemical fertilizers.

The major pulse crops grown in India are tur, gram, arhar, mungbean, urad and beans. Gram, commonly known as chickpea or Bengal gram or garbanzo bean, is an important pulse crop of India. It is world's third food legume crop and fifteenth grain crop **(**FAO, 1972). Chickpea or Bengal gram (*Cicer arietinum* L.) the oldest pulse crop has special significance in the diet of the predominantly vegetarian population of India. Chickpea belongs to family of Fabaceae and subfamily Papillionaceae. Different pulses belong to different tribes. *Cicer arietinum* L. belongs to the tribe fabae (Vicieae). Chickpea seeds are nutritionally rich in protein, phosphorus, calcium, and fiber and relatively low in antinutritional factors such as trypsin inhibitors. Thus it holds good promise for humans as protein source and as component of feed of ruminants and non ruminants (Rathore and Sharma, 2003). There are two types of chickpea: **1) Desi**- With small, dark seeds and a rough coat (prevailing in the Indian subcontinent, Ethiopia, Mexico, Iran). 2) **Kabuli**- With light-colored, larger seeds and a smoother coat (mainly grown in Southern Europe, northern Africa, Afghanistan, Chile and introduced in the 18th century to the Indian subcontinent). Desi type chickpeas are said to have a very low glycemic index making them suitable for many people with blood sugar problems.

FUSARIUM WILT IN CHICKPEA

More than 50 pathogens have been reported to affect chickpea but only a few devastate the crop. The most important are ascochyta blight (*ascochyta rabiei*), Fusarium wilt (*Fusarium oxysporum f.sp. ciceri*), dry root rot *(Macrophomina phaseolina*), stunt *(Bushystunt virus),* botrytis grey mold *(Botrytis cinerea*) and black root rot (*Rhizoctonia solani). Fusarium oxysporum* is a saprophytic fungus growing in soil and organic matter. It can survive as mycelia and also in form of three different spores namely micro conidia, macro conidia and chlamydospores. Fusarium belongs to the group *Hyphomycetes* formely classified in *Deutromycetes*, sub group *Hypocreales (Ascomycetes*).

Like various other plant pathogens, *Fusarium oxysporum* has several specialized forms – known as *formae specialis* (f.sp.) – That infects a variety of hosts causing various diseases. *Fusarium oxysporum* and its various *formae speciales* have been characterized as causing the following symptoms; vascular wilt, yellow corm rot, root rot and damping off. Browning of the vascular tissue is strong evidence of fusarium wilt. These symptoms are generally more apparent during the period between blossoming and fruit maturation.

Fusarium oxysporum f.sp*. ciceri* is a very serious, destructive and wide spread disease on chickpea. It has threatened the cultivation of this crop at many places causing poor yield and caused a major economic loss in the affected areas. Eight distinct physiological races have been determined namely 0, 1A, 1B/C, 2, 3, 4, 5, 6. Races 0 and 1B/C causes yellowing syndrome and 1A, 2, 3, 4, 5, 6 wilting syndrome. Genetics of resistance to two races (1B/C and 6) is yet to be determined. However, for other races resistance is governed either by monogenes or oligogenes. The individual genes of oligogenic resistance mechanisms delay onset of disease.

The genus *Fusarium* is known to produce many mycotoxins that attack plant cells. these are called phytotoxins (substance that are toxic to plants) and they may break down the cell wall of the host plant or release nutrients. fusaric acid is a well known phytotoxin that is produced by several fusarium species, particularly pathogenic strains of *Fusarium oxysporum* causing wilt disease of a great variety of plants. Fusaric acid (5-butylpicolinic acid) first discovered during the laboratory culture of fusarium heterosporum, was one of the first fungal metabolites implicated in the pathogenesis of wilt symptoms of plants especially under adverse conditions. Fusaric acid considered as a wilt toxin has been examined for its production and role in

the wilt of field maize (Bacon, 2006). Correlation between fusaric acid production and virulence of isolates of *Fusarium oxysporum* species has been reported in Lily (Curir, 2000), date palm (Bouizgarne, 2004) and *Arabidopsis thaliana* (Bouizgarne, 2006).

Knowledge about the genetic and biochemical bases involved in the pathogenesis of *Fusarium oxysporum* would be invaluable in getting clues helping to discriminate between pathogenic and nonpathogenic strains and may lead to the development of more effective long term and ecologically safe control against Fusarium wilt. In general, it is now accepted that more than specialized resistance genes, the timing, and rate of accumulation and relative amounts of secondary metabolites synthesized in the plant play crucial roles in plant's resistance to pathogen invasion. Keeping these facts in mind the present investigation was taken up with the evaluation of the response of two distinct varieties of Chickpea (Wilt tolerant and wilt susceptible) to a non-pathogenic fungus, *Fusarium oxysporum* f.sp. *lycopersici*(FOL) and a pathogenic fungus *Fusarium oxysporum* f. sp *ciceris* (FOC) through Biochemical, Histochemical and Molecular parameters.

Materials and Methods:

Plant material:

Seeds of chickpea (*Cicer arietinum* L) variety JG-62 (susceptible) and JCP-27 (tolerant) were received from Junagadh agriculture university.

Fungal strains:

Non pathogenic fungal strain *Fusarium oxysporum* f.sp*. lycopersici* was collected from ITCC. Pathogenic fungal strain *Fusarium oxysporum* f.sp*. ciceri* was obtained from the sick plot of Junagadh Agricultural University.

Maintenance of fungal culture:

Isolated cultures of *Fusarium oxysporum* f.sp*. lycopersici* (causative organism of wilt disease in tomato) and *Fusarium oxysporum* f. sp. ciceri (causative organism of wilt disease in chickpea) were regularly sub cultured on potato dextrose agar and potato dextrose broth (Hi media, India) at an interval of 10 days and incubated at 25˚C ± 2˚C.

Identification of fungus

Macroscopic characters:

Change in the growth pattern of the mycelium i.e. size, colour and texture of mycelia were observed. These were then recorded and photographed with Carl-Zeiss Image Analyzer.

Microscopic characters:

Purity of fungus was checked by using light microscope and was identified by its specific sporulation and mycelial pattern. A small fragment of mycelia from PDA was taken and kept on a clean glass slide. Later on it was stained with lacto phenol cotton blue. Then the cover slip was placed and observed under light microscope.

Bioassay

Preparation of the plants for the experiment:

Seeds of both the varieties of chickpea were surface sterilized with 1% sodium hypochlorite and rinsed thrice with sterile distilled water to remove the excess sterilant. These seeds were soaked in distilled water and kept overnight. Next day seeds were transferred to sterile Petri plates containing moistened Whatman No. 1 filter plate. The seedlings were allowed to grow for 2 -3 days till a uniform root and shoot length was obtained. Later on the seedlings were transferred to plastic cups containing garden soil. Seedlings were grown till the plants were 8-9 days old. The 9 day old plants were used for the bioassay.

Preparation of the fungal inoculum

A non-pathogenic (FOL) and a pathogenic (FOC) fungal strain were used for the bioassay. Fungal inoculum for FOL and FOC was prepared by inoculating a loopful of 1-week old PDA cultured fungal strain into 250 ml PDB flasks. The flasks were kept at 26°C in an incubator. One week old cultured flasks were used for the experiment.

Experimental design

Two experimental designs were set up in the present study. Each design included 4 sets of plants.

I. Experiment using non-pathogenic fungal strain *Fusarium oxysporum* f.sp. *lycopersici* (FOL)

S. NO.	VARIETY	INOCULATION CONDITION
1.	JCP-27 (tolerant)	Unautoclaved garden soil + distilled water
2.	JCP-27 (tolerant)	Unautoclaved garden soil + FOL
3.	JG-62 (susceptible)	Unautoclaved garden soil + distilled water

4.	JG-62 (susceptible)	Unautoclaved garden soil + FOL

II. Experiment using pathogenic fungal strain *Fusarium oxysporum* f. sp. ciceri (FOC)

S. NO.	VARIETY	INOCULATION CONDITION
1.	JCP-27 (tolerant)	Unautoclaved garden soil + distilled water
2.	JCP-27 (tolerant)	Unautoclaved garden soil + FOC
3.	JG-62 (susceptible)	Unautoclaved garden soil + distilled water
4.	JG-62 (susceptible)	Unautoclaved garden soil + FOC

After the experiment was set up, plants were watered daily and weekly observed for disease symptoms. Whole plants from 8 sets were harvested at regular time intervals like 8 hrs, 16 hrs, 40 hrs, 48s, 7th day, 14th day and 21st day after infection. These harvested plants were analysed for various biochemical and anatomical parameters.

RAPD Analysis:

Total genomic DNA was isolated from the two chickpea varieties and the isolated DNA was subjected to RAPD analysis to confirm the presence of a marker linked to resistance gene.

Biochemical assays:

Peroxidase assay was measured according to the method of Guilbault, (1976). Catalase activity was measure according to the method of Beersand Sizer (1952). Total phenols were estimated by following the method of Bray and Thorpe (1954). The total soluble proteins were estimated according to the method of Folin and Lowry's method.

Histopathological study:

Along with the biochemical studies, histochemical localization of peroxidase activity and lignin in the root and shoot tissues were also studied. Localization of Peroxidase was carried out by the the method of Dejong *et al*., (1967). Localization of lignin was done as per the method of (Johansen, 1940; Gibbs, 1958)

Results and Discussion

The pathogen:

Pathogen of Fusarium wilt disease of chickpea has been characterized to be a fungus, *Fusarium oxysporum* f.sp. *ciceri* while the non-pathogenic fungus used in the

present study causes *Fusarium* wilt disease in Tomato (*Fusarium oxysporum* f.sp. *lycopersici*).

Macroscopic characters:

Fusarium oxysporum f.sp. *ciceri*- The mycelia appeared initially white and later turned into pink and finally pigmentation was intensified throughout the plate (Fig. 1A). *Fusarium oxysporum* f.sp. *lycopersic*i- The mycelia appeared white to pink initially which on later stages turned into violet (Fig.1B).

Fig. 1A

Fig. 1B

Microscopic characters:

Fusarium oxysporum produces three types of spores: microconidia, macroconida and chlamydospores. Microconidia are two celled, oval and straight to curved. Macroconidia are sparse to abundant, three to five celled, and chlamydospores are round and thick walled (**Fig. 2**). Branching of mycelia is observed.

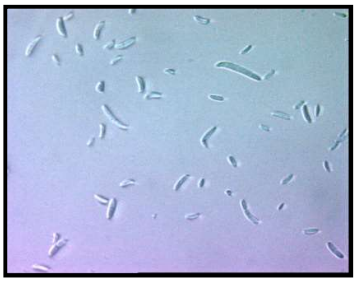


Fig. 2

Symptomatology:

No symptoms were observed in the control and treated plants when inoculated with the non-pathogenic fungus. Whereas when the control and treated plants were inoculated with the pathogenic fungus, symptoms were observed in the treated plants. Initial wilting was observed in the topmost leaves during the pre-infectional stages and

on further prolongation to the fungus, progressive yellowing symptoms were seen throughout the plant and eventually the plants wilted (**Fig. 3**).

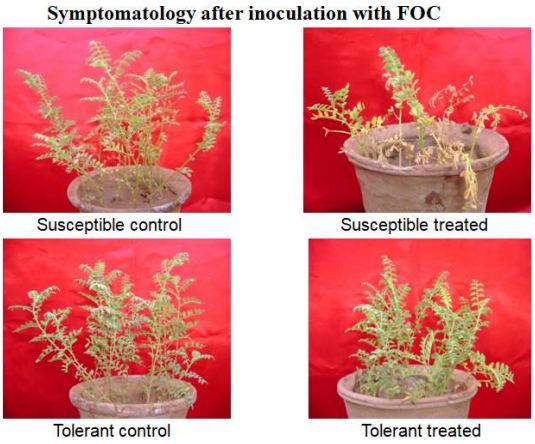
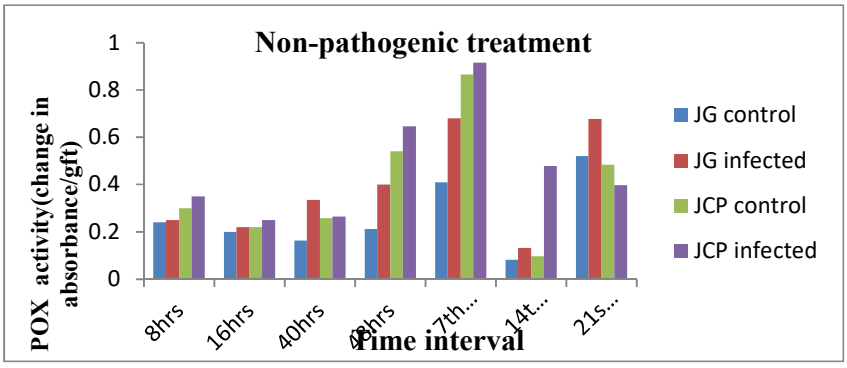


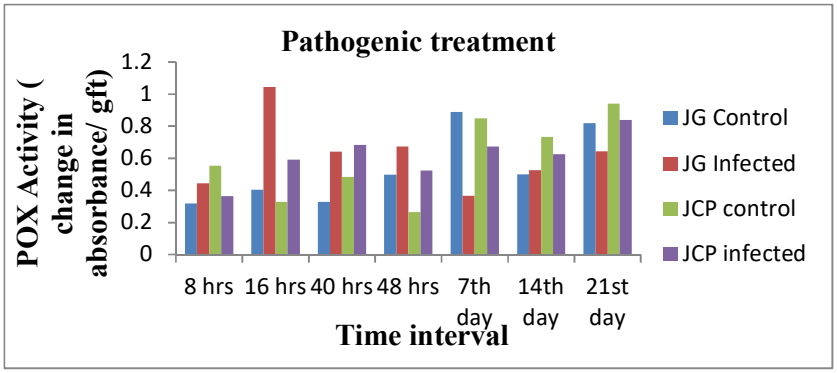
Fig. 3

Biochemical assays:

Peroxidase activity

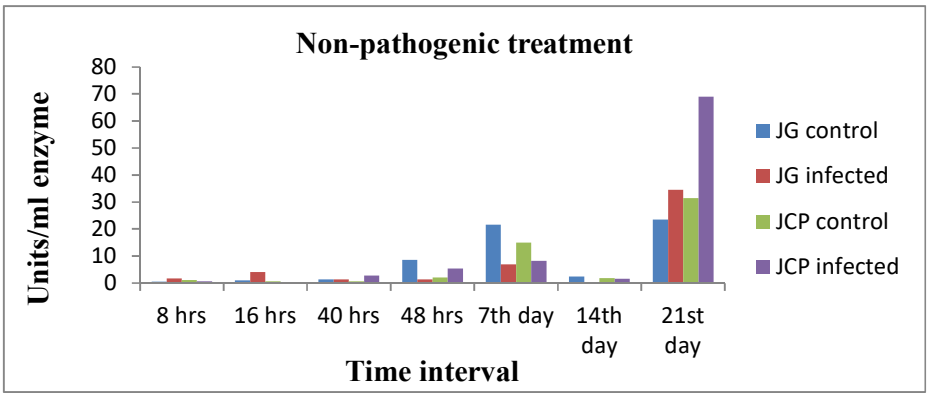
An increase in activity was observed in all inoculation experiments in comparison with control plants. On inoculation with the non-pathogenic fungus, total peroxidase activity got highly increased in the treated plants within 48 hrs and reached maximum levels on the 7th day and 21st day. On treatment with the pathogenic fungus, a rapid oxidative burst was observed in both the treated plants within 48 hrs of infection.

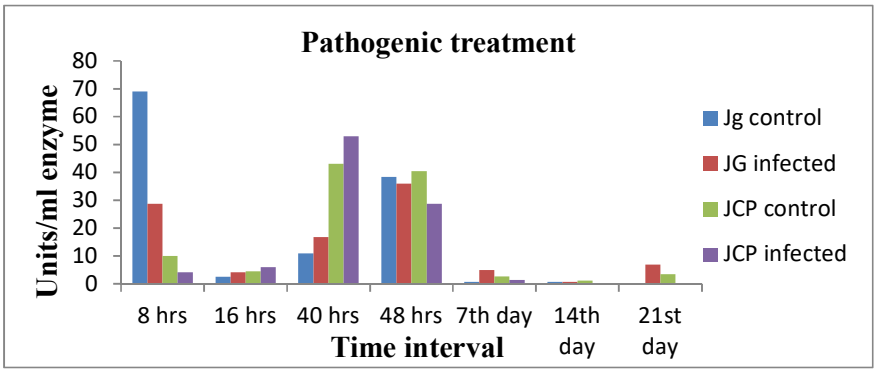




Catalase activity:

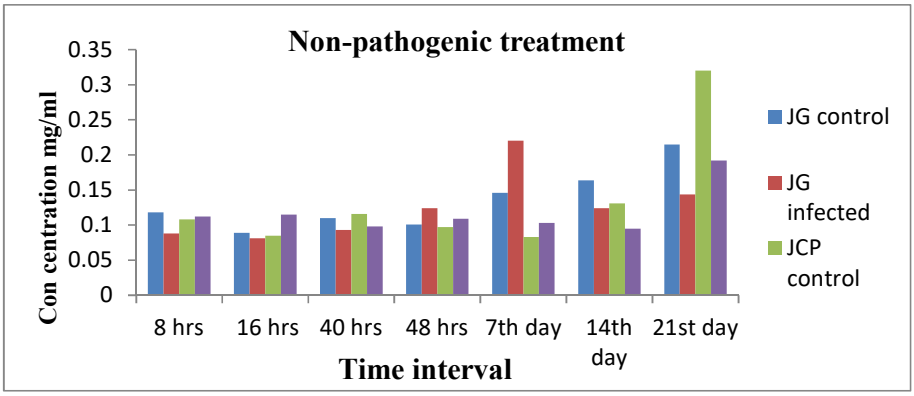
Catalase activity produced a significant 1-2-fold increase in the susceptible and tolerant varieties within 48 hrs after infection with the non-pathogenic fungus which attained maximum levels in resistant variety at 21 DAI. While after infection with the pathogenic fungus an initial rapid oxidative burst was seen within 40 hrs for both the varieties. The maximum activity for tolerant variety was recorded at 40 hrs time interval with reference to control plants while for the susceptible variety the maximum induction occurred on the 7th and 21st day of infection.

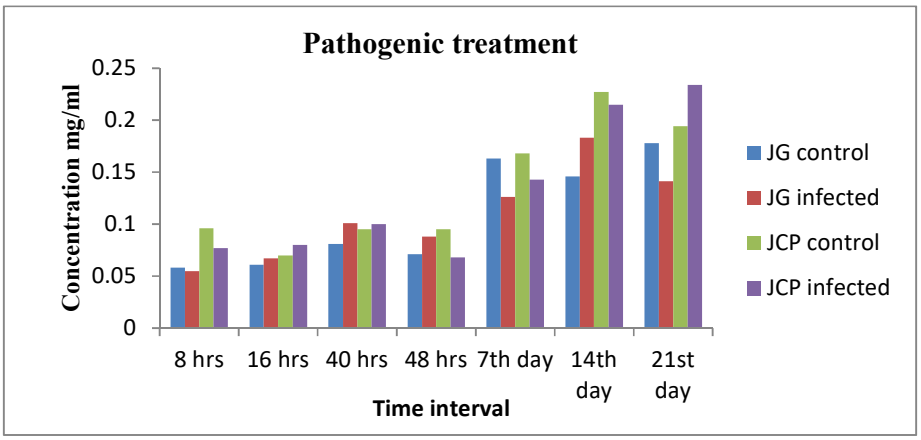




Total Phenolics:

A rapid increase in total soluble phenolics was observed after inoculation with the pathogenic and nonpathogenic fungi. A linear pattern of accumulation of phenolics was observed in both the varieties after fungal treatment. In the case of infection with the non-pathogenic and pathogenic fungus maximum phenolic activity was attained on the 7th day and 14th day of the susceptible variety respectively, while the resistant variety showed maximum activity on the 21st day of infection in both the cases.

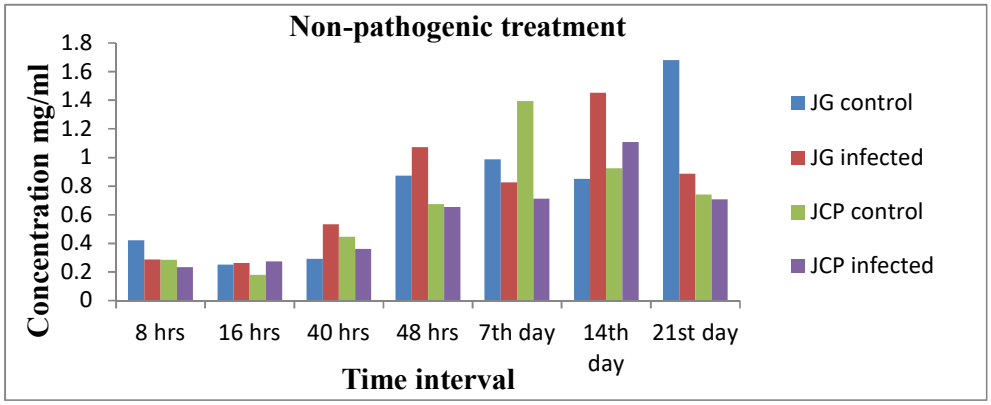


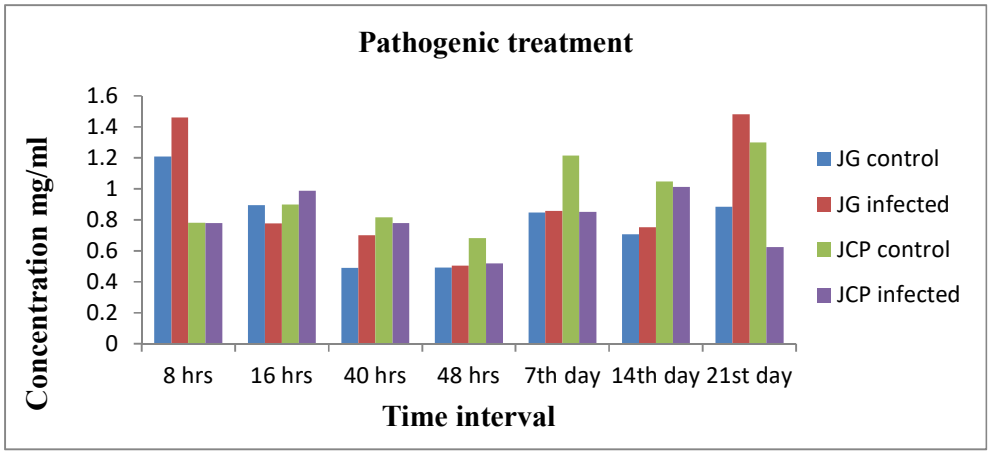


Total soluble proteins:

Relatively low levels of proteins were exhibited in the first 40 hrs of infection with the non-pathogenic fungus as compared with its pathogenic counterpart which was able to induce considerably higher levels of protein in the susceptible and tolerant varieties. Protein expression exhibited in the tolerant variety showed similar levels at the 7th, 14th and 21st day of infection with the two fungi, while in the case of the susceptible variety maximum levels

of protein were attained on the 14th day and 21st day of infection with the non-pathogenic fungus and pathogenic fungus respectively.





Anatomical studies:

Peroxidase localization:

Significant changes were observed in the shoots of chickpea after the 21st day of treatment with FOL and FOC. Higher POX activity in infected plants was also confirmed by histochemical methods. An intensive brown to black precipitate located this enzyme along the cell walls of shoot cells. A dark brown ring was observed in the sections of JG-62 than in JCP-27 treated with the non-pathogenic fungus, while in the case of treatment with the pathogenic fungus, the intensity of peroxidase was more in the control varieties than in the treated. Furthermore, in the treated varieties, the resistant variety showed dense brown color than the susceptible

Lignin localization

The results for lignin deposition and its localization were found to be in correlation with the peroxidase activity and its histochemical localization.

Discussion:

In the present study, Fusarium *oxysporum* f.sp. *lycopersici* which is pathogenic to tomato but non-pathogenic to chickpea has been used to study various parameters involved in plant defense. An attempt has been made in the present investigation to provoke the defense response in chickpea by pre-inoculating the plant with a non-pathogenic strain to induce mitigation of symptoms when the plant was later inoculated with pathogenic *Fusarium*. To monitor the various defense responses in the plant two separate experimental sets were designed and inoculations were done using a pathogenic (FOC) and a non-pathogenic fungus (FOL). Biochemical and anatomical parameters were studied at various time intervals.

Peroxidases are a large family of enzymes with very diverse functions in plant systems. They often increase in response to stress. One of the principle roles of peroxidases appears to be cellular protection from reactive oxygen species generated by various stresses (Siegel, 1993). It has been reported that peroxidases have an antifungal activity against various pathogens (Peng and Kuc, 1992). Increased catalase and peroxidase activity in resistance response has been related to lignifications which have an important role in the reduction and blockage of nutrient diffusion from neighboring host cells to the haustoria (Kuc and Preisig, 1984). Increased activities of the enzymes peroxidase and catalase and lignin deposition are thought to play a major role in local and systemic disease resistance (He et al., 2002). Lignifications also act as physical barriers to prevent the entry of fungal toxin and enzymes in the host systems (Conti et al., 1994).

Lignification is common in healthy plants. Lignin is formed by random dehydrogenate polymerization of precursor produced in the phenylpropanoid pathway. Phenylalanine ammonia lyase (PAL) provides precursor for lignin and for several phenylpropanoid derived secondary metabolites in plants involved in resistance. Several researchers have correlated lignifications with enhanced activities of peroxidase (Bruce and Galstone: 1989). Enhanced lignifications are an induced process which is elicited by release of fungal molecules.

Conclusion:

The macroscopic analysis showed specific cottony mycelial growth of *Fusarium oxysporum* at 25º±2ºC on PDA and PDB which changes from pink to violet colour. Changes in media colour due to pigmentation have been observed. Microscopic

analysis showed typical sickle shaped macroconidia of *Fusarium oxysporum* small and oval microconidia, chlamydospores and branching of the mycelium. A time course study of susceptible and tolerant varieties of chickpea after inoculating with the non-pathogenic and pathogenic fungal treatment was studied with respect to enzymes involved in the initial oxidative burst. Plant peroxidases were found to be released in high amounts within 48 hours of infection with both the fungal treatments. Results obtained with catalase activity highlight the importance of time regulated induction of enzyme levels. After both the fungal treatments, it was observed that initial hours are crucial for inducing maximum levels of enzyme, after which the peak levels are reached at the last stages of infection. Anatomical studies could detect POX activity and presence of lignin in the vascular tissues of shoots of susceptible and tolerant varieties of chickpea after fungal infection.

References:

Bacon C W, Hinton D M (2006). Is fusaric acid a wilt toxin in maize? *Phytopathology* **96:** 57-59.

Beers, R.F. Jr. and Sizer, I.W. (1952): Journal of Biological Chemistry **195**, 133-140

Bouizgarne B, Brault M, Pennarun A M, Rona J P, Ouhdouch Y, El Hadrami I, Bouteau F (2004): Electrophysiological Responses to fusaric acid of root Hairs from seedlings of Date Palm-susceptible and resistant to *Fusarium oxysporum* f.sp. *albedinis*, Journal of *Phytopathology* **152(6)**, 321-324.

Bouizgarne B, EI-Maarouf-Bouteau H, Frankart C, Reboutier D, Madiona K, Pennarum A M, Monestiez M, Trouverie J, Amiar Z, Briand J, Brault M., Rona J P, Ouhdouch Y, El Hadramiand F. Bouteau I (2006): Early Physiological responses of *Arabidopsis thaliana* cells to fusaric acid: toxic and signaling effects. *New Phytologist*, **169(1):** 209-218.

Bouslama M (1980): Chickpea improvement in Tunisia. In: Proc Int Workshop Chickpea Improv (pp 277–280). ICRISAT, Hyderabad, India

Bray, H. G., and Thorpe W. V. (1954). Analysis of phenolic compounds of interest in

Curir P, Guglieri L, Dolci M, Capponi A. and Aurino G (2000): Fusaric acid production by *Fusarium oxysporum* f.sp. *lilii* and its role in the Lily Basal Rot Disease, European *Journal of Plant Pathology*, Vol **106 (9)**,

Dandurand LM and Knudsen GR (1993): Influence of *Pseudomonas fluorescens* on hyphal growth and biocontrol activity of *Trichoderma hamatum* in the spermosphere and rhizosphere of pea. *Phytopathology* **83:** 265–270

Dejong, D.W. (1967): An investigation of the role of plant peroxidase in cell wall development by the histochemical methods*. J. Histochem. Cytochem*. **15**, 335.

Food and Agricultural organization (1972): "Production year book". **25**: pp. 167

Gibbs R.D. (1958): The Maule reaction, lignins and the relationship between woody plants. *In*: "The physiology of forest trees", K.V. Thimann (eds). Ronald Press, New York.

Gozzo F, (2004): Systemic acquired resistance in crop protection. Outlooks on pest management.

Guibault, G. G. (1976): Handbook of enzymatic methods of analysis. Marcel Dekker. Inc., New York. Pp-147

Hulse, J. H. (1976): Production of nutritional quality of pigeon pea and chickpea and prospects of research. International workshop on grain legumes 13-16 Jan. 1975. ICRISAT, Andhra Pradesh. India. pp. 189-207.

metabolism. *Meth. Bio-chem. Anal.*, **1**:27-52.

Peng M and Kuc J (1992): Peroxidase generated hydrogen peroxide as a source of antifungal activity invitro and on tobacco leaf discs. *Phytopathology*, **82**: 696-699.

Rathore, P. S. and Sharma, S. K. (2003): Scientific Pulse Production, Yash Rathore, P. Publishing House. Bikaner, Rajasthan. pp. 92-122.

Siegel B. Z (1993): Plant peroxidases an organism perspective. *Plant growth regulation*, **12:** 303-312.

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10 Curing diseases by Herbal therapy in the Rural area of the Banaskantha District, Gujarat, India

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Abstract:

Herbal therapy is one of the common practices used by local medicine men by rural area of the BanaskanthaDistirct in Gujarat State. The paper deal with 10 plant species which is used in herbal therapy, mainly in joint disorder, swollen, body pain, boils, insect bite, headache and rheumatic pain.

Key Words: Herbal therapy; Banaskantha; Gujarat

Introduction

Banaskantha district situated in the north part of Gujarat, lies between $23^{0}\,^{33}$' to $24^{0}\,^{45}$'N Latitudes and 720 15' to $73^{0}\,^{87}$' E Longitudes. The district takes its name from the river Banas that flows through the district. The Banaskantha district as its name indicates consists of the territories situated on around the river Banas. About 80 percent of its working population is engaged in agriculture and allied activities and they live in rural area. Therefore, they possess rich traditional knowledge on the uses of various plants. They always prefer to use available plants for the treatment of their disease, disorders and ailments. Unfortunately, this valuable knowledge as well as plant species is getting depleted leading to their extinction due to lake of interest among younger generation towards traditional knowledge and urbanization. Through this district is ideal from ethnobotanical study point of view, as rich in floristic as well as in ethnic diversity. The common diseases for which hot fomentation therapy is practiced are contusion, chronic cold, joint disorder, body pain, headache, toothache, back pain, rheumatic pain and insect bite etc.

Material and Methods

The present study was conducted during 2017-2022. Regularly field trips were arranged in different places of the area. The ethnomedicinal information was gathered through interviews and discussion with local informants, Bhagats and elder villagers of

different localities of the area. Data were recorded on the plants part used, local name(s), process of preparation and mode of administration and dosage. Indigenous traditional practitioners and some knowledgeable local informants, who have knowledge about therapeutic values of wild and domestic plant species in the treatment of common ailments, were interviewed in the forest and in their homes.

The collected plant specimens were identified using 'Flora of Gujarat State' (Shah, 1978) and 'A textbook of systematic Botany' (Sutaria 1949). The data considered worth mentioning only when at least 2 to 3 local healers gave similar answers for the same plant.

Enumeration

The botanical names of the plant species have been arranged alphabetically with their family in parentheses, followed by habit, local name, along with ethno medicinal uses are given in detail. Ethno medicinal uses of different plant parts, mode of preparation and method of uses are given.

1. *Curcuma longa* L. (Zingiberaceae) 'HALDAR'.

Turmeric powder and salt mixed with water and heating in a pan this warm paste is applied externally to cure contusion, common swollen, body pain and remove thorn in the body part.

2. *Calotropisprocera* R. Br. (Asclepiadaceae) 'AAKDO'.

Apply castor oil on mature leaves slightly roasted on burning coal this warm leaves bandaged to cure chronic cold, painful rheumatic joints, headache, and body pain. Root branches foment on burning coals and chewed for relive toothache.

3. *Cassia auriculata* Linn. (Caesalpiniaceae) 'AAVAL'.

The hot poultice of fresh leave is applied to cure back pain and spinal cord disorder.

4. *Citrulluscolocynthis* (L.) Soland. (Cucurbitaceae) 'KADVA KALINGDA'.

The fruit is cut into two parts, the inner pulp is removed and then roasted in the burning coals. This warm fruit is tied to the heel of the foot to cure heel pain.

5. *Euphorbia neriifolia* L. (Euphorbiaceae) 'THOR'.

Prepare a shallow pipe of the stem and then cork the holes at both the ends with cloth. Then foment it on burning coals. After that remove the cloth and finally blow through the pipe on the body pain, contusion, and joint disorder for curing.

6. *Ephedra karumanchiana* (Ephedraceae) 'VADVEL/SOMVEL'.

Fresh twig boiled with water in pot and inhale vapor to cures asthma and respiratory diseases and allergies.

7.*Madhukaindica* J.F. Gmel. (Sapotaceae) 'Mahudo'

Flowers boiled in water this warm flowers bandage to cure body injuries

and swelling.

8. *Prosopisspicigera* L. (Mimosaceae) 'KHINJADO'.

The warm ash applies on the body to cure skin disease.

9. *Trachyspermumammi* (L.) Sprague (Apiaceae) 'AJAMO'.

Seeds boiled with water in pot and inhale vapor to cure blocked nose and this warm seed apply externally on the nose for relive pain.

10. *Vitexnegundo* L. (Verbenaceae) 'NAGOD'.

Fresh leaves boiled in water this warm leaves are bandage on rheumatic joints and swelling for relief body pain.

RESULT AND DISCUSSION

In this paper there are 10 plants species belonging to 9 families of Angiosperms and one family from Gymnosperm, used to cure various ailments such as contusion, chronic cold, joint disorder, body pain, headache, toothache, back pain, rheumatic pain and swelling.

These ethnomedicinal uses were compared with well-known Indian ethnobotanical literatures and found that only 3 plant species have reported earlier. The people of area have a vast wealth of plants, which are sources of medicinal compounds. Therefore, more concerted efforts are needed for the documentation of all the local medicines and their health practices useful in the treatment of different disorders. The above reported ethnomedicinal plants also require a proper chemical, pharmacological experiments and clinical trials for the validation of the traditional claims.

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References

Ant, H. M. (2001) The Ethnobotanical studies of Angiosperms of Aravally hills, DistBanaskantha., Ph.D. thesis, Bhavnagar University, Bhavnagar.

Punjani, B. L. (1997) An Ethnobotanical study of Tribal area of district Sabarkantha (North Gujarat). Ph.D. thesis, North Gujarat University, Patan.

Shah, G. L. (1978) The Flora of Gujarat State, Part I and II.

Bhatt, D. C., Nurani, M. A., Mitaliya, K. D. and Baxi, U. S. (2001) Some ethnomedicinal plants of Barda hills of Saurashtra, Gujarat, India. Ad. Plant Sci. 14 (II): 427-431.

Tintisara, M.P. 2014. Studies on Floristic Diversity and Traditional uses of plants in Bhiloda (E) forest range, District Sabarkantha, Gujarat., Ph.D. Thesis, HNG University, Patan.

Jadhav D, Ethno-medicinal survey of Maalgamdi in Ujjain District, Madhya Pradesh, India, Ethnobotany 18 (2006): 157-159.

Jadhav D, Ethnomedicinal plants used in leaf therapy in Ratlam district (Madhya Pradesh), Ethnobotany 21 (2009): 84-90.

Chauhan M G and Pillai a P G, Impact of Gender variation on quality of herbal drugs, Indian J. Nat. Prod., 19(1) (2003):30-34.

Goel A K and Mudgal V, A Survey of medicinal plants used used by the tribals of SanthalParagana, Bihar. J. Econ. Tax. Bot. 12 (1988):329-335. Maheshwari J K, Singh K K and Saha S, Ethnobotany of tribals of Mirzapur district, U.P. Nation. Bot. Res. Inst. Lucknow (1986). (1984):41-48.

Katewa S S and Galav P K, Additions to the traditional folk herbal medicines from Shekhawati region of Rajasthan, Indian J. Traditional Knowledge 5(4) (2006):494-500.

Punjani B L, some less known ethnomedicinal plants used by Maldharis of SasanGir forest in Junagadh district, Gujarat, *Ethnobotany* 19 (2007): 116-119.

Pandey, V.B. 2011. Plant species Diversity and their Ethnobotanical Study in Tribal Area of Bhiloda (West) Forest Range of Sabarkantha District (North Gujarat). A Ph.D. Thesis submitted to HNG Uni. Patan.

Parajapati, M.M. 2002. Survey of plant community of Shamlaji forest hillock. A Ph.D. Thesis submitted to HNG Uni. Patan.

Desai, P.R. 2013. Studies on floristic diversity, ethanobotany and resources survey I Jessor wildlife Sanctuary of Banaskantha district, Gujarat. A Ph.D. Thesis submitted to HNG Uni. Patan.

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28

GC-MS ANALYSIS OF ETHANOLIC EXTRACT FROM TWIG OF EPHEDRA KARUMANCHIANA IN NORTH GUJARAT

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Abstract

The present investigation was carried out to determine the bioactive compounds present in twig of *Ephedra karumanchiana*. GC-MS analysis in ethanolic extract of this twig bioactive 63 compound were identified. The major bioactive compounds are 4H-pyran-4- one,2,3-dihydro-3,5-dihydroxy-6-methyl-, 1,3- Dioxane, 2,4-dimethyl-, Pentanoic acid,2-(Aminooxy)-, 1-octadecyne, Phytol, N-Hexadecanioc acid, Tridecanoic acid, Octadecanoic acid, Cis-11-Hexadecanal. Further investigations are necessary to isolate and characterize bioactive and to evolute its therapeutical potential.

Key words: *Ephedra karumanchiana*, Ethanolic extracts, GC-MS Analysis.

INTRODUCTION

Ephedra genus contains bronchial dilator, ephedrine, and other ephedrine alkaloids. It hasbeen also used for many years in traditional medicine to treat allergies, bronchial asthma, chills, colds, coughs, edema, fever, flu, headaches, and nasal congestion and has been a natural source of alkaloids such as ephedrine, pseudoephedrine, pseudoephedrine, and other related compounds (Parsaeimehr, Sargsyan & Javidnia et al.,2010). Similarly, the extracts with various chemicals from medicinal plants have been tested and showed the effectiveness of traditional herbs against microorganisms; as a result, plants are one of the bedrocks for modern medicine to attain new principles (Evans & Banso et al.,2002). Thus, medicinal plants can offer a wealth for their biological activities, such as antimicrobial, antioxidants, antimalarial, and anticancer activities (Rahman et al.,2016).

Gas Chromatography Mass Spectroscopy, a hyphenated system is a very compatible technique and the most commonly used technique for

the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra. There are at least two significant advantages for using GC-MS in the analysis of herbal medicines. First with the capillary column, GC-MS has in general very good separation ability, which can produce a chemical fingerprint of high quality and secondly with the coupled mass spectral database, quantitative composition information of the herb investigated could be provided by GC-MS, which will be extremely useful for the further research for elucidating the relationship between chemical constituents in the herbal medicine and its pharmacology in further research. Hence the present study investigated that GC-MS analysis of bioactive compounds in twig of Ethanoic extract of *Ephedra karumanchiana*.

MATERIAL AND METHOD

Study area

Various species of *Ephedra* are wide spread in many arid and semi-arid region of the world. Species grow on shores or in sandy soils with direct sunlight exposure. *Ephedra* located in north Gujarat and widely spread in some part of Banaskantha district for they specialty of our area.

Collection and drying of plant Material

The plant material of *Ephedra* was collected from Different villages of Dhanera and Deesa taluka of Banaskantha district in north Gujarat. It was air dried in shape avoiding exposure to direct sunlight and pulverized in grinder.

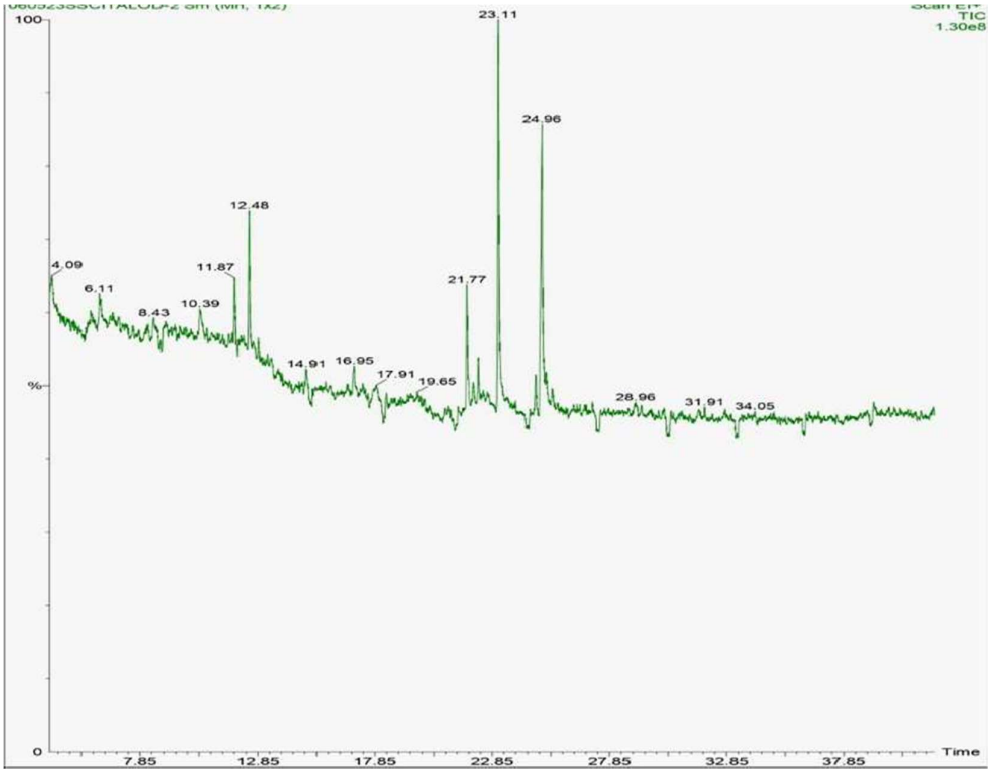
Plant extracts for GC-MS Analysis

The organic extraction was performed by Soxhlet extraction method. This extraction was done by taking 20 gm of dried plant powder and was placed into a glass thimble then extracted with 250 ml of ethanolic solvents. The extraction processes carry on till the solvent in siphon tube of Soxhlet apparatus become colorless. After that the extract was heated on hot water bath at 35 ºC until all the solvent evaporated. The dried plant crude extract was kept in refrigerator at 2-8 ºC for their

future use. The ethanolic extraction was done by taking five grams of the plant powder and mixed with 200 ml of distilled waterin a beaker. The mixture was heated on a hot plate at 30º-40 ºC and mixed with continuous stirring for 20 minutes. The mixture was filtered using whatman filter paper filter and thefiltrate was used for the further GC-MS analysis. (Mukherjee et al, 2002).

Gas Chromatography–Mass Spectrometry

Ephedra karumanchiana extracts were analyzed by gas chromatography (Agilent 7890A GC System). A Hp-5 ms fused silica capillary column was used (5% phenyl/95% dimethyl siloxane 30 M × 0.25 mm film thickness 0.32 Lm). The oven temperature was 110° C (isothermal for 2 minutes), with an increase to 200°C (10° C/min) and then 280° C (5° C/ min), with final temperature (isothermal for 9 minutes). The carrier gas used washelium (flow rate of 1 mL/min). GC–MS analyses were performed using an Agilent Technologies 7000 GC/MS Triple Quad coupled to an Agilent Technologies 7693 Auto sampler. The capillary column and GC conditions were calculated as described above. MS spectra were recorded at 70 eV, and the scanning rate was 1 scan/s with a run time of 90 minutes (Shljooghianpour & Javaran et.al,2013).



RESULTS AND DISSCUSSION

Fig.1- GC-MS chromatograms of *Ephedra karumanchiana* twig Ethanolic extract

GC-MS Analysis of Bioactive Compounds in *Ephedra karumanchiana* TwigEthanolic Extract

Sr. No	Compound Name	Molecular Weight	Molecular Formula	RT	Area %
1	4H-PYRAN-4-ONE,2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-	144	$C_6H_8O_4$	4.09	3.55
2	1,3-DIOXOLANE,2,4,5-TRIMETHYL-	116	$C_6H_{12}O_2$	4.32	0.87
3	2,4-DIHYDROXY-2,5-DIMETHYL-3(2H)-FURAN-3-ONE	144	$C_6H_8O_4$	4.81	1.32
4	1,3-DIOXANE, 2,4-DIMETHYL-	116	$C_6H_{12}O_2$	5.0	1.01
5	1,3-DIOXANE, 4-METHYL-	102	$C_5H_{10}O_2$	5.05	3.25
6	TRANS-4-NITROSO-2,3-MORPHOLINDIOL,3-ACETATE	190	$C_6H_{10}O_5N_2$	5.62	2.36
7	2-DIHYDROXY-3-HEXANONE	116	$C_6H_{12}O_2$	5.94	0.32
8	SUCCINAMIC ACID	117	$C_4H_7O_3N$	5.99	2.18
9	2-PROPYL-TETRAHYDROPYRAN-3-OL	144	$C_8H_{16}O_2$	6.11	2.38

10	4-ETHOXY-2-BUTANONE	116	$C_6H_{12}O_2$	6.23	3.15
11	1-BUTANOL,4-(1-METHYLETHOXY)-	123	$C_7H_{16}O_2$	6.91	6.20
12	PENTANOIC ACID,2-(AMINOOXY)-	133	$C_5H_{11}O_3N$	7.54	1.21
13	1(HYDROXYMETHYL)PROPYLACETATE	132	$C_6H_{12}O_3$	8.06	0.38
14	N,N-DIMETHYLLSUCCINAMIC ACID	145	$C_6H_{11}O_3N$	8.43	0.65
15	3-NITROPROPANOIC ACID	119	$C_3H_5O_4N$	8.46	0.91
16	NEOPHYTADIENE	278	$C_{20}H_{38}$	8.99	0.97
17	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	296	$C_{20}H_{40}O$	9.08	1.35
18	1-OCTADECYNE	250	$C_{18}H_{34}$	9.71	3.37
19	PHYTOL, ACETATE	338	$C_{22}H_{42}O_2$	10.2	4.08
20	PHYTOL	296	$C_{20}H_{40}O$	10.39	1.24
21	1-HEXADECYNE	222	$C_{16}H_{30}$	10.60	2.31
22	18-NONADECEN-1-OL	282	$C_{19}H_{38}O$	10.69	1.58
23	1-PENTADECYNE	208	$C_{15}H_{28}$	11.25	0.66
24	Z-2-OCTADECEN-1-OL	268	$C_{18}H_{36}O$	11.87	5.14
25	1-OCTADECYNE	250	$C_{18}H_{34}$	12.36	0.24
26	CYCLODODECANOL	184	$C_{12}H_{24}O$	12.48	10.88
27	1-HEPTADECYNE	236	$C_{17}H_{32}$	13.01	1.33
28	PENTADECANAL-	226	$C_{15}H_{30}O$	13.96	0.37
29	HEXADECANAL	240	$C_{16}H_{32}O$	14.65	1.84
30	3,7,11,15-TETRAMETHYL-2-EN-1-YL ACETATE	338	$C_{22}H_{42}O_2$	14.91	0.51
31	1-HEXADECYNE	222	$C_{16}H_{30}$	15.65	0.17
32	1-TETRADECYNE	194	$C_{14}H_{26}$	15.79	2.69
33	CIS-9,10-EPOXYOCTADECAN-1-OL	284	$C_{18}H_{36}O_2$	16.27	0.20
34	E-2-TETRADECEN-1-OL	212	$C_{14}H_{28}O$	16.95	0.79
35	1-TRIDECYNE	180	$C_{13}H_{24}$	17.05	2.36
36	N-HEXADECANOIC ACID	256	$C_{16}H_{32}O_2$	17.60	1.04
37	EICOSANOIC ACID	312	$C_{20}H_{40}O_2$	17.91	1.00
38	PENTADECANOIC ACID	242	$C_{15}H_{30}O_2$	18.25	2.16
39	TERTADECANOIC ACID	228	$C_{14}H_{28}O_2$	19.65	4.31
40	OCTADECANOIC ACID	284	$C_{18}H_{36}O_2$	20.10	0.78
41	TRIDECANOIC ACID	214	$C_{13}H_{26}O_2$	20.26	0.62
42	DODECANOIC ACID	200	$C_{12}H_{24}O_2$	21.03	1.15
43	NONADECANOIC ACID	298	$C_{19}H_{38}O_2$	21.77	8.76
44	9,12-OCTADECADIENOYL CHLORIDE, (Z,Z)	298	$C_{18}H_{31}OCl$	21.98	1.36
45	(Z)6,(Z)9-PENTADECADIEN-1-OL	224	$C_{15}H_{28}O$	22.65	1.01
46	7-TETRADECENAL,(Z)-	210	$C_{14}H_{26}O$	22.78	0.96
47	2-METHYL-Z, Z-3,13-OCTADECADIENOL	280	$C_{19}H_{36}O$	23.11	33.68
48	CIS-9-HEXADECENAL	238	$C_{16}H_{30}O$	23.58	0.65
49	9-TETRADECENAL,(Z)-	210	$C_{14}H_{26}O$	24.69	2.23
50	9,12-OCTADECADIEN-1-OL,(Z,Z)-	266	$C_{18}H_{34}O$	24.96	30.1

					5
51	CIS-11-HEXADECENAL	238	$C_{16}H_{30}O$	25.15	1.18
52	13-TETRADECE-11-YN-1-OL	208	$C_{14}H_{24}O$	28.65	0.21
53	9-OCTADECENAL,(Z)-	266	$C_{18}H_{34}O$	28.96	1.33
54	Z, Z-4,16-OCTADECADIEN-1-OL ACETATE	308	$C_{20}H_{36}O_2$	29.03	0.27
55	1-HEXYL-2-NITROCYCLOHEXANE	213	$C_{12}H_{23}O_2N$	29.36	0.47
56	3,11-TETRADECADIEN-1-OL	210	$C_{14}H_{26}O$	29.48	0.68
57	9,12-OCTADECADIENOIC ACID (Z,Z)-	280	$C_{18}H_{32}O_2$	30.17	0.29
58	Z,E-3,13-OCTADECADIEN-1-OL	266	$C_{18}H_{34}O$	31.91	0.91
59	7,11-HEXADECADIENAL	236	$C_{16}H_{28}O$	32.11	7.77
60	13-OCTADECENAL,(Z)-	266	$C_{18}H_{34}O$	32.67	1.39
61	CIS,CIS-7,10,-HEXADECADIENAL	236	$C_{16}H_{28}O$	33.12	1.06
62	Z,E-2,13-OCTADECADIEN-1-OL	266	$C_{18}H_{34}O$	33.65	0.14
63	(R)-(-)-14-METHYL-8-HEXADECYN-1-OL	252	$C_{17}H_{32}O$	34.05	0.73

Activity of major compound found in Ethanoic extract of *Ephedra karumanchiana*twig by GC-MS

SR. NO	COMPOUND NAME	ACITIVITY
1	Tetradecanoic acid	Larvicidal and repellent activity, antifungal, antioxidant, cancer preventive, nematicide, hypercholesterolemic
2	Pentadecanoic acid	Antimicrobial
3	6-octadecanoic acid	Antifungal, antibacterial, analgesic, anti-infammatory and antipyre
4	Hexadecanoic acid	Anti –inflammatory, Hypocholesterolemic, Nematicide, Antioxidant.
5	9,12,-Octadecatrienole acid, (Z,Z,)	Anti-inflammatory, Hypocholesterolenic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistamininic, Antieczemic, Anticancer, 5-alpha reductase inhibitor, Antiandrogenic, anti-rthritic, Anti-coronary, Insectifuge.
6	9-octadecanoic acid(Z)-	Emulsifying agent
7	Phytol, acetate	Antimicrobial; Anti-inflammatory, Anticancer; diuretic
8	(2E)-3,7,11,15-Tetramethyl-2-Hexadecene	Cancer-Preventive Antimicrobial anti-inflammatory anti-diuretic
9	4 Nonadecanoic acid	Antioxidant
10	Phytol	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antifungal, resistant gonorrhea, joint dislocation, headache, hernia, stimulant and antimalarial.

.

The GC-MS analysis of Ethanolic extract of *Ephedra karumanchiana* twig revealed the presence of sixty-three constituents. The GC-MS running time 42.05 minutes. The GC- MS chromatogram is presented in fig.1.The active principles with their retention time (RT), molecular weight, molecular formula and peak area are presented in the table 1. Theactivity of phytoconstituents are presented in table 2.

The major phytoconstituents compounds and its pick area are 2-Methyl-Z, Z-3,13- octadecadienol (33.68%); 9,12-Octadecadien-1-ol, (Z, Z) -(30.15%); Cyclododecanol (10.88%); Nonadecanoic acid (8.76%); 7,11-Hexadecadienal (7.77%); 1-Butanol,4-(1-methylethoxy)- (6.20%); Z-2-octadecen-1-ol, (5.14%); Tertadecanoic acid (4.31%); Phytol, Acetate (4.08%); 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (3.55%);1-Octadecyne (3.37%); 1,3-Dioxane, 4-methyl-(3.25%); 4-Ethoxy-2-butanone (3.15%); 1-Tetradecyne (2.69%); 2-Propyl-tetrahydropyran-3-ol (2.38%); 1-Tridecyne (2.36%);1-Hexadecyne (2.31%);9- Tetradecenal, (Z) 2.23%); pentadecanoic acid (2.16%). Among the identified compound 9,12-octadecadien-1-ol, (Z, Z)-, methyl ester which is a linoleic acid coumpound and reported to have an Anti-inflammatory, Hypocholesterolenic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistamininic, Antieczemic, Anticancer, 5-alpha reductase inhibitor, Antiandrogenic, anti-rthritic, Anti-coronary and Insectifuge properties (Ajayi et al.,2011). Hexadecanoic acid is also known as palmatic acid ester in effective in the treatment of Anti – inflammatory, Hypocholesterolemic, Nematicide, flavouring agent (Pramitha et al., 2016). 9-octadecenoic acid(Z)- used in Emulsifying agent.

CONCLUSION

The present study helps to predict the molecular formula and molecular weight of biomolecules of *Ephedra karumanchiana* which can be used as a drugs. It enhances the traditional usage of which possesses some known and unknown bioactive compound.

However, isolation of individual Phytochemical compounds and subjecting it to pharmacological activity will definitely give faithful results.

REFERENCES

Ajayi GO, Olagunju JA, Ademuyiwa O, Martins OC. GC- MS analysis and phytochemical screening of ethanolic root of Plumbago zeylanica (Linn.). J. Med. Plants Res, 2011; 5(9):1756-1761

Akpuaka A, Ekwenchi MM, Dashak DA, Dildar A. Biological Activities of Characterized Isolates of nHexane Extract of Azadirachta Indica A. Juss (Neem) Leaves, Nature and Science 2013, 11(5):141-147.

Bradford P.G. and Awad A.B. Phytosterols as anticancer compounds. Mol Nutr Food Res, 51 (2): 161-70, (2007).

Evans.W.C, "Treaseand Evans Pharmacognosy", Harcourt Brace and company. Asia pvt.Ltd.Singapore, 1997.

Igwi, O.U. and D.E. Okwa (2013). GC-MS evaluation of bioactive compounds and antibacterial activity of the oil fraction from the stem bark of Brachystegia eurycoma Harms. International Journal of Chemical Sciences, 11: 357-371.

James.E, K. Robbers, m. speedie and E. Varro Tyler, Pharmacognosy and pharmaco Biotechnology, Williams and Willkins, New York, U.S.A, 1996.

Khan, I.H. and A. Javaid (2022). Hexane soluble bioactive components of leaf extract of quinoa. Journal of Animal and Plant Sciences, 32(2): 309-314.

M. Saljooghianpour and T. A. Javaran, "Identifcation of phytochemical components of aloe plantlets by gas chromatography-mass spectrometry," African Journal of Biotechnology, vol. 12, no. 49, pp. 6876–6880, 2013.

Mukherjee PK. Quality Control of Herbal Drugs. An approaches to evaluation of botanicals, edition 1st Business Horizons, New Delhi. 2002, 390-403.

Naqvi, S.F., I.H. Khan and A. Javaid (2020). Hexane soluble bioactive components of Chenopodium murale stem. Pakistan Journal of Weed Science Research, 26(4): 425-432.

Parsaeimehr, A., Sargsyan, E., Javidnia, K., (2010): A comparative study of the antibacterial, antifungal and antioxidant activity and total content of phenolic compounds of cell cultures and wild plants of three endemic species of Ephedra. Molecules, 15: 1668-1678

Pramitha VS, Sree Kumari N. Anti-inflammatory, antioxidant, phytochemical and GC- MS analysis of marine brown macroalga, sargassum wighti, International Journal of Pharmaceutical, Chemical and Biological sciences. 2016; 6(1):7-15

Samuelsen, A.B., 2000. The traditional uses, chemical constituents and biological activities of Plantago major L. A review. Journal of Ethnopharmacol., 71(1-2): 1-21.

Sermakkani M, Thangapandian V. GC-MS Analysis of Cassia italica leaf methanolextract, Asian J Pharm Clin Res 2012, 5(2):90-94

Subrahmanyam DK, Mooney T, Raveendran R, Zachariah B. A Clinical and Laboratory profile of Cleistanthus collinus poisoning. J Assoc Physicians India 2003; 51:1052-54.

Thakur, N.; Bashir, S.F.; Kumar, G. Assessment of Phytochemical Composition, Antioxidant and Anti-Inflammatory Activities of Methanolic Extracts of Morus nigra and Artocarpus heterophyllus Leaves. Plant Cell Biotechnol. Mol. Biol. 2020, 21, 83–91–91.

Vijisaral ED and Subramanian A, GC – MS Analysis of Ethanol Extract of Cyperus rotundus Leaves, International Journal of Current Biotechnology 2014, 2(1):19-23.

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5 Leaves Extract Of *Papaya (Carica Papaya)* As A Corrosion Inhibitor For Copper In Trichloroacetic Acid

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ABSTRACT:
The present work investigates the corrosion of copper in Trichloroacetic acid containing leaves extract by mass loss measurement and electrochemical techniques. The inhibitor efficiency of Papaya (Carica Papaya) extract was found to very with concentration was kept uniform for 24 hours and temperatures was kept uniform for 2 hours. Experimental results revealed that inhibition efficiency (I.E %) increased with increasing inhibitor concentration. As temperatures increased, percentage of inhibition decreases. The value of free energy of adsorption (ΔG^0_{ads}), energy of activation (E_a), enthalpy of adsorption (ΔH^0_{ads}) and entropy of adsorption (ΔS^0_{ads}) were calculated. The result also showed that, adsorption of inhibitor molecules on the surface of copper followed Temkin and Langmuir adsorption isotherm model.Potentiodynamic study and Electrochemical Impedance Spectroscopy (EIS) implies that film developed on copper using the both of Trichloroacetic acid exhibits good corrosion resistance.
Key word: Papaya (Carica Papaya), Copper, Trichloroacetic acid, Corrosion, Inhibitor.

INTRODUCTION
Corrosion is defined as destruction of metals or detrition of its physical properties due to chemical or electrochemical reaction with its surrounding atmosphere. Copper and it´s alloy show high resistivity towards a wide variety of corrosive environments. This is may be due to the formation as protective and at times invisible oxide film on the metal surface. The film is generally stable in solution of pH 4.5-8 [1].

Inhibitor are frequently used for controlling corrosion of metals and alloys in acidic media for removing scales and rusts in metal finishing industries, cleaning of boilers and heat exchangers. The use of inhibitors is one of the most practical methods for protection against corrosion especially in acid solution to prevent metal dissolution and acid consumption. The hazardous effect of most synthetic corrosion inhibitors has motivated scientists to use naturally occurring product as corrosion inhibitors as they are inexpensive, readily available and renewable sources of materials, environmentally friendly and ecologically acceptable. Plant products are organic in nature, and some of the constituents including tannins, organic and amino acids, alkaloids and pigments are known to exhibit inhibiting action. Moreover, they can be extracted by simple procedures with low cost [2].

Previous research showed the inhibition of corrosion with ethanolic extract of African bush pepper (Piper guinensis) and neem leaves extract (Azadirachta indica) on copper in H_2SO_4.

The present work carried out to investigation the inhibition efficiency of an extract of *CP*for controlling corrosion of Copper in TCA solution by Mass loss, Temperatures, Potentiodynamic polarization and Electrochemical impedance spectroscopic (EIS) method.

Material and Method

2.1Metal specimen and surface pretreatment

The Copper plate, which was used for the experiment having elemental composition: Zn (0.69%)Specimens were prepared polished aluminum sheet by cutting into rectangular shaped pieces having dimension of 5.13cm × 2.55cm × 0.094cm (Cu – 99.31%) with a small hole of 2mm diameter near the upper edge, were used for the determination of the corrosion rate.

2.2 Preparation of test solution

All the chemicals and reagents used were of analytical grade and used as such without further purification. The aggressive media were, respectively, 0.5M, 1M and 2M TCA with and without inhibitor solution. *CP* leaves extract was used as inhibitor in 6, 8, 10, 12 mM concentration. Only one specimen was suspended by a V shaped glass hook, in each beaker containing 230ml of the test solution and was open to air at room temperature for 24 hours duration.

2.3 Preparation of inhibitor

Stock solution prepared by extraction of *CP* by refluxing 100 gm of dry material in 500 ml distilled water for 2 hours. The refluxed solution was filtered to remove any contaimination. The concentration of the stock solution was calucated in terms of mM.

Result and Discussion

3.1 Mass loss measurement

The value of percentage inhibition efficiency (I.E. %) and corrosion rate (C.R) obtained from mass loss method at different concentration.

The inhibition efficiency was determined using the below given relationship.

$$\textbf{Inhibition efficiency}(\mathbf{I.E}\%) = \frac{W_0 - W_1}{W_0} \times 100 \qquad (1)$$

The results of inhibition efficiency (I.E. %) were calculated and are presented in Table – 1

The inhibition efficiency decreases with the increase in 0.5M, 1M and 2M TCA. Maximum inhibition efficiency of 12 ml *CP* inhibitor is 94.40, 88.42 and 72.31 % with respect to 0.5M, 1M and 2M TCA after 24 hours exposure time. For example, 1M TCA the inhibition efficiency was found to be 69.11, 73.36, 81.85 and 88.42 % with respect to 6, 8, 10 and 12 ml inhibitor concentration. (Figure-2). Mass loss Vs Acid concentration of copper of TCA. (Figure-1).

Degree of Surface coverage (θ) for different concentration of the inhibitor in acidic media have been evaluated from mass loss experiments using this relation (Table-2).

$$\boldsymbol{\theta} = \left(\frac{W_0 - W_1}{W_1}\right) \qquad (2)$$

TABLE-1

Mass loss (mg/sq.m^2) and inhibition efficiency (I.E. %) for copper in TCA containing with given inhibitor addition of *CP*.
Temperature 301±1K Immersion period: 24h Effective specimen area: 0.2727 sq.m^2

Acid concentration							
Inhibitors	Inhibitor Conc. (mM)	0.5 M		1 M		2 M	
		Mass loss (mg/sq.m^2)	I.E. (%)	Mass loss (mg/sq.m^2)	I.E. (%)	Mass loss (mg/sq.m^2)	I.E. (%)
Blank	-	458.38	-	949.76	-	1191.79	-
Papaya (Carica Papaya)	6ml	69.67	84.80	293.36	69.11	469.38	60.61
	8ml	58.67	87.20	253.03	73.36	396.04	66.77
	10ml	40.34	91.20	172.35	81.85	359.37	69.85
	12ml	25.67	94.40	110.01	88.42	330.03	72.31

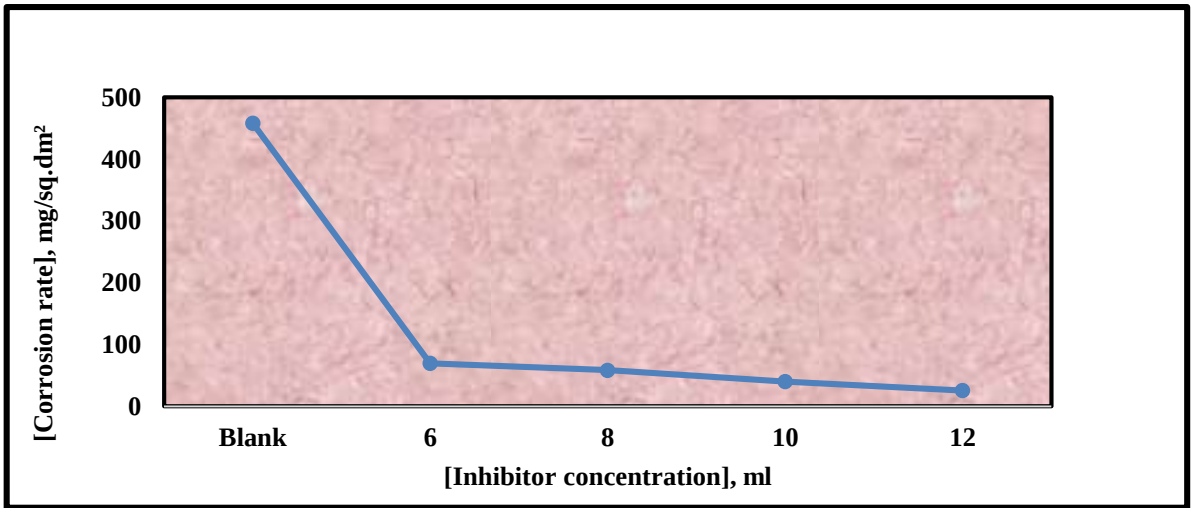
The plot of C_{inh} versus Cinh/θ (inhibitor concentration) shows straight line Figure-3, which indicate that the inhibition action appears to be the chemisorption and inhibitors cover both anodic and cathodic region through general adsorption following Langmuir isotherm.

$$\frac{\mathbf{Cinh}}{\boldsymbol{\theta}} = \frac{\mathbf{1}}{\boldsymbol{Kads}} + \mathbf{Cinh} \qquad (3)$$

TABLE-2
Corrosion rate (Log ρ) of aluminum in 2M TCA in absence and presence of *CP*for an immersion period of 24 h.

Inhibitor Conc. (mM)	C.R (ρ)	Log ρ	I.E (%)	Surface coverage (θ)	1-θ	Log (θ/1−θ)
Blank	1191.79	3.0762	-	-	-	-
6ml	469.38	2.6715	60.61	0.6061	0.3939	0.1871
8ml	396.04	2.5977	66.77	0.6677	0.3323	0.3030
10ml	359.37	2.5555	69.85	0.6985	0.3015	0.3648
12ml	330.03	2.5186	72.31	0.7231	0.2769	0.4168

Figure 1 & 2: Corrosion rate in 0.5M, 1M and 2M TCA and I.E (%) of copper in presence of different concentration of *CP* extract for an immersion period of 24 h.



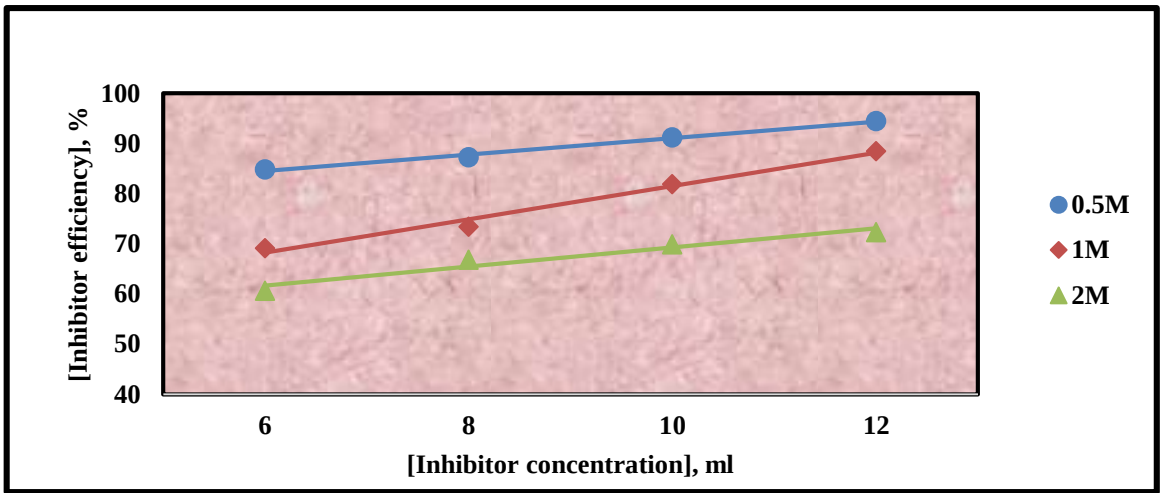


Figure 3: Langmuir adsorption isotherm for corrosion of copper in 0.5M TCA Solution containing different concentration of *CP* extract for an immersion period of 24 h.

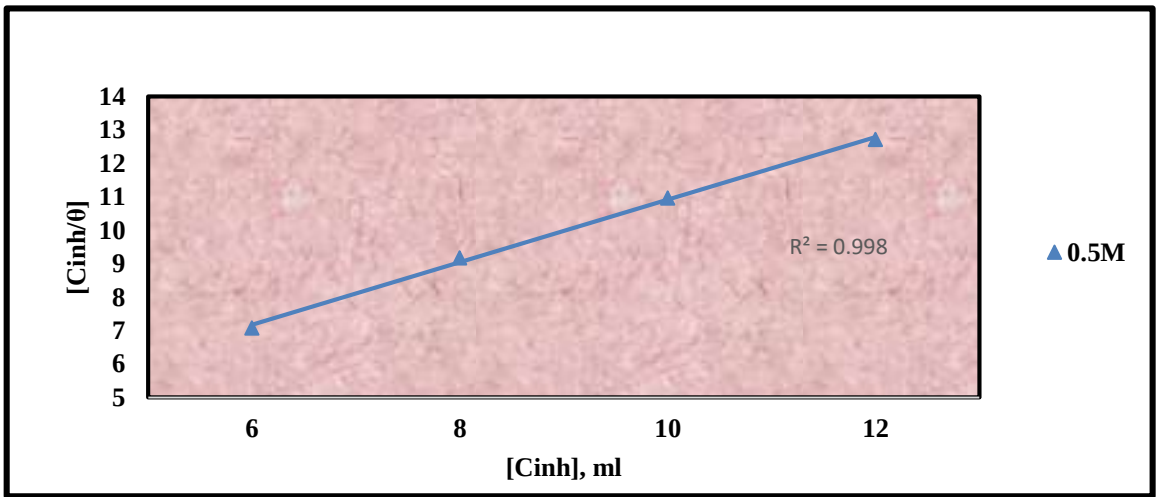
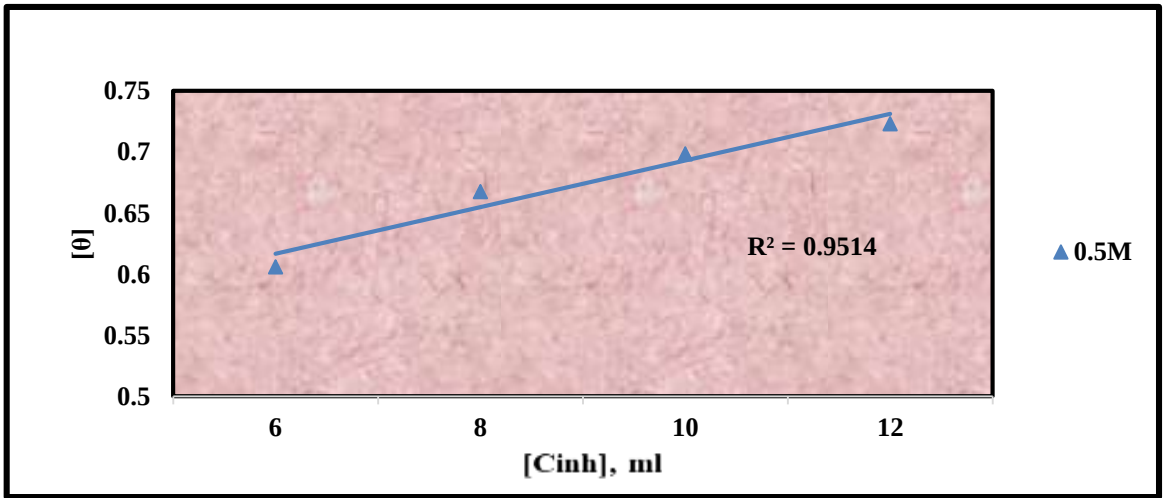


Figure 4: Temkin adsorption isotherm for corrosion of copper in 0.5M TCA Solution containing different concentration of *CP* extract for an immersion period of 24 h.



3.2 Effect of temperature

The study the effect of temperature on corrosion rate, the specimen were immersed in 230 ml of 0.5 M TCA solution with *CP* inhibitor. Corrosion rate was measured in 0.5 M TCA containing temperature of 313, 323 and 333 K at 6, 8, 10 and 12 ml inhibitor concentration for 2 hours exposure time. The effect of temperature was used thermostat assembly with an accuracy of ± 0.5.

$$\log \frac{P_1}{P_2} = \frac{Ea}{2.303R}\left[\left(\frac{1}{T_1}\right) - \left(\frac{1}{T_2}\right)\right] \quad (4)$$

Where P_1 and P_2 are corrosion rates at temperature T_1 and T_2 respectively.

TABLE – 3

Effect of temperature on corrosion rate (C.R) inhibition efficiency (I.E. %) and activation energy (Ea) for copper in 0.5 M TCA containing with given inhibitor addition of *CP*.

Immersion period: 2h Effective specimen area: 0.2727 sq.m^2

Inhibitor Concentration	Temperature K						Mean(Ea) from eqation(4) (kJ/Mol)
	313K		323K		333K		
	C.R mg/sq.m²	I.E. %	C.R mg/sq.m²	I.E. %	C.R mg/sq.m²	I.E. %	
Blank	290.01	-	333.70	-	396.04	-	13.56
6 ml	77.00	73.45	106.34	68.13	146.68	62.96	27.09
8 ml	62.34	78.50	84.34	74.23	121.01	69.45	28.85
10 ml	40.34	86.09	55.01	83.52	91.68	76.84	32.86
12 ml	29.34	89.88	40.34	87.91	62.68	84.26	35.88

The effect of change in temperature on corrosion rate of copper in 0.5 M TCA. Previous investigators showed that the corrosion rate increase with increase in temperature [17]. Effect of inhibition efficiency and activation energy (Ea) were calculated in (Table - 3) of *CP* extract for copper at 0.5 M acid and 6, 8, 10 and 12 ml inhibitor concentration. (Figure-5 & 6).

Figure 5: Effect of temperature on I.E (%) for copper corrosion in 0.5 M TCA at different inhibitor concentration of *CP* extract for immersion period of 2 h.

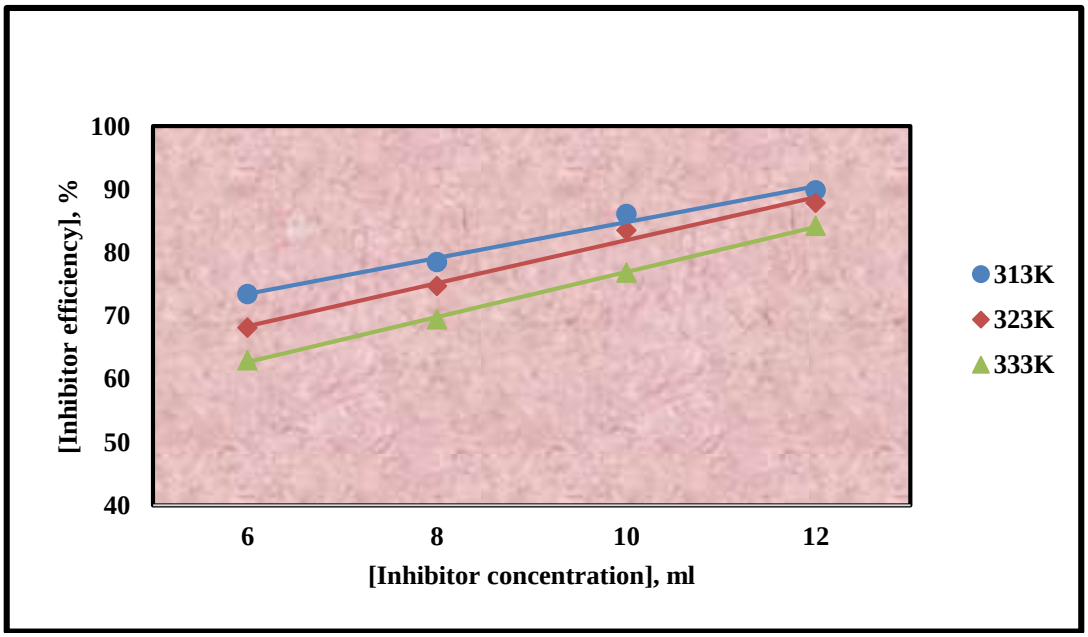
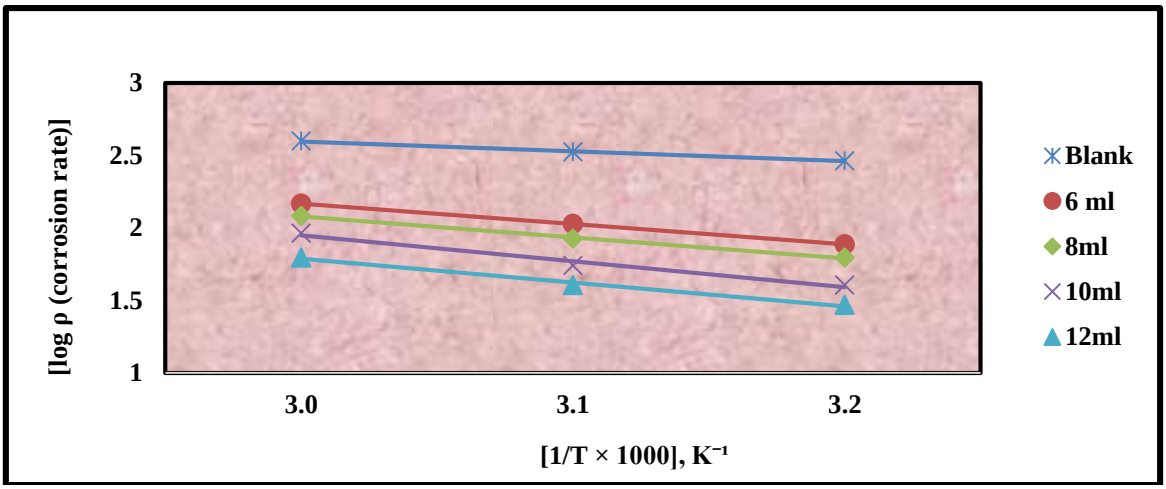


Figure 6: Effect of Arrhenius plots for copper in 0.5 M TCAin absence and presence of the different temperature of *CP* extract.



3.4 Potentiodynamic study

Corrosion behavior of anodized copper sample were study as per standards in 12 ml inhibitor and 0.5 M TCA solution using potentiostat Gamry reference 600. Corrosion cell which consists of calomel electrode as reference electrode graphite rod as counter electrode and test sample as working electrode. The important corrosion potential (E_{corr}), cathodic and anodic Tafel slop (β_a and β_c) value were obtained by extrapolating the Tafel straight line on the Tafel plot.

$$\eta\ \% = \frac{\mathbf{icorr - icorr(inh)}}{\mathbf{icorr}} \times \mathbf{100} \quad (5)$$

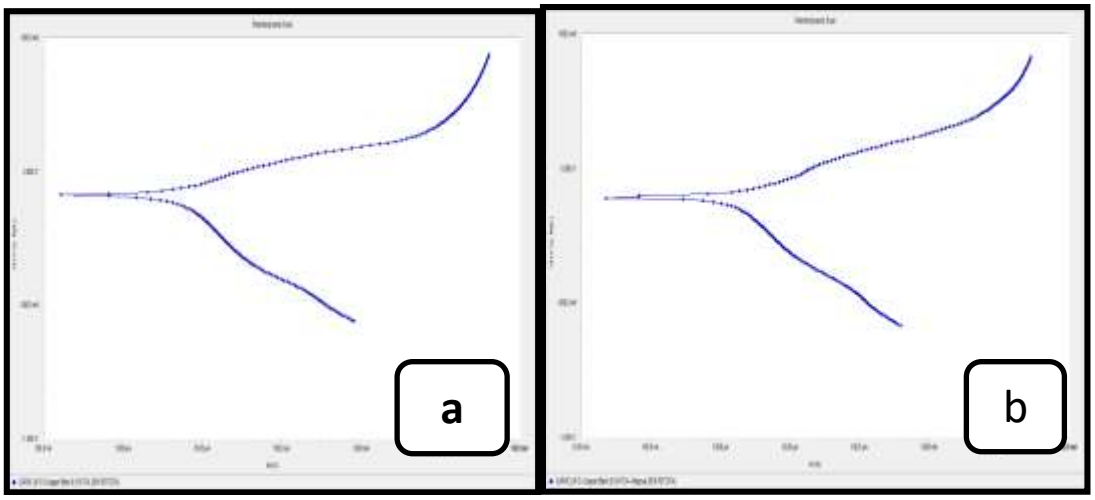
Potentiodynamic curve of *CP* extract in 0.5 M solution with 12 ml of *CP* extract are shown. (Figure-7). In anodic value of β_a decreases with presence of *CP*extract. The inhibition efficiency (η %) increased with *CP* extract concentration reaching a maximum value 94.40 % at 12 ml.

TABLE-4

Potentiodynamic data and inhibition efficiency I.E (%) for copper in 0.5 M TCA at 12 ml *CP* inhibitor.

System	E_{corr}(mV)	I_{corr}(μA)	Tafel Slop			Inhibition efficiency (I.E %)	
			Anodic +βa	Cathodic -βc	β (mV)	By Polarization Method	By Mass Loss Method
Blank	-88.10	5.860	111.80	327.20	36.18	-	-
CP	-110.00	0.399	67.40	117.30	18.56	93.19	94.40

Figure 7: Potentiyodynamic polarization curve for copper in (a) 0.5 M TCA and (b) 0.5 M TCA in the presence of 12 ml *CP* extract.



3.5 Electrochemical Impedance spectroscopy measurement (EIS)

EIS were carried over the frequency range from 10 kHz to 0.01 Hz at open circuit potential. The capacitive semicircle at higher frequencies is attributed to the redox Al-Al^{+3} reaction since it was assumed to be the rate determining step in the charge transfer process [11]. Therefore, the resistance value obtained from intercept of the first capacitive semicircle with real axis corresponds to the Al-Al^{+3} charge transfer resistance.

Nyquist plots of copper in 0.5 M TCA solution in the presence of 12 ml concentration of*CP* extract are given in (Figure-8), where it can be observed that the diameter of the semicircles increase with increasing *CP* extract concentration. The increase capacitive semicircles suggests that the inhibition action of these inhibitor is due to their adsorption on the metal surface with altering the corrosion mechanism.

$$I.E\% = \frac{\mathbf{Rct(inh) - Rct}}{\mathbf{Rct(inh)}} \times \mathbf{100} \quad (6)$$

$$Al\text{-}Al^{+3} + 3e^{-} \text{ (anode)}$$

$$H^{+} + e^{-} \rightarrow H \text{ (cathode)}$$

Followed by the reaction,

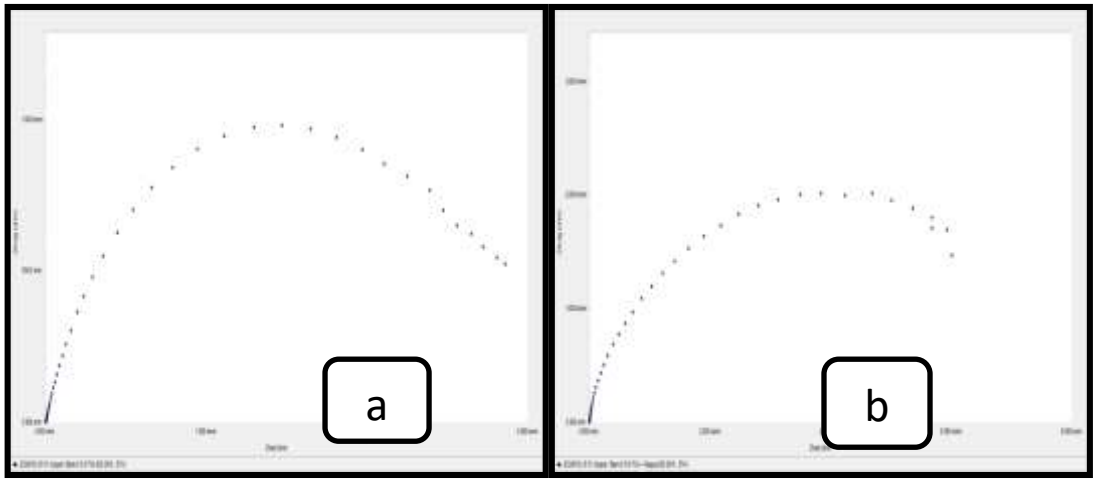
$$H + H \rightarrow H_2$$

The following secondary reaction can also take place in TCA solution [18].

$$2M + 2H^{+} \rightarrow H_2 + 2M^{+} \text{ (anode)}$$

$$O_2 + 4H^{+} + 4e^{-} \rightarrow 2H_2O \text{ (cathode)}$$

Figure 8: Nyquist plot for copper in (a) 0.5 M TCA and (b) 0.5 M TCA in presence of 12 ml *CP*.



APPLICATIONS

This study is useful for prevention of corrosion of copper by Papaya (Carica Papaya*)*leaves extract as green inhibitor in TCA media. The corrosion of copper is mainly controlled by charge transfer process.

Conclusions

From the present study, it is concluded that *CP* extract can be used as an effective inhibitor for copper corrosion in TCA medium. At all concentration of acid, as the inhibitor concentration increases inhibition efficiency increases and corrosion rate decreases.As the temperature increases corrosion rate increases in plain acid.It has also been found that the inhibitive action of *CP* extract is basically controlled by temperature and the concentration of the inhibitor.

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Referance

1. J. Alam, M. Kashif, S. Ahmad and A. W. Mohammad. (2010), Lawsonia Inermis as green inhibitor for corrosion protection of aluminium alloy, World Appl. Sci. J. Vol. 9, 01-05
2. El Hosary A A, Saleh R M and Shams El Din A M. (1972), "Corrosion inhibition by naturally occurring substance-I. The effect of Hibiscus Subdariffa (karkade) extract on the dissolution of Al", Corrosion Science. Vol.12, 897-904
3. Molina-Ocampo L B, M G valladares-Cisneros and J G Gonzalez-Rodriguez. (2014), "Using Hibiscus Sabdariffa as corrosion inhibitor for Al in 0.5M H_2SO_4"International Journal Electrochemical Science. Vol.10, 388-403
4. Ating E I, S A Umoren, I I Udousoro, E E Ebenso and A P Udoh. (2010), "Leaves extract of Ananas Sativum as green corrosion inhibitor for aluminum in HCL acid solution", Green Chemistry Letter and Review. Vol.3, 61-68
5. El- Etre. (2003), "Inhibition of aluminum corrosion using Opuntia extract", Corrosion Science. Vol.45, 2485-2495
6. Sharma P, Chaturvedi A, Upadhyay R K and Parashar P. (2008), "Study of corrosion inhibition efficiency of naturally occurring Argenmone Mexicana on aluminum in HCL solution", Journal of Technology Research Chemistry. Vol.15(1), 21
7. Al-Turkustani A M, Arab S T and Aldahiri R H. (2010), "Aloe plant extract as an environmentally friendly inhibitor on the corrosion of aluminum in HCL in absence and presence of Iodide ions", Journal of Modern Applied Science. Vol.4, 105-124
8. James A O and Etela A O. (2008), "Aloe-vera an inhibitor of aluminum corrosion in HCL", Journal of Pure and Applied Chemistry. Vol.3(3), 159-163
9. Abdel-Gaber A, Abd-El-Nabey B and Saadaway M. (2009), "Inhibitive action of Cannabis plant extract on the corrosion of copper in 0.5M H_2SO_4", Corrosion Science. Vol.51, 1038

10. Oguzie E E. (2007), "Corrosion inhibition of aluminum in acidic and alkaline media by Sansevieria Trifasciata extract", Corrosion Science. Vol.49, 1527-1539
11. Nnanna L A, Obasi V U, Nwadiuko O C, Mejeh K I, Ekekwe N D, Udensi S C. (2012), "Inhibition by Newbouldia leavis leaf extract of the corrosion of aluminum in HCL and H_2SO_4 solution", Archives of Applied Science Research. Vol. 4(1), 207-217
12. Deng S and X Li. (2012), "Inhibition effect of Dendrocalamus Brandisii leaves extract on aluminum in HCL solution", Corrosion Science. Vol.65, 299-308
13. Desai P S and Vashi R T. (2010), "Inhibitive efficiency of Xylenol Orange as corrosion inhibitors for aluminum in TCA", Indian Journal of Chemical Technology. Vol.17, 50-55
14. Desai P S and Kapopara S M. (2014), "Inhibitory action of Xylenol Orange on aluminum corrosion in HCL solution", Indian Journal of Chemical Technology. Vol.21(2), 139-145
15. Desai P S and Vashi R T. (2011), "Inhibitive efficiency of Sulphathiazole for aluminum corrosion in TCA", Anti Corrrosion Methods and Materials. Vol.58(2), 70-75
16. El-Etre A Y, Abdallah M and El-Tantawy Z E. (2005), Corrosion inhibition of some metals using Lowsonia extract, Corrosion Science. 47(2), 385-395
17. Prassan Singh Pratihar, Monika, Verma P Sand Alka Sharma. (2015), "Cappris Decidua Seed: Potential green inhibitor to combat acid corrosion of copper" Rasayan Journal of Chemistry. Vol.8(4), 411-421
18. Abdallah Y M and Shalabi K. (2015), "Comprehensive study of the behavior of copper inhibition in 1 M HNO_3 by Euphorbia helioscopia linn.extract as green inhibitor" Protection of Metals and Physical Chemistry of Surface. Vol.51(2), 275-284
19. Zuo Y, Zhao P H and Zhao J M. (2003), "The influences of sealing method on corrosion behavior of Anodized aluminum alloys in NaCl solution", Surfactant Coatings Technology. Vol.166, 237-242
20. Desai P S and Desai S A, Vashi R T. (2008), "Anisidine as corrosion inhibitors for aluminum in TCA", Acta Ciencia Indica. Vol.2, 209-217

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28 Photocatalytic Degradation of Acid brown dye in wastewaters

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Abstract

In this research work Titanium dioxide (TiO_2) semiconductor is used for degradation of Acid Brown 349 dye in presence of hydrogen peroxide (H_2O_2) under direct solar light. The % degradation reaction is affected by many parameters such as concentration of dye, pH of solution, amount of semiconductor, concentration of hydrogen peroxide, and light intensity. Maximum solar light degradation was observed at 60 ppm concentration of Acid Brown 349 dye solution, 5 mg of TiO_2, 5 ml of H_2O_2, 2.0 pH, under 350 Lux after 180 minutes were found as optimal conditions. After this experiment we perform the quencher effect in typical run. In the quencher effect there is no effect on degradation of dye.

Introduction

Clean water is the important and valuable thing to every living creature but pollutants are the major challenge to obtain clean water. Dyes are the major pollutants of water of rivers, lake and underground water. Pollutants of waste water mainly come from industries such as; textile, printing, painting, leather, agro allied companies – pesticides, insecticides and fertilizers. [1,2] Water is the most important natural resource for life on earth, and it is usually contaminated with materials including organic pollutants. Some serious problems and diseases have been created due to an inadequacy of clean water. [3,4] In recent years, Pollution of clean water has become an alarming situation due to the discharge of industrial effluents, which mostly includes non-degraded dyes, at an elevated rate. The annual production of dyes is estimated to be 70,000 tons [5]. Dyes and coloured pigments are widely used in the textiles, paper, plastics, leather, food and cosmetic industry to colour their products.[6] Textile effluents can affect the ecosystem by decreasing water transparency and sunlight penetration, thereby changing photosynthetic activity and gas solubility of clean water [7]. Colour-containing wastewater originated from textile industries is one of the most important sources of pollution that causes dangerous effects on environment and human beings health [8,9]. "A dye is a coloured organic compound that strongly absorbs light in the visible region.[10] Dye molecules comprise of two key components Chromophores and Auxochromes. The chromophores, responsible for producing colour, and the auxochromes, which cannot only supplement the chromophore but also render the molecule soluble in water and give enhanced affinity (to attach) toward the fibres [10]. Chromophores determine the colour of the dye while the auxochromes determine the intensity of the colour [11]. Some common auxochrome groups include $–NH_3$, –COOH, $–SO_3H$, and –OH [12].

Acid dyesare water-soluble anionic dyes that are applied to fibres such as silk, wool, nylon and modified acrylic fibres using neutral to acid dye baths. Acid dye used in present study is Acid Brown 349.

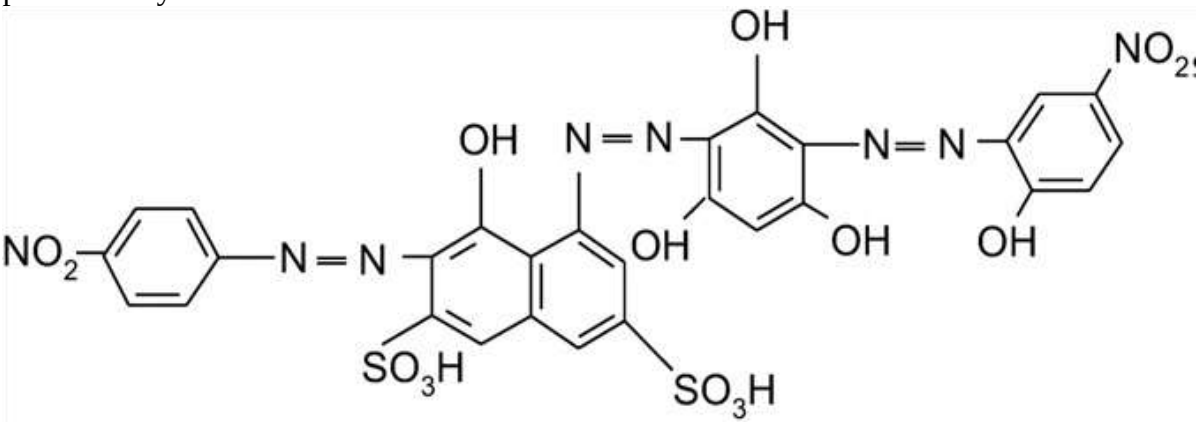

Fig.1 Structure of Acid Brown349 dye

Molecular formula of this dye is $C_{28}H_{18}O_{15}N_8S_2$ and molecular weight of this dye is 770 g/mol. This dye is supplied by Shree Balaji processor G.I.D.C, Ankleshwar, Gujarat, India.

This is a trisazo dye because it is an azo dye containing three azo groups(-N=N-), two nitro groups(-NO_2-), five hydroxyl groups(-OH) and two sulphonic acid(-SO_3H) groups. Other name of this dye is Jotzadope Brown SG.

This dye is highly soluble in water and colour of dye is reddish light brown. $\lambda_{max}$of this dye is 438 nm. This is used for wool, dyeing and printing and leather shading etc.

Many types of dye and textile industries are located in India, many of them are present in Gujarat. Many companies are based in ankleshwar in Gujarat manufacture different dyes and use them to dye fabrics. As the dye is dissolved in water and then the fabrics are dried and washed in water. So, this washing water is known as wastewater. This type of water will go into the river and pollute the water as well as the land. So, this problem is a serious issue. So, it was dealed to find a method for removing dye from wastewater.

In the late 90's, dye removal methods include only preliminary water purification method such as sedimentation and equalisation due to the fact that there was no dye effluent discharge limit [13]. After permissible dye effluent release standards were established, improvements were made by introducing more effective dye removal methods such as dye degrading filter beds and activated sludge processes [14] This system, known as the traditional dye removal method, was implemented by the concerning industries for some time till it was stopped due to its high cost of operation and maintenance [15].

Photochemical Method

Photocatalyst is explain as a species, which motivate photochemical reaction and photocatalysis is used for those chemical reactions, which occur in the presence of photocatalyst and light. In this photochemical reaction, light is never act as a catalyst but act as reactant. Light is absorbed by a catalyst or substrate or semiconductor. The energy of photon is used to generate electronically excited molecule which never comes out in the same from after the reaction and further, it cannot be used in photo assisted or photo induced reactions. The rate of reaction as

per explanation of catalyst is enhanced by reducing activation energy (Ea) where the light absorbed cannot alter the energy level of the product or reactant, or the free energy change of overall reaction. So, the concept of photocatalyzed reaction seems to be basically improper [16].

Photocatalysis

The word 'Photocatalysis' was originated from Greek word which is made up of two parts. The first one is "photo" means (phos: light) and second one is "catalysis" means (Katalyo: Decompose). It has been a widely studied technology since the 1970s [17], A Photocatalysis process need solid semiconductor which is activated when absorbs near UV light or UV [18]. The role of semiconductor is as a sensitizer for processes due to their electronic configurations or light- induced redox reactions, which is characterized by an empty conduction band (CB) and a filled valence band (VB) [19]. The band gap energy is the energy difference between the valence band and conduction band [21]. In which, absorption of EMR by the semiconducting materials can excites an electron (e^-) from the valence band to the conduction band if the photon energy, hυ, vary close or equal to the threshold energy or band gap of photocatalyst or semiconductor [22]. At the same time, an electron vacancy or a positive charge r called a hole (h^+) has also been generated in a valence band. The lifetime of holes (h^+) pair and an electron (e^-) is few nanoseconds but it is sufficient to promote redox reactions in the gas or solution phase in presence of the semiconductor [17-20].

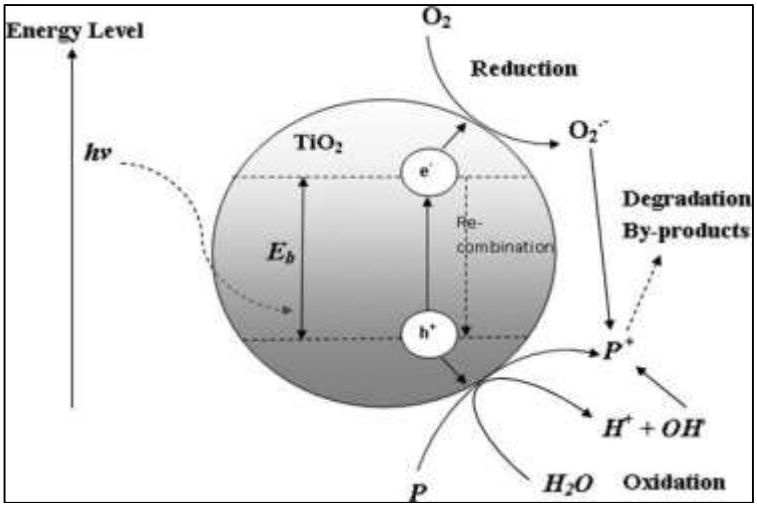

Fig. 2. The mechanism of photoinduced formation of an electron-hole pair in a semiconductor TiO_2 particle in the presence of water pollutant (P).

1.Types of photocatalysis

Mainly two types of photocatalysis are known.

(i) Homogenous photocatalysis

In homogeneous photocatalysis process, the photocatalysts and reactant are in the same phase. The most commonly used homogeneous photocatalyst are the transition metal oxide, ozone and the photo-Fenton system.

(ii) Heterogeneous photocatalysis

Heterogeneous photocatalysis can be defined as the reaction in which reactants and products both are in a different phase. Heterogeneous reactions are two phase

systems where the reactants are in liquid or gas phase and catalyst in the solid phase. The initiation of the photocatalytic reaction it requires also the activation by photons, the absorbance on the surface of the catalyst.[23]

Importance of work

Water pollution due to influence addition of dye effluents is a major problem faced by India, in specially Gujrat is a big centre producing dye intermediates that are banned various other countries of world.

Dyes are used in many types of industries such as plastic, food, cosmetics and textile in order to colour their products. Among various industries, textile industry ranks first in usage of dye where the main source of wastewater is generated from the washing and bleaching of natural fibres and 100,000 different commercial dyes and pigments; about 7×10^5 tons of dyestuff produced annually. It is estimated that 10-15% of the overall production of dyes is released into the environment via waste water. So, technology is needed where in dye bath effluents are treated before they are mixed with other textiles effluents. Acid Brown 349 dye is used for wool dyeing, leather and textile industry. In Gujarat GIDC Ankleshwar is synthesising and applying on to textiles.

Plan of Work

From detailed literature survey it has been found that Acid Brown 349 textiles dyes that are not studied for degradation process. So, it is planned to study this dye. Since Solar energy is easily available throughout India. So, solar energy was considered to degrade this dye.

The photocatalytic method will be used to optimization the photocatalytic process effect of concentration, effect of TiO_2, effect of pH, effect of H_2O_2 will be considered.

Considering the effect of various constraints, a tentative mechanism will be proposed for this selected degradation method.

This developed method will be applied to hypothetical waste water containing Acid brown 349 dye.

EXPERIMENTAL

In this experiment all reagents and chemicals used in present study were purchased among the product of high purity (Analytical Grade). Deionized water was used throughout the work.

Some chemicals and instruments are used in present study like Acid Brown 349 dye, Titanium dioxide (TiO_2), Hydrogen peroxide (H_2O_2), Sodium hydroxide (NaOH), Hydrochloric acid (HCl), Methanol (CH_3OH), Ethanol (C_2H_5OH) and Iso propyl alcohol etc. and some instruments are weight machine, LUX meter, pH meter, Centrifuge instrument, stop watch, UV-Vis Spectrophotometer and HPTLC instrument.

Stock solution of Acid Brown 349 dye is 200 ppm and it was prepared in double distilled water.

In this present work advance oxidation processes (AOPs) are applied for the study of the photocatalytic degradation of Acid Brown - 349 dye, H_2O_2 and TiO_2 are used as oxidant. All the solutions were prepared in deionised water. The intensity of solar light was measured with the help of a digital Lux meter in units of $mWcm^{-2}$. The pH meter was used to measure the pH of dye solutions at specific values.

Establishment of protocols

(i) Control Experiments
Beaker 1: - 60 ppm Acid Brown 349 dye solution was kept in visible light.
Beaker 2: - 60 ppm Acid Brown 349 dye solution and 5 ml of (30%) H_2O_2 was kept invisible light.
Beaker 3: - 60 ppm Acid Brown 349 dye solution and 5 mg TiO_2 was kept in visible light.
Beaker 4: - 60 ppm Acid Brown 349 dye solution, 5 ml of (30%) H_2O_2 and 5 mg TiO_2 was kept visible light.
Beaker 5: - 60 ppm Acid Brown 349 dye solution was kept in UV light.
Beaker 6: - 60 ppm Acid Brown 349 dye solution and 5 ml of (30%) H_2O_2 was kept inUV light.
Beaker 7: - 60 ppm Acid Brown 349 dye solution and 5 mg TiO_2 was kept in UV light.
Beaker 8: - 60 ppm Acid Brown 349 dye solution, 5 ml of (30%) H_2O_2 and 5 mg TiO_2 was kept UV light.
Beaker 9: - 60 ppm Acid Brown 349 dye solution was kept in solar light.
Beaker 10: - 60 ppm Acid Brown 349 dye solution and 5 ml of (30%) H_2O_2 was kept insolar light.
Beaker 11: - 60 ppm Acid Brown 349 dye solution and 5 mg TiO_2 was kept in solar light.
Beaker 12: - 60 ppm Acid Brown 349 dye solution, 5 ml of (30%) H_2O_2 and 5 mg TiO_2 was kept solar light.
All the beakers were checked for absorbance after 180 minutes with the help of UV-visible spectrophotometer (Thermo scientific-evolution 201). There was no specific change in absorbance of first eight beakers. But the 9 to 11 beakers were showed nominal changes in absorbance and beaker 12 was showed remarkable changes in absorbance at specific wavelength.
(ii) Reaction mixture Preparation
20, 40, 60, 80, 100 ppm dye concentration is used in the present experiment. For each sample preparation 60 ppm 20ml dye solution is added to H_2O_2 (30%) 5 ml and 5 mg TiO_2 in a 50 ml beaker.
(iii) Progress of Reaction
The progress of reaction was observed by measuring optical density of the reaction mixture containing dye at different time intervals during exposure. A decrease in optical density indicates that the dye was bleached during this photochemical process. λ_{max} for Acid Brown 349 dye is 438 nm.

Results & Discussion

In this experiment first we show the typical run and after show the different parameter like effect of concentration, effect of pH, effect of TiO_2, effect of H_2O_2, effect of quencher and HPTLC analysis of degraded samples.

Preparation of Typical run;

To perform this photochemical reaction 20ml of dye solution of fixed concentration (60 ppm), volume of H_2O_2 (5 ml), amount of photocatalyst TiO_2 (5mg), pH was maintained at 2 using pH meter and was taken in 50 ml beaker added to it. This reaction mixture was put into sunlight using lux meter to measure the intensity of light

provided energy to animated TiO_2 loading. At every 30 minutes time intervals, solution was centrifuged for 5-7 min. From this centrifuged solution, about 5 ml of the dye solution was take out after a specific 30 minutes interval and measured its absorbance using UV-Vis Spectrophotometer at 438 nm. The rate of decreases of colour with time was constantly monitored. The result of photocatalytic degradation of Acid Brown 349 dye is graphically showed in Table 1. Control these experiments confirm the required photocatalyst, oxygen and light to follow the photocatalytic path for the degradation of dye. Photocatalytic degradation of Acid Brown 349 dye was studied at 438 nm. The optimum condition for the removal of dye concentration = 60 ppm, pH = 2.0, Volume of H2O2 (30%) = 5 ml, Amount of TiO2 = 5 mg, Light Intensity = 350×10^2 Lux.

Table 1

Volume of Dye = 60 ppm (20 ml), pH = 2, Volume of H_2O_2 (30%) =5 ml
Amount of TiO_2 = 5 mg, Light Intensity = 350×10^2 Lux.

Time	O.D.	2+log O. D	R^2
0	1.284	2.1085	0.9751
30	1.117	2.0480	
60	0.971	1.9872	
90	0.834	1.9212	
120	0.655	1.8162	
150	0.576	1.7604	
180	0.403	1.6053	

Effect of concentration

In this effect I tried the different concentration of dye solution and other parameters are same. In different concentration I tried 20 ppm to 80 ppm dye concentration and H_2O_2 is 5 ml and TiO_2 is 5 mg. The result shows the maximum degradation at30 ppm and 60 ppm are 75.55 % and 68.61 % respectively after 180 min. But for optimal condition we take 60 ppm dye concentration because I tried degrade this at higher concentration.

Table 2

Volume of Dye = 20-80ppm, pH = 3.12,Volume of H_2O_2 (30%) = 5 ml
Amount of TiO_2 = 5 mg, Light Intensity = 350×10^2Lux

Time	20 ppm	30 ppm	40 ppm	50 ppm	60 ppm	70 ppm	80 ppm
0	0.487	0.716	0.988	1.043	1.284	2.026	2.443
30	0.425	0.591	0.895	0.918	1.117	1.965	2.440
60	0.358	0.429	0.725	0.803	0.971	1.891	2.247
90	0.300	0.337	0.611	0.695	0.834	1.549	1.843
120	0.215	0.238	0.490	0.571	0.655	1.450	1.781
150	0.185	0.178	0.397	0.498	0.576	1.291	1.542
180	0.159	0.175	0.300	0.404	0.403	1.060	1.377

Effect of pH

I have also studied the degradation of dye under different pH interval. In this study the pH is measure by pH meter and pH interval is 2-10 pH. And other parameters are same. The result obtained at maximum degradation of dye at 2.0 pH. The maximum percentage of degradation is 88.07 %. Optimal condition for pH is 2.

Table 3

Volume of Dye = 60ppm, Amount of TiO_2 = 5 mg, pH = 2 – 10.
Volume of H_2O_2 (30%) = 5 ml, Light Intensity = 350×10^2Lux.

Time	2 pH	4 pH	6 pH	8 pH	10 pH
0	1.510	1.428	1.546	1.205	0.470
30	0.744	1.183	1.256	0.765	0.176
60	0.340	1.055	1.194	0.709	0.177
90	0.184	0.419	0.816	0.522	0.113
120	0.182	0.417	0.810	0.510	0.108
150	0.181	0.415	0.807	0.506	0.106
180	0.180	0.414	0.805	0.504	0.105

Effect of TiO_2

We have also studied the degradation of dye under different amount of TiO_2. In this study the different amount of TiO_2 is 3 mg to 15 mg. The result obtained at maximum percentage of degradation 68.61 % at 5 mg. So, optimal condition for the degradation of dye at 5 mg TiO_2.

Table 4

Volume of Dye = 60ppm, Amount of TiO_2 = 3 -12 mg, pH = 3.12.
Volume of H_2O_2 (30%) = 5 ml, Light Intensity = 350×10^2Lux.

Time	3 mg	5 mg	7 mg	10 mg	12 mg	15 mg
0	1.380	1.284	1.482	1.741	1.681	1.840
30	1.176	1.117	1.176	1.527	1.611	1.761
60	1.139	0.971	1.110	1.460	1.601	1.743
90	1.050	0.834	1.068	1.304	1.576	1.683
120	0.909	0.655	0.916	1.298	1.328	1.463
150	0.678	0.576	0.622	0.945	1.057	1.168
180	0.641	0.403	0.582	0.940	1.043	1.156

Effect of H_2O_2

We have also studied the degradation of dye under different volume of H_2O_2. In this study the different amount of H_2O_2 is 3 ml to 13 ml. The result obtained that maximum 68.61 % percentage of degradation at 5 ml H_2O_2. So, optimal condition for the degradation of dye at 5 ml H_2O_2.

Table 5

Volume of Dye = 60ppm, Amount of TiO_2 = 5 mg, pH = 3.12
Volume of H_2O_2 (30%) = 3 - 13 ml, Light Intensity = 350×10^2Lux.

Time	3 ml	5 ml	7 ml	9 ml	11 ml	13 ml
0	1.746	1.284	1.598	1.522	1.378	1.330
30	1.506	1.117	1.336	1.241	1.185	1.127
60	1.355	0.971	1.230	1.186	1.015	1.032
90	1.179	0.834	1.062	1.048	0.958	0.924
120	1.025	0.655	0.956	0.974	0.828	0.811
150	0.964	0.576	0.796	0.800	0.661	0.765
180	0.892	0.403	0.790	0.795	0.656	0.760

Effect of radical Quencher

We have also studied the quencher effect on typical run. So, we can used methanol, ethanol and isopropanol are used to radical quenching. After this process we can conclude that the not effect on degradation of quencher. Because dye sample is sensitive.After effect of quencher, we have study the HPTLC analysis of the degraded samples.After all this observation the degraded samples will be subjected to HPTLC to find out the end product.This method can be applied to treatment of effluent samples taken from dye industry.

Table 6

Volume of Dye = 60 ppm, Amount of TiO2 = 5 mg, pH = 2.
Volume of H2O2 (30%) = 5 ml, Light Intensity = 350×10^2 Lux.

Time in Min	2 ml Methanol	4 ml Methanol	2 ml Ethanol	4 ml Ethanol	2 ml Iso Propanol	4 ml Iso Propanol
0	1.187	1.450	1.443	1.420	1.775	2.018
30	0.863	1.038	1.147	1.225	1.150	1.069
60	0.689	0.988	1.141	1.205	1.083	1.026
90	0.368	0.469	0.777	0.770	0.790	0.802
120	0.317	0.424	0.757	0.735	0.774	0.791
150	0.234	0.282	0.611	0.613	0.695	0.723
180	0.161	0.183	0.466	0.492	0.639	0.648

Mechanism

The results of above experiment, A mechanism have been progress further degradation of dye using titanium dioxide as semiconductor. This mechanism is same as proposed light absorbed by dye molecules and the photons gets excited to its first singlet state [24]. This singlet state gets converted to triplet state via intercrossing. Thesemiconductor also absorbs a light get in excited state.

$$1_{Dye0} \rightarrow 1_{Dye1} \text{ (Singlet excited state)}$$
$$1_{Dye1} \rightarrow 3_{Dye1} \text{ (Triplet excited state)}$$
$$SC \rightarrow e^{+}h^{+} \text{ or } SC^{+}$$
$$h^{+}+OH^{-}\ (from\text{acid}) \rightarrow \dot{O}H$$
$$3_{Dye1} + \dot{O}H \rightarrow \text{Products}$$

An electron which excited from its valance bond jumps to conduction band, it leaves a hole there this hole takes away an electron from OH^{-} ions generating $\dot{O}H$ free radical. This is confirmed by the use of scavenger.

Conclusion

Titanium dioxide (TiO_2) semiconductor is used for degradation of Acid Brown 349 dye in presence of hydrogen peroxide (H_2O_2) under direct solar light. The % of degradation reaction is affected by may parameters such as concentration of dye, pH of solution,amount of semiconductor, concentration of hydrogen peroxide, and light intensity. Maximum solar degradation was observed at 60 ppmconcentration of Acid Brown 349 dye solution,5 mg of TiO_2, 5 mlof H_2O_2, 2.0 pH, under 350 Lux after180 minutes were found as optimal conditions. After this experiment we perform the quencher effect and HPTLC analysis of the dye solution and typical run. In the quencher effect there is no effect on degradation of dye.

References

[1] Shameem, A., P. Devendran, V. Siva, M. Raja, S. Asath Bahadur, and A. Manikandan. "Preparation and characterization studies of nanostructured CdO thin films by SILAR method for photocatalytic applications." Journal of Inorganic and Organometallic Polymers and Materials 27, no. 3 (2017): 692-699.

[2] Ahmed, M. A., Emad E. El-Katori, and Zarha H. Gharni. "Photocatalytic degradation of methylene blue dye using Fe2O3/TiO2 nanoparticles prepared by sol–gel method." Journal of Alloys and Compounds 553 (2013): 19-29.

[3] Fernández, Cristina, M. Soledad Larrechi, and M. Pilar Callao. "An analytical overview of processes for removing organic dyes from wastewater effluents." TrAC Trends in Analytical Chemistry 29, no. 10 (2010): 1202-1211.

[4] Ahmed, M. A., Emad E. El-Katori, and Zarha H. Gharni. "Photocatalytic degradation of methylene blue dye using Fe2O3/TiO2 nanoparticles prepared by sol–gel method." Journal of Alloys and Compounds 553 (2013): 19-29.

[5] Reemtsma, Thorsten, and Martin Jekel, eds. Organic pollutants in the water cycle: properties, occurrence, analysis and environmental relevance of polar compounds. John Wiley & Sons, 2006.

[6] Leena, R., and R. D. Selva. "Bio-decolourization of textile effluent containing Reactive Black-B by effluent-adapted and non-adapted bacteria." African Journal of Biotechnology 7, no. 18 (2008).

[7] Bergamini, Raphael BM, Eduardo B. Azevedo, and Lucia R. Raddi De Araújo. "Heterogeneous photocatalytic degradation of reactive dyes in aqueous TiO2 suspensions: Decolorization kinetics." Chemical Engineering Journal 149, no. 1-3 (2009): 215-220.

[8] Radha et al., 2009

[9] Nguyen, Thai Anh, and Ruey-Shin Juang. "Treatment of waters and wastewaters containing sulfur dyes: a review." Chemical engineering journal 219 (2013): 109-117.

[10] Gupta, V. K. "Application of low-cost adsorbents for dye removal–a review." Journal of environmental management 90, no. 8 (2009): 2313-2342.

[11] Moussavi, Gholamreza, and Maryam Mahmoudi. "Removal of azo and anthraquinone reactive dyes from industrial wastewaters using MgO nanoparticles." Journal of hazardous materials 168, no. 2-3 (2009): 806-812.

[12] Al-Ghouti, M. A. "Mechanisms and chemistry of dye adsorption on ditomite and modified ditomite." (2002): 1064-1064.

[13] Robinson, Tim, Geoff McMullan, Roger Marchant, and Poonam Nigam. "Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative." Bioresource technology 77, no. 3 (2001): 247-255.

[14] Mezohegyi, Gergo, Frank P. van der Zee, Josep Font, Agustí Fortuny, and Azael Fabregat. "Towards advanced aqueous dye removal processes: a short review on the versatile role of activated carbon." Journal of environmental management 102 (2012): 148-164.
[15] Adegoke, Kayode Adesina, and Olugbenga Solomon Bello. "Dye sequestration using agricultural wastes as adsorbents." Water Resources and Industry 12 (2015): 8-24.
[16] Abo-Farha, S. A. "Photocatalytic degradation of monoazo and diazo dyes in wastewater on nanometer-sized TiO2." Journal of American science 6, no. 11 (2010): 130-42.
[17] Kabra, Kavita, Rubina Chaudhary, and Rameshwar L. Sawhney. "Treatment of hazardous organic and inorganic compounds through aqueous-phase photocatalysis: a review." Industrial & engineering chemistry research 43, no. 24 (2004): 7683-7696.
[18] Ibhadon, Alex Omo, and Paul Fitzpatrick. "Heterogeneous photocatalysis: recent advances and applications." Catalysts 3, no. 1 (2013): 189-218.
[19] Chen, Dingwang, Fengmei Li, and Ajay K. Ray. "Effect of mass transfer and catalyst layer thickness on photocatalytic reaction." AIChE journal 46, no. 5 (2000): 1034-1045.
[20] Anandan, S., Y. Ikuma, and K. Niwa. "An overview of semi-conductor photocatalysis: modification of TiO2 nanomaterials." In Solid State Phenomena, vol. 162, pp. 239-260. Trans Tech Publications Ltd, 2010.
[21] Hoffmann, Michael R., Scot T. Martin, Wonyong Choi, and Detlef W. Bahnemann. "Environmental applications of semiconductor photocatalysis." Chemical reviews 95, no. 1 (1995): 69-96.
[22] Schiavello, M., and A. Sclafani. "Thermodynamic and kinetic aspects in photocatalysis." Photocatalysis: Fundamentals and Applications. John Wiley & Sons (1989): 159-173.
[23] Herrmann, Jean-Marie. "Heterogeneous photocatalysis: fundamentals and applications to the removal of various types of aqueous pollutants." Catalysis today 53, no. 1 (1999): 115-129.
[24] Panwar, O. P., Anil Kumar, Mukesh Paliwal, Rameshwar Ameta, and Suresh C. Ameta. "Use of zirconium phosphate as photocatalyst in photobleaching of some dyes." *Bull. Cat. Soc. India* 7 (2008): 105-110.

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33 Synthetic Approach, Structural Investigation and Antifungal Studies of 2-hydrazino Benzothiazole containing Schiff Base derivatives

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Abstract

The benzothiazole and Schiff base moieties are crucial functionalities due to their wide variety of antimicrobial activities and have a wide range of therapeutic properties. Keeping in view the importance of these organic moieties, Substituted 2-hydrazino-1,3-benzothiazole was synthesized via bromination of substituted aniline with ammonium thiocyanate and this product were refluxed with conc. HCl and hydrazine hydrate in presence of ethylene glycol. This new heterocyclic Organo substituted 2-hydrazino-1,3-benzothiazole derivative was a starting material, which condensed and tethered with different aromatic aldehyde pendant arm in presence of ethanol and glacial acetic acid isolating an interesting sequence of tridentate Schiff bases. Structures of the all the synthesized Schiff bases were established basis on melting point, TLC, FT-IR, 1H NMR, and MASS spectral data. Allthe synthesized Schiff bases were examined for antifungal activity when tested against four fungal strains: Candida albicans (MTCC 183), Aspergillus niger(MTCC 228), Candida tropicalis (MTCC 6192), and Fusarium oxysporium (MTCC 3656).The results obtained justify the importance of benzothiazole inthe field of therapeutics.
Keywords: Benzothiazole derivatives, Schiff base, antifungal studies

Introduction:

In recent years heterocyclic compounds and derivatives have fascinated strong interest due to their effective biological and pharmacological assets. Benz-fused compounds have been employed in the synthesis of number of pharmaceutical compounds because of the significant activities possessed by them (Benzotriazole, Benzoxazoles, Benzimidazole and benzothiazole etc.) Benzothiazole, a multifaceted nucleus, has been under research for the last two decades. Being a heterocyclic compound, benzothiazole finds use in research as a starting material for the synthesis of larger bioactive structures. Its aromaticity makes it relatively stable, although as a heterocycle, it has reactive sites which allow for functionalization [1]. Many dyes, such as Thioflavin, and pharmaceutical drugs, such as Riluzole, Zolantidine have benzothiazole analogs. From the literature survey, it has been found that extensive work has been reported on 2- substituted benzothiazole derivatives in past and evaluated for different activities like antibacterial[1], anticancer [2], antiviral [3], antitumor [4], antifungal [5], anti-inflammatory[6] antioxidative and antidiabetic [7], anticonvulsant [8]. Benzothiazole Schiff's base is a nitrogen analogue of an aldehyde/ketone in which the carbonyl group has been switched by an imine or azomethine group. They also have been used widely as antimicrobial activity. Taking this into view, certain new derivatives were synthesized taking benzothiazole asthe basic moiety.

EXPERIMENTAL:

2.1 Material and methods

All the chemicals and solvents used during the experimental studies were of analytical grade and commercially available reagents used without further purification. Melting points of all synthesized compounds were determined using open capillary tube and are uncorrected. The completion of the reaction and purity of the compounds were checked by TLC. IR spectra were recorded in KBr pellets on Perkin Elmer IR spectrophotometer (4000-400cm^{-1}) and^{1}H NMR spectra were recorded with Bruker 300 MHz using DMSO-d_6 as solvent and TMS as internal reference.Mass spectra were recorded onMass spectrophotometer by LC- MS and the spectra were interpreted.

2.2. Synthesis of 2-hydrazino benzothiazole derivative in following steps:

2.2.1 Synthesis of 6-chloro-2-benzothiazolamine (1)

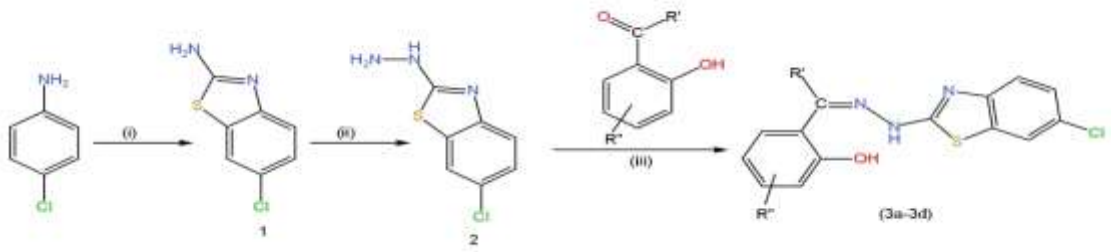
P-Chloro aniline (0.01mol) and potassium thiocyanate (0.08mol) were dissolved in glacial acetic acid (20 mL), cooled and stirred for 15 min at 2–4 °C. Cold bromine solution (0.01mol, 1.6 mL in 6 mL acetic acid) was added drop wise. Stirring was continued for 2 h and then at room temperature for 10 h. Separated hydrochloride salt was filtered off, washed with acetic acid, dissolved in hot water and neutralized with aqueous ammonia solution (25%) The resulting precipitate was filtered off, washed with water and recrystallized from ethanol.

2.2.2. Synthesis of 6-chloro-2-benzothiazol-2-yl-hydrazine (2)

With stirring to hydrazine hydrate (99%, 6 mL) solution conc. HCl (6 mL) was added drop wise at 5–10 °C temperature. To it ethylene glycol (24 mL) and compound (1) (0.03 mol) were added in portion and refluxed for 3 h. Reaction mixture cooled to room temperature and was poured to crushed ice to afford a solid which was filtered and recrystallized from ethanol to yield compound 2.

2.2.3. Procedure for the synthesis of Schiff bases (3a-3b)

6-chloro-2-benzothiazol-2-yl-hydrazine(2) (1.5mmol) and 2,4-dihydroxy benzaldehyde, o-hydroxyacetophenone, o-hydroxybenzophenone, o-vanillin (2.2mmol) and glacial acetic acid (2-3 drops) were taken in absolute ethanol (20ml) and refluxed on water bath for 5-6 hours till a different spots on TLC may appears. On cooling, solid get separated which was filtered and wash with little water and recrystallized with absolute ethanol to get the pure final compound (3a-3d). Physical and analytical data of synthesized compounds is given in Table 1. Synthetic pathway for preparation of title compounds is shown in Scheme 1.



- Scheme 1 Reagents and conditions: (i) CH3COOH, KSCN, BR2, stirring 10h; (ii) NH2NH2.H2O, ethylene glycol, reflux 3 h; (iii) ethanol, reflux 5 h.

Table 1: Some Physical Characteristics of the Synthesized Compounds.

Comp.	R'	R"	M.P(°C)	Yield (%)	Mol. form. & Wt.	Elemental analysis calcd. /found (%)					
						C	H	N	O	S	Cl
1	-	-	160-163	76	$C_7H_5ClN_2S$ 184	45.5	2.7	15.2	-	17.4	19.2
2	-	-	220-223	80	$C_7H_6ClN_3S$ 199	42.11	3.03	21.1	-	16.0	17.8
3a	H	p-OH	215-225	73	$C_{14}H_{12}O_2ClN_3S$ 319.02	52.59	3.15	13.14	10.0	10.3	11.1
3b	CH_3	-	225-228	74	$C_{15}H_{12}OClN_3S$ 317.79	56.7	3.81	13.2	5.03	10.09	11.1
3c	C_6H_5	-	192-195	78	$C_{20}H_{14}OClN_3S$ 379	63.2	3.72	11.1	4.21	8.44	9.33
3d	H	m-OCH_3	230-235	70	$C_{15}H_{12}O_2ClN_3S$ 334	53.98	3.62	12.6	9.59	9.60	10.6

2.3. Spectral data

2.3.1. 6-Chloro-2-benzothiazolamine (1)

IR ϑ_{max}cm^{-1}(KBr): 3417 (NH), 3086 (CH-Ar), 1512 (C=N), 1263, 1280 (C–N), 1082 (C–Cl), 615 (C–S–C). ^{1}H NMR (300 MHz, DMSO-d6, δ ppm): 7.12 (m, 7H, Ar-H), 7.10 (s, 1H, NHN–, D_2O exchangeable). Mass analysis MS (LC-MS) :(Calcd. For m/z[M+]183.99) Found:184.

2.3.2. 6-Chloro-2-benzothiazol-2-yl-hydrazine (2)

IR ϑ_{max}cm^{-1}(KBr): 3484 (NH), 3084 (CH-Ar), 1548 (C=N), 1257 (C–N), 1112 (C–Cl), 604 (C–S–C). 1H NMR (300 MHz, DMSO-d6, δ ppm): 7.87 (m, 7H, Ar-H), 4.0 (s, 1H, NHN–, D2O exchangeable). Mass analysis MS (LC-MS):(Calcd. For m/z [M+] 199) Found:199.

2.3.2. 4-[2-(6-chloro-1,3-benzothiazol-2-yl)hydrazin-1-ylidene]benzene-1,3-diol (3a)

IR ϑ_{max} cm^{-1} (KBr): 3309 (OH), 3042 (CH-Ar), 1604 (azomethine C=N), 1267 (C–N), 1114 (N–N), 1068 (C–Cl), 578 (C–S–C). ^{1}H NMR (300 MHz, DMSO-d6, δ ppm): 8.34–7.45 (m, 7H, Ar-H), 8.027 (s, 1H, Ar-CH=N), 5.0 (s, 1H, Ar-OH), 7.12 (s, 1H, NHN–, D2O exchangeable).Mass analysis MS (LC-MS):(Calcd. For m/z [M+] 319.02) Found:319

2.3.3. 1-(2-Hydroxyphenyl)ethanone(6-chloro-1,3- benzothiazol-2-yl)hydrazine (3b)

IR ϑ_{max} cm^{-1} (KBr):3315 (OH), 3042 (CH-Ar), 2981, 2805 (CH Aliph.), 1558 (azomethine C=N), 1267 (C–N), 1114 (N–N), 1086 (C–Cl), 611 (C–S–C). ^{1}H NMR (300 MHz, DMSO-d6, δ ppm): 0.91 (s, 3H, CH3), 7.48 (m, 7H, Ar-H), 5.0 (s, 1H, Ar-OH), 7.12 (s, 1H, NHN–, D2O exchangeable). Mass analysis MS (LC-MS) :(Calcd. For m/z [M+] 317.04) Found: 317.

2.3.4. 2-[2-(6-chloro-1,3-benzothiazol-2-yl)hydrazine-1-ylidene](phenyl)methyl]phenol (3c)

IR ϑ_{max} cm^{-1} (KBr): 3316 (OH), 3301 (N-H), 3016 (CH-Ar), 1494 (C=N), 1288 (C–N), 1016 (C–Cl), 653 (C–S–C). ^{1}H NMR (300 MHz, DMSO-d6, δ ppm): 8.17–6.7 (m, 7H, Ar-H), 5.0(s, 1H, Ar-OH) 7.0 (s, 1H, NHN–, D2O exchangeable).Mass analysis MS (LC-MS) :(Calcd. For m/z [M+] 379) Found: 379

2.3.5. 2-[(1E)-[2-(6-chloro-1,3-benzothiazol-2-yl)hydrazine-1-ylidene]methyl]-6-methoxyphenol (3d)

IR ϑ_{max} cm^{-1} (KBr):3433 (O-H and N-H), 3163 (Ar C-H), 1610 (C=N), 1415 (Ar C=C), 1540 (cyclic C=N),1068 (C–Cl), 578 (C–S–C).^{1}H NMR (300 MHz, DMSO-d6, δ ppm): 8.17–6.7 (m, 7H, Ar-H), 5.0(s, 1H, Ar-OH), 4.5(s, 3H, CH3), 7.28 (s, 1H, NHN–, D2O exchangeable).Mass analysis MS (LC-MS) :(Calcd. For m/z [M+] 333) Found:333

Antifungal activity: For present work efficacy of four compounds (3a-3d) were detected against four fungal strains Candida albicans (MTCC 183), Aspergillus niger (MTCC 228), Candida tropicalis (MTCC 6192), and Fusarium oxysporium (MTCC 3656). The concentration of the test compound used was 1mg/ml, Clotrimazole were taken as the

standard drug and Acetone was used as solvent control. The zones of inhibition obtained in different strains of fungi are shown in Table2.

Table 2: Comparison of Zone of Inhibition of various compounds derivatives

Compound	Antifungal activity in (mm)			
	C.albicans (MTCC 183)	A. niger (MTCC 228)	F. oxysporium (MTCC 3656)	C. Tropicalis (MTCC 6192)
STD	21	20	20	21
3a	17	18	15	14
3b	15	14	17	11
3c	17	13	15	14
3d	18	19	15	14

Results and conclusion:

The efficient synthetic route for the synthesis of benzothiazole containing Schiff base derivatives was shown in Fig 1. It involves the cyclization of aromatic amine and formation of compound 1, through KSCN and Br_2 in gl.Acetic acid then involves the reaction of hydrazine hydrate with compound 1 in the presence of ethylene glycol and finally formation of the compounds (3a–3d) through reaction of substituted ketones in the presence of ethanol and compound 2 by refluxing for 5-6 h. The synthesized compounds were evaluated for their antifungal activity against four fungal strains. Compounds 3a,3d showed significant activity against Candida albicans (MTCC 183), Aspergillus niger (MTCC 228), while 3b,3c showed comparable activity against Candida albicans (MTCC 183), Fusarium oxysporium (MTCC 3656)when tested at 1mg/ml concentration taking Clotrimazole as the standard. The rest of the synthesized derivatives showed low to moderate activity. From the above results, it may be concluded that the derivatives of benzothiazole possess moderate to potent antifungal activity[1,5] when compared to standard Clotrimazole. Therefore, the experimental study justifies the therapeutic application of the benzothiazole moiety in the present era.

References

1. Bhawsar SB, Mane DV, Sinde DB, Shingare MS, Deokate AS and Congwane LV. Synthesis of some 8-[6'-substituted1', 3'-benzothiazol-2'-yl) amino methyl] substituted hydroxyl coumarins and Evaluation of their antibacterial activity. Indian J Het Chem 1996; 8: 23.
2. Suvarna K, Swain SP and Gandhi AM. Synthesis and evaluation of Novel Benzothiazole Derivatives against Human Cervical Cancer Cell Lines. Indian J Pharm Sci 2007; 69: 46-50.
3. Nagarajan SR, De CGA, Getman DP, Lu HF, Sikorski JA, Walker JL et al. Replacement of the ureas moiety by benzothiazolesulfonamide provided inhibitors of HIV-1 protease with improved potency and antiviral activities. Bioorg and Med Chem Lett 2003;11: 4769.
4. Wells G, Bradshaw TD, Diana P, Seaton A, Shi DF, Westwell AD et al. Synthesis and Anti-tumor activity of Benzothiazole Substituted Quinol Derivatives. Bioorg Med Chem Lett 2000; 10: 513-515.
5. Latrofa A, Franco M, Lopedota A, Rosato A, Carone D and Vitali C. Structural modification and antimicrobial activity of N-cycloalkylidene-2,3-dihydro-1,3-benzothiazoles, N-cycloalkyl-2-acylalkylidene-2, 3-dihydro-1,3-benzothiazoles. IL Farmaco 2005; 60: 291-297.

6. Dogruer DS, Unlu S, Sahin MF and Yesilada E. Synthesis of (2-benzothiazolone-3-yl and 2- benzothiazolone-3-yl) acetic acid derivatives and Evaluation of their Antinocioceptive and Anti-inflammatory Activity. IL Farmaco 1998; 53: 80.
7. Cressier D, Prouillac C, Hernandez P, Amourette C, Diserbo M, Lion C, Rima G. Synthesis, antioxidant properties and radioprotective effects of new benzothiazoles and thiadiazoles. Bioorg Med Chem 2009; 17: 5275.
8. Siddiqui N, Rana A, Khan SA, Haque SE, Alam MS and Ahsana W. Anticonvulsant and Toxicity Evaluation of Newly Synthesized 1-[2-(3,4-disubstitutedphenyl)-3-chloro-4-oxoazetidin-1-yl]-3-(6-substituted-1,3-benzothiazol-2-yl)ureas. Acta Chim Slov 2009; 56: 462-469.

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36 Advances and Perspectives in Eco-Friendly Synthesis of Magnetic Nanoparticles: A Comprehensive Review.

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Abstract

This review paper explores a sustainable approach for synthesizing magnetic nanoparticles through the green methodology, utilizing Plant extract as a reducing and stabilizing agent. The optimized synthesis conditions yield magnetite nanoparticles with a nanoscale dimension, as confirmed by X-ray diffraction and transmission electron microscopy. The nanoparticles exhibit superparamagnetic behavior, rendering them suitable for various applications. Biocompatibility studies demonstrate their potential for biomedical use, showcasing minimal cytotoxic effects. The eco-friendly synthesis process significantly reduces energy consumption and waste generation compared to conventional methods, aligning with principles of green nanotechnology. Stability assessments in different media emphasize their practical utility. Preliminary scalability investigations suggest the potential for large-scale production. This study contributes to sustainable nanotechnology, offering a versatile platform for environmental remediation and biomedical applications. The findings underscore the importance of environmentally conscious practices in nanomaterial synthesis, paving the way for the integration of green technologies into mainstream applications.

Introduction:

Nanotechnology is the process of manipulating matter through specific chemical or physical processes to produce materials with specific properties that can be used in specific applications [1]. A nanoparticle is a microscopic particle with at least one dimension smaller than 100 nanometers [2]. They differ from bulk materials in terms of optical properties, thermal properties, electrical properties, chemical properties, and physical properties[3]. As a result, they have a wide range of applications in the fields of medicine, chemistry & environment, energy & agri-culture, information & communication, heavy industry & consumer goods [4]. Traditional nanoparticle synthesis methods (attrition, pyrolysis) have drawbacks such as poor surface formation, low manufacturing rate, highmanufacturing costs, and high energy requirements [2]. Chemical synthesis (e.g., chemical reduction, solvent gel technique) involve the use of toxic chemicals, the production of hazardous by-products, and contamination by precursor chemicals. Therefore, the need for clean, non-toxic, and environmentally friendly nanoparticle synthesis processes is increasing.

Green synthesis alludes to naturally inviting strategies of synthesizing different materials, counting nanoparticles. Within the setting of nanoparticle union, the green synthesis strategy has picked up noteworthy significance due to its various focal points over routine strategies. Green synthesis strategies ordinarily utilize generous and eco-friendly materials, diminishing the natural affect related with dangerous chemicals and by-products. Green synthesis strategies regularly utilize characteristic sources, such as plant extricates or microorganisms, which can lead to the generation of nanoparticles with enhanced biocompatibility. This can be especially imperative for applications in pharmaceutical, where biocompatible nanoparticles are alluring for medicate conveyance and imaging purposes. Green synthesis strategies can offer better control over the estimate, shape, and composition of nanoparticles. This can be significant for fitting the properties of nanoparticles for particular applications, such as catalysis, hardware, or biomedical employments.

A wide range of biological resources can be used for the synthesis of nanoparticles, such as microorganisms (bacteria, yeast, fungi, algae and viruses) and plants [5]. Although microbe-based protocols have been developed based on the cumulative research of several authors, the importance of plant-mediated biological synthesis of nanoparticles has only increased in recent years [6]. Plant extracts reduce metal ions in a shorter time than microbes. Depending on the type of plant and concentration of phytochemicals, nanoparticlesare synthesized in a few minutes or hours, while methods based on microorganisms require a longer time [7]. The main disadvantage of microbial-mediated nanoparticle synthesis is the mandatory limitation of aseptic conditions, which requires trained personnel and increases the cost of scale-up [8]. All these reasons, as well as the easy availability of plants in nature, make them biologically cheaper than microbes.

Nanotechnology, particularly the synthesis of magnetic nanoparticles (MNPs), has revolutionized diverse fields, ranging from biomedical sciences to environmental remediation. The unique properties of MNPs, such as their magnetic responsiveness and high surface area, make them valuable in applications like targeted drug delivery, imaging, and pollutant removal. However, the conventional methodologies employed in their synthesis often involve the use of hazardous reagents and energy-intensive processes, raising concerns regarding the environmental impact and sustainability of these practices. The exponential growth in the utilization of MNPs underscores the urgency to develop eco-friendly synthesis methods that address the shortcomings of traditional approaches. In this context, the current study delves into the prospect of green synthesis as a sustainable alternative for the production of magnetic nanoparticles. Green synthesis, characterized by its reliance on environmentally benign precursors and energy-efficient processes, emerges as a promising strategy to mitigate the ecological footprint associated with nanoparticle synthesis. The motivation behind adopting green synthesis methods lies in the imperative to align scientific advancements with sustainable practices. The synthesis of MNPs using plant-mediated approaches, specifically, offers a holistic and eco-friendly route. Plants, with their inherent richness in bioactive compounds, not only act as reducing agents but also provide a biocompatible environment for nanoparticle stabilization. This dual functionality of plant extracts positions them as ideal candidates for green synthesis, ensuring that the production process is not only environmentally conscious but also yields nanoparticles with desirable properties.

Till presently, iron nanoparticles have been generally synthesized utilizing diverse plant extricates. Plant extricates act as low-cost decreasing and stabilizing

specialists. Attractive nanoparticle mixture is carried out at room temperature or by the aqueous course by blending plant extricate with metal salt arrangement in a settled proportion.

The paradigm shift towards green synthesis methods for MNPs has gained attraction due to their potential to overcome the limitations of conventional approaches. Green synthesis involves the utilization of environmentally benign precursors, often derived from natural sources, to create MNPs under mild reaction conditions. Among the various green synthesis methods, plant-mediated synthesis has emerged as a particularly promising avenue. Plants, with their diverse array of bioactive compounds, offer an attractive alternative for green synthesis. Various studies have explored the use of plant extracts rich in phytochemicals, such as flavonoids and polyphenols, as both reducing and stabilizing agents in the synthesis of MNPs. The dual functionality of these extracts not only facilitates the reduction of metal ions but also imparts stability to the resulting nanoparticles. The versatility of plant-mediated synthesis is evident across multiple plant species, including but not limited to Aloe vera, green tea, and neem. The phytochemical composition of these plants plays a crucial role in determining the properties of the synthesized MNPs. This method not only ensures eco-friendliness but also offers the potential for large-scale, cost-effective production.

Figure: Green synthesis of iron oxide nanoparticles

Biosynthesis of magnetic nanoparticles using plants:

The most commonly used plant resource for the synthesis of iron nanoparticles is tea extract. nZVI was synthesized on the reaction of Camellia sinensis (green tea) extract with 0.1 M FeCl3 soln. [9]. These nanoparticles were synthesized in minutes at room temperature, where thepolyphenols act as a reducing and capping agent. These nanoparticles were found to have a higher activity in degrading bromothymol blue compared to two commonly used iron chelates.

synthesized polydisperse iron nanoparticles using eucalyptus leaf extract obtained from its leaf litter[12]. nZVI, Fe_3O_4 and Fe_2O_3 were different forms of nanoparticles synthesized during the process. Due to the presence of different phytochemicals, each with a different reducing power in extract form, the nanoparticles were polydisperse unlike nanoparticles synthesized by chemical reducing agents. For the first time, biologically synthesized nanoparticles were used to treat eutrophic wastewater. After 21 days, the total nitrogen removal percentage was 71.7%, total P and COD percentage was 84.5%. The very low P removal was due to the lack of calcium, magnesium or aluminum acting as precipitants.

Huang et al. [11] used oolong tea extract to synthesize iron nanoparticles. The polyphenol/caffeine content of the extract acted as a reducing and thickening agent. XRD and FTIR characterization showed that valent iron, maghemite and magnetite nanoparticles were not present. Thanks to the organic coating of biomolecules, the synthesized nanoparticles remained dispersed and also showed good reactivity. Interestingly,is mediated by an oolong extract.green tea Fe NPs at 50,000 magnificationiron nanoparticles effectively degraded (75.5% in 60 min, steady state) an otherwise difficult to degrade dye, malachite green, with a degradation rate of± 0.045 min 1. Malachite green degradation followed pseudo-first-order kinetics.

Shahwan et al. [10] synthesized iron nanoparticles, GT-Fe NPs (mainly composed of iron oxide/oxohydroxide) using green tea extracts. These nanoparticles act as a Fenton-like catalyst for the degradation of cationic dyes such as methylene blue (MB) and anionic dyes such as methyl orange (MO). Almost complete removal of both dyes was achieved for MB and MO at 200 and 350 min. For GT-Fe NPs, almost 100% removal of MB and MO was observed at initial dye concentrations of 10 mg/L and 100 mg/L. Efficiency was slightly lower for MB (96.3% at 10 mg/L and 86.6% at 100 mg/L) and significantly lower for MO (61.6% at 10 mg/L and 47.1% at 100 mg/L). iron nanoparticles were synthesized by a conventional borohydride reduction method.

In another work, the synthesis was carried out at room temperature using different volumes of tea extract and $Fe(NO_3)_3$ solution to check the effect of tea extract concentration on the resulting nanoparticle size; it was observed that the particle size decreased with increasing concentration. The size of the nanoparticles synthesized by the borohydride reduction method was found to vary between 50 nm and 500 nm. The biocompatibility of nZVIs synthesized using green tea and borohydride as reductant was evaluated by methyl tetrazolium (MTS) and lactate dehydrogenase (LDH) assay by exposing cell lines to nZVIs for 24–48 hours. LDH leakage increased with increasing particle size, which stresses the cell membrane. Thus, nZVI synthesized by green tea, with a much smaller size, was shown to be non-toxic to human keratinocytes compared to nanoparticles synthesized by the borohydride reduction process [13].

When navigating research into the green synthesis of magnetic nanoparticles, it is important to assess the limitations of conventional methods. Commonly used chemical routes often involve the use of toxic reagents, leading to dangerous byproducts and challenging large-scale production. The move to green synthesis solves these problems and envisions a future where nanotechnology not only advances the frontiers of science but also adheres to the principles of environmental responsibility. In the following sections of this paper, we present a comprehensive study on the green synthesis of magnetic nanoparticles. Our methodology

TABLE: Size and morphology of iron nanoparticles synthesized by plant extracts.

PLANT	MORPHOLOGY	SIZE	REFERENCE
Green Tea	Spherical	5-17	[9]
Eucalyptus	Spherical	10-20	[12]
Oolong Tea	Spherical	40-50	[11]
Green tea	Irregular clusters	40–60	[10]

Tea powder	Differs according to the quantity of tea extract		[13]
Plantain peel	Spherical	Less than 50 nm	[67]
Banana peel	ND	10–25	[68]
Sorghum bran	Spherical	40–50	[69]
Pomegranate	ND	100–200 nm	[70]
Stevia rebaudiana	Spherical	20	[71]
Punica granatum	Spherical	25-55	[72]
Cynara cardunculus	Semi-spherical	13.5	[73]
Tamarix aphylla	Spherical		[74]
K. alvarezii	Hexagonal	10–30	[75]
Moringa oleifera	Irregular spherical	18-20	[76]
Amaranthus dubius	Oval	58-530	[77]
Rhamnella gilgitica	Spherical	21-25	[78]

uses plant-mediated synthesis and we delve into the complexities of this process with the aim of demonstrating its efficiency, reproducibility and potential for scalable production. The study not only advances the expanding range of green synthetic methods, but also heralds a paradigm shift in the synthesis of magnetic nanoparticles, ushering in an era of sustainable nanotechnology.

In conclusion, the literature review highlights the transformative potential of green synthesis methods for MNPs. The shift to environmentally friendly practices not only addresses environmental problems associated with traditional methods, but also opens up opportunities for innovative applications in medicine and environmental science. As we delve into the experimental details and results in the following sections, this study contributes to the growing body of knowledge supporting sustainable nanotechnology practices.

Characterization technique:

A few methods have been utilized for the assessment of the properties of incorporated IONPs. The affirmation of IONPs development is analyzed with UV spectroscopy [14]. Fourier change infrared spectroscopy (FTIR) is utilized for the useful gathering identification [15]. The morphological not entirely settled by microscopy procedures like transmission electron microscopy instrument (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM) [16]. The crystallinity of synthesized not entirely settled with X-ray diffraction [17] while the purity and composition of component are estimated with EDX [18] and total reflection X-ray fluorescence (TXRF) [19].

Ultraviolet-visible (UV-Vis) spectroscopy serves as a valuable technique for verifying the creation of metal nanoparticles (NPs) and metal oxide NPs, including iron oxide nanoparticles (IONPs) [21]. This involves measurement of its surface plasmon resonance and estimating the oscillations of conduction band electrons induced by electromagnetic radiation.

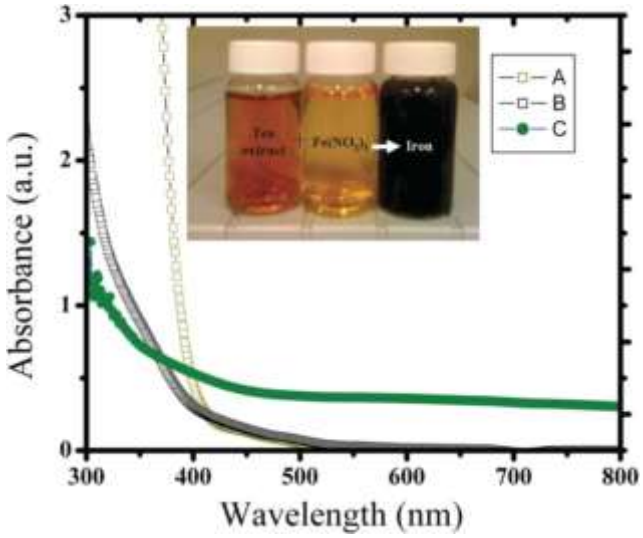

Figure: UV spectra of synthesized iron oxide nanoparticles

UV analysis has been instrumental in examining the stability, size, aggregation, and structure of NPs, as emphasized in the work [20]. Each type of metal oxide and metal NP possesses a distinctive absorbance wavelength discerned from the UV spectrum when incident light interacts with conduction band electrons at the NP surface, a phenomenon elucidated [22]. Specifically, the absorbance band indicative of IONPs falls within the 280 to 450 nm range on the UV spectrum, as reported by [20].

Fourier transform infrared spectroscopy (FTIR) proves valuable in identifying the functional groups within plant extracts and iron oxide nanoparticles (IONPs), as highlighted in[23]. Additionally, it plays a crucial role in determining the functional groups involved in the bio-reduction of iron precursors. FTIR analysis is versatile, capable of generating absorption and infrared emission spectra for solids, liquids, and gases. The distinctive combination of atoms in biomolecules enhances the identification of functional groups in synthesized IONPs using FTIR spectra, as elucidated in[24]. The spectra derived from FTIR analysis present a unique fingerprint containing absorption peaks corresponding to the vibration wavelengths within the bounds of the NP atoms, as noted in[25]. Researchers have extensively utilized FTIR spectroscopy to confirm the presence of biomolecules such as flavonoids, tannins, alkaloids, and saponins in plant extracts used for the bio-reduction of iron precursors during IONP formation, as demonstrated in[26].

The transmission electron microscope (TEM) is a frequently employed tool for characterizing the shape, size, and morphology of iron oxide nanoparticles (IONPs), as highlighted in[27]. However, the preparation process for TEM analysis is intricate due to the necessity for an extremely thin sample (IONPs) to facilitate electron transmittance.The procedure involves encapsulating IONPs in thin films created on carbon-coated copper grids. This is achieved by dispensing a small quantity of IONPs solution onto the grid, followed by the removal of excess solution using blotting papers, as explained in[28]. To enhance the penetration of a monochromatic electron

beam through the sample, producing an image on the viewing screen, the prepared samples are dried under a mercury lamp, as outlined in[29].Numerous researchers have utilized TEM for morphological assessments of IONPs synthesized from various plant materials.

Scanning electron microscopy (SEM) stands out as an electron microscopy-based technique extensively employed for the direct visual assessment of various nanoparticles (NPs), as discussed in[30]. This technique offers unique advantages in morphological and size analysis compared to other available methods. In preparing iron oxide nanoparticles (IONPs) for SEM analysis, the IONPs solution undergoes evaporation to dryness. The resulting powder is then mounted on a sample holder within the SEM machine and coated with a conductive metal using a sputter coater, as detailed in[31]. Following this, a focused beam of high-energy electrons is directed towards the IONPs, generating multiple signals on their surface, as explained in[32]. These signals are captured by electron beams and recorded by the detector, providing information about the crystalline structure, external morphology, orientation, and chemical composition of the IONPs. However, a limitation of SEM lies in its inability to offer precise and sufficient details regarding the average size distribution of IONPs, as noted in[33]. The collective findings concerning the morphological determination of synthesized IONPs using various plant extracts through TEM analysis are summarized in table.

Numerous studies have highlighted the utility of Atomic Force Microscopy (AFM) in the morphological assessment of nanoparticles (NPs), as documented in[34]. This technique involves scanning samples, such as iron oxide nanoparticles (IONPs), with a submicron-level probe tip, aided by software-based image processing [35]. AFM provides essential information about NP morphology, including surface texture, length, width, and height, by analyzing forces between the sample surface and the tip. For AFM sample preparation, a small quantity of IONPs solution is placed on a glass slipcover attached to the AFM stub and dried over nitrogen gas at ambient temperature. Multiple images are then recorded for comprehensive interpretation [36]. The instrument operates by utilizing forces between the surface and the tip to generate a topographical map scanned in contact mode [37]. Notably, AFM offers advantages such as not requiring sample pretreatment before image production, applicability for evaluating the morphological features of nonconducting samples, and providing information about the volume and height of NPs [38]. In addition to AFM, X-ray Diffraction (XRD) has been recognized as a valuable technique for assessing the crystallinity of synthesized NPs. This involves analyzing lattice and structure parameters of diffracted IONPs powder, with the crystal size determined based on the width of X-ray peaks using the Scherrer formula [39]. Moreover, the elemental composition of IONPs can be determined through X-ray emission obtained after bombarding the nanoparticles with an electron beam [40]. The use of an attached Energy-Dispersive X-ray Spectroscopy (EDS) detector to Scanning Electron Microscopy (SEM) has also been employed for elemental composition determination by estimating the number of emitted X-rays, providing both quantitative and qualitative analysis [41].

To assess the purity and elemental composition of nanoparticles (NPs) synthesized using plant extracts, researchers have employed the Energy-Dispersive X-ray Spectroscopy (EDX) technique, as demonstrated in[35]. The elemental composition analysis of iron oxide nanoparticles (IONPs) involves capturing X-ray emissions emitted when the IONPs are subjected to an electron beam bombardment,

as explained in[40]. Furthermore, the integration of an Energy-Dispersive X-ray Spectroscopy (EDS) detector with Scanning Electron Microscopy (SEM) has been utilized for determining the elemental composition of IONPs. This process estimates the number of X-rays emitted, balancing the difference in energy of the two electrons, as reported in[41]. The emitted X-ray energy serves as a distinctive identifier of the element when subjected to both quantitative and qualitative analyses, highlighting the attainability of comprehensive information about the composition of IONPs, as discussed in[42].

X-ray Diffraction (XRD) has been acknowledged as a valuable technique for the evaluation of crystallinity in synthesized nanoparticles (NPs). The assessment of crystallinity involves the analysis of lattice and structural parameters of diffracted iron oxide nanoparticles (IONPs) powder, accomplished by measuring the diffraction angle when subjected to an incident X-ray beam. Utilizing the Scherrer formulathe determination of crystal size is facilitated based on the width of X-ray peaks, as explained in[39]. This approach enables a quantitative assessment of the crystal size of the synthesized NPs through the analysis of their X-ray diffraction patterns.

Applications of MNPs synthesized from plant extract:

1. Antimicrobial Application

Numerous investigations have been conducted to augment the antimicrobial efficiancy of existing antibiotic drugs and to develop novel antimicrobial agents addressing microbial resistance to available antibiotics and antiseptics [43]. In vitro antimicrobial studies of metal and metal oxide nanoparticles (NPs) against diverse microbial species have revealed significant inhibitory effects compared to conventional antibiotics and antiseptics [44]. The antimicrobial activities of iron oxide nanoparticles (IONPs) and other NPs are predominantly influenced by particle size and synthesis material. In recent decades, IONPs functionalized with therapeutic agents, including antimicrobials, have garnered scientific and industrial interest due to their notable antimicrobial and antibiofilm activities [45]. Agglomerated IONPs surfaces are modified with essential biological molecules and polymers to prevent agglomeration and enhance their utility in biomedical applications [46]. The use of metal NPs is considered a promising strategy for mitigating microbial drug resistance due to their diverse interaction mechanisms with microbial cells. These mechanisms include generating pits in bacterial cells, causing fragmentation of the cell wall, denaturation of microbial outer membranes, and reacting with enzymes' disulfide groups to impede metabolic processes, leading to cell death [47]. Similarto other metal and metal oxide NPs (e.g., silver, gold, zinc oxide, and copper oxide), IONPs exhibit significant antimicrobial activities [48]. Studies have demonstrated synergistic antibacterial effects of IONPs synthesized with plant extracts, such as those from corn plants [49]. Bactericidal actions of IONPs synthesized using Couroupita guianensis fruit extract and Argemone mexicana L. leaf extract have shown effectiveness against various human pathogens [50,51]. IONPs synthesized with plant extracts, particularly from Argemone mexicana L. leaf extract, have been reported to limit the growth of Proteus mirabilis and Escherichia coli [51]. The potential of IONPs in combating both gram-positive and gram-negative bacteria has been substantiated, prompting the development of synergistic IONPs platforms as potential carrier systems for treating microbial infections in the future [52].

2. Anticancer Application:

The challenging issues of lack of selective targets and multidrug resistance pose significant obstacles in the effective treatment of tumors and cancer [53]. Advances in nanotechnology and nanoscience have demonstrated the potential of nanoparticles (NPs) in cancer treatment due to their unique features and interactions with cancerous cells [54]. Among metal oxide NPs, iron oxide nanoparticles (IONPs) are recommended for anticancer therapy due to their large surface area for grafting targeting substrates and moieties, high resistance to in vivo degradation, and potential synergistic activity influencing drug sensitivity in cancer treatment [55]. The cytotoxicity effects of biosynthesized IONPs against human HepG2 liver cell lines indicate the ability of IONPs to inhibit cancer cell growth with increasing concentrations [56]. In a study evaluating the cytotoxicity efficiency of lead oxide NPs and IONPs against HepG2 cells, IONPs exhibited higher cytotoxicity efficiency (38.49%) compared to lead oxide NPs (20.88%), attributed to the smaller particle size of IONPs [57]. In vitro cytotoxicity evaluation of IONPs synthesized using flaxseed against MCF-7 cells demonstrated high toxicity efficacy at concentrations of 4.7 μg mL–1 and above [26]. The heightened cytotoxicity efficiency of IONPs against MCF-7 cells was linked to their ability to disrupt the cell membrane by interacting with phospholipid molecules in the cell layer. Psoralea corylifolia-mediated IONPs exhibited outstanding cytotoxicity against renal tumor cells, highlighting significant anticancer activity [58]. These findings underscore the promising role of IONPs in cancer therapy, leveraging their unique properties and interactions with cancer cells for potential therapeutic applications.

3. Environmental applications:

The extensive utilization of anionic and cationic dyes in various industries, including textiles, plastics, pharmaceuticals, leather, printing, and paper milling, has resulted in a significant global demand and supply [59]. Studies indicate that more than 20% of the total dyes used are wasted and discharged into the environment following manufacturing processes in these industries, contributing to various forms of environmental pollution [60]. The release of dyes and other toxic waste into ecosystems has led to adverse effects such as the mortality of aquatic animals, water turbidity, and various human health issues [61]. Managing and effectively controlling effluents containing dyes pose significant challenges. In response to the environmental issues associated with industrial effluents, investigations have focused on the catalytic degradation and oxidation of dyes using metal and metal oxide nanoparticles (NPs) [62]. Metal oxides, including zinc, copper, titanium, and iron oxides, have demonstrated efficient dye degradation capabilities [63,64]. The photodegradation of dyes by iron oxide nanoparticles (IONPs) is attributed to their high surface area to mass ratio and many surfaces reactive sites facilitating easy dye adsorption [65]. Parameters such as catalyst loading, pH, temperature, and time are reported as major factors influencing the photocatalytic activities of IONPs [66]. providing valuable insights into the efficacy of IONPs in addressing environmental pollution challenges associated with dye-containing effluents.

4. Other applications:

The intrinsic magnetic properties of nanoparticles render them excellent candidates for biomedical imaging. Eco-friendly synthesized magnetic nanoparticles exhibit high magnetic moments, enabling enhanced contrast in magnetic resonance imaging (MRI). Their uniform size distribution and biocompatibility make them valuable contrast agents, offering improved spatial resolution and signal sensitivity for precise anatomical visualization. Functionalized magnetic nanoparticles play a

pivotal role in targeted drug delivery. Surface modifications with bioactive molecules enable site-specific drug delivery, enhancing therapeutic efficacy while minimizing systemic side effects. The superparamagnetic behavior of these nanoparticles facilitates controlled drug release under external magnetic fields, ensuring localized treatment with increased precision. The unique magnetic properties of nanoparticles find application in magnetic hyperthermia therapy for cancer treatment. Under the influence of an alternating magnetic field, eco-friendly synthesized magnetic nanoparticles generate localized heat, inducing hyperthermia in cancer cells. This targeted thermal effect proves beneficial for controlled destruction of malignant cells, presenting a promising avenue for cancer therapy. In industrial processes, magnetic nanoparticles find application in magnetic separation techniques. Their magnetic susceptibility allows for efficient separation and purification of target molecules or substances from complex mixtures. This is particularly advantageous in fields such as biotechnology, where precise separation is crucial for downstream processes. In material science, incorporating eco-friendly magnetic nanoparticles into polymer matrices leads to the development of advanced nanocomposites. The magnetic nanoparticles act as reinforcing agents, imparting enhanced mechanical and thermal properties to the resulting materials. This application broadens the scope of eco-friendly synthesized nanoparticles in industries requiring lightweight, high-strength materials. In agriculture, eco-friendly magnetic nanoparticles contribute to the development of smart nano pesticides and nutrient delivery systems. Targeted delivery of agrochemicals using magnetic nanoparticles enhances efficacy while minimizing environmental impact. Additionally, these nanoparticles can facilitate soil remediation and nutrient uptake in plants.

In summary, the diverse and evolving applications of eco-friendly synthesized magnetic nanoparticles underscore their significance in various scientific and technological domains. From biomedical applications to environmental remediation, these nanoparticles continue to pave the way for sustainable and innovative solutions in diverse fields.

Conclusion:

In this comprehensive review, various green synthesis methods for iron nanoparticles derived from different plants and microorganisms were examined. A comparative analysis revealed that the plant extract method is recommended due to its ease, rapid reaction time, and safety, contrasting with microorganism-based synthesis methods that may pose infection risks or yield products of high toxicity. The green synthesis approaches demonstrated diverse shapes and morphologies of nanoparticles, proving versatile for numerous applications. The highlighted green methods exhibited enhanced stability compared to many chemical synthesis approaches. The widespread adoption of green synthesis methods can be attributed to their simplicity, safety, cost-effectiveness, and eco-friendliness. The synthesized iron nanoparticles have found utility in diverse applications, including bioremediation, antibacterial effects, and dye removal, contributing significantly to environmental preservation. Furthermore, the review delved into the intricate methods and techniques involved in the synthesis and characterization of iron oxide nanoparticles (IONPs). The bioactive constituents in plant extracts used for IONP synthesis were identified not only as reducing and stabilizing agents but also as contributors to improved biological activities and prevention of agglomeration. Optimal conditions for synthesis and enhanced yields of

IONPs with desirable properties were extensively discussed. Literature review emphasized that plant-based synthesis is environmentally friendly, scalable for industrial production, non-toxic, rapid, and energy-efficient. Despite the diverse applications of biosynthesized IONPs in effluent treatment, catalysis, and biomedicine, future studies should address the challenges in understanding subsurface mechanistic pathways, transport of IONPs in environments, and their toxicological consequences. This synthesis approach holds promising potential and warrants further exploration for sustainable and safe applications in various fields. In conclusion, the eco-friendly synthesis of magnetic nanoparticles presented in this study exemplifies the potential of green nanotechnology. The integration of sustainable practices into material synthesis not only addresses environmental concerns but also propels the development of advanced materials with applications spanning across various domains. As we navigate towards a future marked by sustainable technologies, this research contributes to the ongoing discourse on responsible and eco-conscious approaches in the field of nanotechnology.

References:

1. Dhawan, Alok, Rishi Shanker, Mukul Das, and Kailash C. Gupta. "Guidance for safe handling of nanomaterials." *Journal of biomedical nanotechnology* 7, no. 1 (2011): 218-224.
2. Thakkar, Kaushik N., Snehit S. Mhatre, and Rasesh Y. Parikh. "Biological synthesis of metallic nanoparticles." *Nanomedicine: nanotechnology, biology and medicine* 6, no. 2 (2010): 257-262.
3. Panigrahi, Sudipa, Subrata Kundu, Sujit Ghosh, Sudip Nath, and Tarasankar Pal. "General method of synthesis for metal nanoparticles." *Journal of nanoparticle Research* 6 (2004): 411-414.
4. TER, I. "Nanotechnology development in India: building capability and governing the technology." (2010).
5. Mohanpuria, Prashant, Nisha K. Rana, and Sudesh Kumar Yadav. "Biosynthesis of nanoparticles: technological concepts and future applications." *Journal of nanoparticle research* 10 (2008): 507-517.
6. Gardea-Torresdey, Jorge L., J. G. Parsons, E. Gomez, J. Peralta-Videa, H. E. Troiani, P. Santiago, and M. Jose Yacaman. "Formation and growth of Au nanoparticles inside live alfalfa plants." *Nano letters* 2, no. 4 (2002): 397-401.
7. Rai, Mahendra, Alka Yadav, and Aniket Gade. "Current trends in phytosynthesis of metal nanoparticles (vol 28, pg 277, 2008)." *Critical reviews in biotechnology* 29, no. 1 (2009): 78-78.
8. Sathishkumar, Muthuswamy, Krishnamurthy Sneha, and Yeoung-Sang Yun. "Immobilization of silver nanoparticles synthesized using Curcuma longa tuber powder and extract on cotton cloth for bactericidal activity." *Bioresource technology* 101, no. 20 (2010): 7958-7965.
9. Hoag, George E., John B. Collins, Jennifer L. Holcomb, Jessica R. Hoag, Mallikarjuna N. Nadagouda, and Rajender S. Varma. "Degradation of bromothymol blue by 'greener'nano-scale zero-valent iron synthesized using tea polyphenols." *Journal of Materials Chemistry* 19, no. 45 (2009): 8671-8677.
10. Shahwan, Talal, S. Abu Sirriah, Muath Nairat, Ezel Boyacı, Ahmet E. Eroğlu, Thomas B. Scott, and Keith R. Hallam. "Green synthesis of iron nanoparticles

and their application as a Fenton-like catalyst for the degradation of aqueous cationic and anionic dyes." *Chemical Engineering Journal* 172, no. 1 (2011): 258-266.

11. Huang, Lanlan, Xiulan Weng, Zuliang Chen, Mallavarapu Megharaj, and Ravendra Naidu. "Synthesis of iron-based nanoparticles using oolong tea extract for the degradation of malachite green." *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 117 (2014): 801-804.
12. Wang, Ting, Xiaoying Jin, Zuliang Chen, Mallavarapu Megharaj, and Ravendra Naidu. "Green synthesis of Fe nanoparticles using eucalyptus leaf extracts for treatment of eutrophic wastewater." *Science of the total environment* 466 (2014): 210-213.
13. Nadagouda, Mallikarjuna N., Alicia B. Castle, Richard C. Murdock, Saber M. Hussain, and Rajender S. Varma. "In vitro biocompatibility of nanoscale zerovalent iron particles (NZVI) synthesized using tea polyphenols." *Green Chemistry* 12, no. 1 (2010): 114-122.
14. Ramesh, A. V., Dharmasoth Rama Devi, Satish Mohan Botsa, and K. Basavaiah. "Facile green synthesis of Fe3O4 nanoparticles using aqueous leaf extract of Zanthoxylum armatum DC. for efficient adsorption of methylene blue." *Journal of Asian Ceramic Societies* 6, no. 2 (2018): 145-155.
15. Devi, Henam Sylvia, Muzaffar Ahmad Boda, Mohammad Ashraf Shah, Shazia Parveen, and Abdul Hamid Wani. "Green synthesis of iron oxide nanoparticles using Platanus orientalis leaf extract for antifungal activity." *Green Processing and Synthesis* 8, no. 1 (2019): 38-45.
16. Lassoued, Abdelmajid, Brahim Dkhil, Abdellatif Gadri, and Salah Ammar. "Control of the shape and size of iron oxide (α-Fe2O3) nanoparticles synthesized through the chemical precipitation method." *Results in physics* 7 (2017): 3007-3015.
17. Akintelu, Sunday Adewale, Seyifunmi Charles Olugbeko, and Aderonke Similoluwa Folorunso. "A review on synthesis, optimization, characterization and antibacterial application of gold nanoparticles synthesized from plants." *International Nano Letters* 10 (2020): 237-248.
18. Rufus, Alex, N. Sreeju, Vidya Vilas, and Daizy Philip. "Biosynthesis of hematite (α-Fe2O3) nanostructures: Size effects on applications in thermal conductivity, catalysis, and antibacterial activity." *Journal of Molecular Liquids* 242 (2017): 537-549.
19. Kulesh, N. A., I. P. Novoselova, A. P. Safronov, I. V. Beketov, O. M. Samatov, G. V. Kurlyandskaya, M. Morozova, and T. P. Denisova. "Total reflection x-ray fluorescence spectroscopy as a tool for evaluation of iron concentration in ferrofluids and yeast samples." *Journal of Magnetism and Magnetic Materials* 415 (2016): 39-44.
20. Aisida, Samson O., Ngozi Madubuonu, M. Hisham Alnasir, Ishaq Ahmad, S. Botha, M. Maaza, and Fabian I. Ezema. "Biogenic synthesis of iron oxide nanorods using Moringa oleifera leaf extract for antibacterial applications." *Applied Nanoscience* 10 (2020): 305-315.
21. Madubuonu, Ngozi, Samson O. Aisida, Ishaq Ahmad, S. Botha, Ting-kai Zhao, M. Maaza, and Fabian I. Ezema. "Bio-inspired iron oxide nanoparticles using Psidium guajava aqueous extract for antibacterial activity." *Applied Physics A* 126 (2020): 1-8.

22. Akintelu, Sunday Adewale, Seyifunmi Charles Olugbeko, Femi Adekunle Folorunso, Abel Kolawole Oyebamiji, and Aderonke Similoluwa Folorunso. "Characterization and pharmacological efficacy of silver nanoparticles biosynthesized using the bark extract of Garcinia kola." *Journal of Chemistry* 2020 (2020): 1-7.
23. Kumar, Brajesh, Kumari Smita, Luis Cumbal, Alexis Debut, Salome Galeas, and Victor H. Guerrero. "Phytosynthesis and photocatalytic activity of magnetite (Fe3O4) nanoparticles using the Andean blackberry leaf." *Materials Chemistry and Physics* 179 (2016): 310-315.
24. Vasantharaj, Seerangaraj, Selvam Sathiyavimal, Palanisamy Senthilkumar, Felix LewisOscar, and Arivalagan Pugazhendhi. "Biosynthesis of iron oxide nanoparticles using leaf extract of Ruellia tuberosa: antimicrobial properties and their applications in photocatalytic degradation." *Journal of Photochemistry and Photobiology B: Biology* 192 (2019): 74-82.
25. Ibraheem, Fatima, Muhammad Hammad Aziz, Mahvish Fatima, Fozia Shaheen, Syed Mansoor Ali, and Qing Huang. "In vitro Cytotoxicity, MMP and ROS activity of green synthesized nickel oxide nanoparticles using extract of Terminalia chebula against MCF-7 cells." *Materials Letters* 234 (2019): 129-133.
26. Rahmani, Reyhaneh, Mohsen Gharanfoli, Mehran Gholamin, Majid Darroudi, Jamshidkhan Chamani, and Kayvan Sadri. "Green synthesis of 99mTc-labeled-Fe3O4 nanoparticles using Quince seeds extract and evaluation of their cytotoxicity and biodistribution in rats." *Journal of Molecular Structure* 1196 (2019): 394-402.
27. Ebrahiminezhad, Alireza, Alireza Zare-Hoseinabadi, Aydin Berenjian, and Younes Ghasemi. "Green synthesis and characterization of zero-valent iron nanoparticles using stinging nettle (Urtica dioica) leaf extract." *Green Processing and Synthesis* 6, no. 5 (2017): 469-475.
28. Wahab, Rizwan, Farheen Khan, and Abdulaziz A. Al-Khedhairy. "Hematite iron oxide nanoparticles: apoptosis of myoblast cancer cells and their arithmetical assessment." *RSC advances* 8, no. 44 (2018): 24750-24759.
29. Gang, L. I. U., Wei-jia Sun, Sha-sha Tang, Shu-quan Liang, and L. I. U. Jun. "Synthesis of α-Fe2O3@ SnO2 core-shell nanoparticles via low-temperature molten salt reaction route." *Transactions of Nonferrous Metals Society of China* 25, no. 11 (2015): 3651-3656.
30. Akintelu, Sunday Adewale, Bo Yao, and Aderonke Similoluwa Folorunso. "Green synthesis, characterization, and antibacterial investigation of synthesized gold nanoparticles (AuNPs) from Garcinia kola pulp extract." *Plasmonics* 16 (2021): 157-165.
31. Ebrahiminezhad, Alireza, Marziyeh Zare, Sedigheh Kiyanpour, Aydin Berenjian, Seyyed Vahid Niknezhad, and Younes Ghasemi. "Biosynthesis of xanthangum-coated INPs by using Xanthomonas campestris." *IET Nanobiotechnology* 12, no. 3 (2018): 254-258.
32. Ranmadugala, Dinali, Alireza Ebrahiminezhad, Merilyn Manley-Harris, Younes Ghasemi, and Aydin Berenjian. "Reduced biofilm formation in Menaquinone-7 production process by optimizing the composition of the cultivation medium." *Trends in Pharmaceutical Sciences* 3, no. 4 (2017): 245-254.

33. Rajiv, P., B. Bavadharani, M. Naveen Kumar, and P. Vanathi. "Synthesis and characterization of biogenic iron oxide nanoparticles using green chemistry approach and evaluating their biological activities." *Biocatalysis and Agricultural Biotechnology* 12 (2017): 45-49.
34. Adio, Salawu Omobayo, Mohamed Hussein Omar, Mohammad Asif, and Tawfik A. Saleh. "Arsenic and selenium removal from water using biosynthesized nanoscale zero-valent iron: a factorial design analysis." *Process Safety and Environmental Protection* 107 (2017): 518-527.
35. Yadav, Virendra Kumar, and M. H. Fulekar. "Biogenic synthesis of maghemite nanoparticles (γ-Fe2O3) using Tridax leaf extract and its application for removal of fly ash heavy metals (Pb, Cd)." *Materials Today: Proceedings* 5, no. 9 (2018): 20704-20710.
36. Jagathesan, G., and P. Rajiv. "Biosynthesis and characterization of iron oxide nanoparticles using Eichhornia crassipes leaf extract and assessing their antibacterial activity." *Biocatalysis and agricultural biotechnology* 13 (2018): 90-94.
37. Bishnoi, Shahana, Aarti Kumar, and Raja Selvaraj. "Facile synthesis of magnetic iron oxide nanoparticles using inedible Cynometra ramiflora fruit extract waste and their photocatalytic degradation of methylene blue dye." *Materials Research Bulletin* 97 (2018): 121-127.
38. SEED, NANOPARTICLESUSING AESCULUS HIPPOCASTANUM. "GREEN SYNTHESIS AND CHARACTERIZATION OF IRON NANOPARTICLESUSING AESCULUS HIPPOCASTANUM SEED EXTRACT." (2018).
39. Truskewycz, Adam, Ravi Shukla, and Andrew S. Ball. "Iron nanoparticles synthesized using green tea extracts for the fenton-like degradation of concentrated dye mixtures at elevated temperatures." *Journal of Environmental Chemical Engineering* 4, no. 4 (2016): 4409-4417.
40. Khalil, Ali Talha, Muhammad Ovais, Ikram Ullah, Muhammad Ali, Zabta Khan Shinwari, and Malik Maaza. "Biosynthesis of iron oxide (Fe2O3) nanoparticles via aqueous extracts of Sageretia thea (Osbeck.) and their pharmacognostic properties." *Green Chemistry Letters and Reviews* 10, no. 4 (2017): 186-201.
41. Badni, N., F. Z. Benheraoua, B. Tadjer, A. Boudjemaa, H. El Hameur, and K. Bachari. "Green synthesis of α-Fe2O3 nanoparticles using Roman nettle." (2016).
42. Akintelu, Sunday Adewale, Yao Bo, and Aderonke Similoluwa Folorunso. "A review on synthesis, optimization, mechanism, characterization, and antibacterial application of silver nanoparticles synthesized from plants." *Journal of Chemistry* 2020 (2020): 1-12.
43. Ansari, Shakeel Ahmed, Mohammad Oves, Rukhsana Satar, Anish Khan, Syed Ismail Ahmad, Mohammad Alam Jafri, Syed Kashif Zaidi, and Mohammad Husein Alqahtani. "Antibacterial activity of iron oxide nanoparticles synthesized by co-precipitation technology against and." *Polish Journal of Chemical Technology* 19, no. 4 (2017): 110-115.
44. Rana, P.; Sharma, S.; Sharma, R.; Banerjee, K. Apple pectinsupported superparamagnetic (γ-Fe2O3) maghemitenanoparticles with antimicrobial potency. Materials Science

45. Maria Holban, Alina. "Magnetite nanoshuttles for fighting Staphylococcus aureus infections: a recent review." *Current Topics in Medicinal Chemistry* 15, no. 16 (2015): 1589-1595.
46. Seabra, Amedea B., Milena T. Pelegrino, and Paula S. Haddad. "Antimicrobial applications of superparamagnetic iron oxide nanoparticles: Perspectives and challenges." *Nanostructures for Antimicrobial Therapy* (2017): 531-550.
47. Rai, Mahendra, Avinash P. Ingle, Indarchand R. Gupta, Sonal S. Birla, Alka P. Yadav, and Kamel A. Abd-Elsalam. "Potential role of biological systems in formation of nanoparticles: mechanism of synthesis and biomedical applications." *Curr Nanosci* 9, no. 6 (2013): 576-587.
48. Taghizadeh, Seyedeh-Masoumeh, Aydin Berenjian, Saeed Taghizadeh, Younes Ghasemi, Azadeh Taherpour, Ajit K. Sarmah, and Alireza Ebrahiminezhad. "One-put green synthesis of multifunctional silver iron core-shell nanostructure with antimicrobial and catalytic properties." *Industrial Crops and Products* 130 (2019): 230-236.
49. Patra, Jayanta Kumar, Md Sarafat Ali, In-Gyung Oh, and Kwang-Hyun Baek. "Proteasome inhibitory, antioxidant, and synergistic antibacterial and anticandidal activity of green biosynthesized magnetic Fe3O4 nanoparticles using the aqueous extract of corn (Zea mays L.) ear leaves." *Artificial cells, nanomedicine, and biotechnology* 45, no. 2 (2017): 349-356.
50. Gao, Lizeng, Kelong Fan, and Xiyun Yan. "Iron oxide nanozyme: a multifunctional enzyme mimetic for biomedical applications." *Theranostics* 7, no. 13 (2017): 3207.
51. Arokiyaraj, S., M. Saravanan, NK Udaya Prakash, M. Valan Arasu, B. Vijayakumar, and S. Vincent. "Enhanced antibacterial activity of iron oxide magnetic nanoparticles treated with Argemone mexicana L. leaf extract: an in vitro study." *Materials Research Bulletin* 48, no. 9 (2013): 3323-3327.
52. Nehra, P., R. P. Chauhan, N. Garg, and K. Verma. "Antibacterial and antifungal activity of chitosan coated iron oxide nanoparticles." *British journal of biomedical science* 75, no. 1 (2018): 13-18.
53. El-Boubbou, Kheireddine. "Magnetic iron oxide nanoparticles as drug carriers: clinical relevance." *Nanomedicine* 13, no. 8 (2018): 953-971.
54. Sathishkumar, G., V. Logeshwaran, S. Sarathbabu, Pradeep K. Jha, M. Jeyaraj, C. Rajkuberan, N. Senthilkumar, and S. Sivaramakrishnan. "Green synthesis of magnetic Fe3O4 nanoparticles using Couroupita guianensis Aubl. fruit extract for their antibacterial and cytotoxicity activities." *Artificial cells, nanomedicine, and biotechnology* 46, no. 3 (2018): 589-598.
55. Bahrami, Behdokht, Mohammad Hojjat-Farsangi, Hamed Mohammadi, Enayat Anvari, Ghasem Ghalamfarsa, Mehdi Yousefi, and Farhad Jadidi-Niaragh. "Nanoparticles and targeted drug delivery in cancer therapy." *Immunology letters* 190 (2017): 64-83.
56. Rajendran, Kumar, Vithiya Karunagaran, Biswanath Mahanty, and Shampa Sen. "Biosynthesis of hematite nanoparticles and its cytotoxic effect on HepG2 cancer cells." *International journal of biological macromolecules* 74 (2015): 376-381.
57. Muhammad, Wali, Muhammad Aslam Khan, Munazza Nazir, Aisha Siddiquah, Sadaf Mushtaq, Syed Salman Hashmi, and Bilal Haider Abbasi. "Papaver somniferum L. mediated novel bioinspired lead oxide (PbO) and iron oxide (Fe2O3) nanoparticles: In-vitro biological applications, biocompatibility

and their potential towards HepG2 cell line." *Materials Science and Engineering: C* 103 (2019): 109740.
58. Nagajyothi, P. C., Muthuraman Pandurangan, Doo Hwan Kim, T. V. M. Sreekanth, and Jaesool Shim. "Green synthesis of iron oxide nanoparticles and their catalytic and in vitro anticancer activities." *Journal of Cluster Science* 28 (2017): 245-257.
59. Fowsiya, J., G. Madhumitha, Naif Abdullah Al-Dhabi, and Mariadhas Valan Arasu. "Photocatalytic degradation of Congo red using Carissa edulis extract capped zinc oxide nanoparticles." *Journal of Photochemistry and Photobiology B: Biology* 162 (2016): 395-401.
60. Padhi, B. S. "Pollution due to synthetic dyes toxicity & carcinogenicity studies and remediation." *International journal of environmental sciences* 3, no. 3 (2012): 940-955.
61. Jin, Xiaoying, Yong Liu, Jeanette Tan, Gary Owens, and Zuliang Chen. "Removal of Cr (VI) from aqueous solutions via reduction and absorption by green synthesized iron nanoparticles." *Journal of Cleaner Production* 176 (2018): 929-936.
62. Thandapani, Keerthika, Manikandan Kathiravan, Elangovan Namasivayam, Indira Arulselvi Padiksan, Geetha Natesan, Manish Tiwari, Benelli Giovanni, and Venkatachalam Perumal. "Enhanced larvicidal, antibacterial, and photocatalytic efficacy of TiO 2 nanohybrids green synthesized using the aqueous leaf extract of Parthenium hysterophorus." *Environmental Science and Pollution Research* 25 (2018): 10328-10339.
63. Gonawala, Kartik H., and Mehali J. Mehta. "Removal of color from different dye wastewater by using ferric oxide as an adsorbent." *Int J Eng Res Appl* 4, no. 5 (2014): 102-109.
64. Stan, Manuela, Adriana Popa, Dana Toloman, Adriana Dehelean, Ildiko Lung, and Gabriel Katona. "Enhanced photocatalytic degradation properties of zinc oxide nanoparticles synthesized by using plant extracts." *Materials Science in Semiconductor Processing* 39 (2015): 23-29.
65. Dutta, Amit Kumar, Swarup Kumar Maji, and Bibhutosh Adhikary. "γ-Fe2O3 nanoparticles: an easily recoverable effective photo-catalyst for the degradation of rose bengal and methylene blue dyes in the waste-water treatment plant." *Materials Research Bulletin* 49 (2014): 28-34.
66. Ahmed, Adeel, Muhammad Usman, Bing Yu, Xin Ding, Qiaohong Peng, Youqing Shen, and Hailin Cong. "Efficient photocatalytic degradation of toxic Alizarin yellow R dye from industrial wastewater using biosynthesized Fe nanoparticle and study of factors affecting the degradation rate." *Journal of Photochemistry and Photobiology B: Biology* 202 (2020): 111682.
67. Venkateswarlu, Sada, Y. Subba Rao, T. Balaji, B. Prathima, and N. V. V. Jyothi. "Biogenic synthesis of Fe3O4 magnetic nanoparticles using plantain peel extract." *Materials Letters* 100 (2013): 241-244.
68. Thakur, Suman, and Niranjan Karak. "One-step approach to prepare magnetic iron oxide/reduced graphene oxide nanohybrid for efficient organic and inorganic pollutants removal." *Materials Chemistry and Physics* 144, no. 3 (2014): 425-432.
69. Njagi, Eric C., Hui Huang, Lisa Stafford, Homer Genuino, Hugo M. Galindo, John B. Collins, George E. Hoag, and Steven L. Suib. "Biosynthesis of iron

and silver nanoparticles at room temperature using aqueous sorghum bran extracts." *Langmuir* 27, no. 1 (2011): 264-271.

70. Rao, Ashit, Ashok Bankar, Ameeta Ravi Kumar, Suresh Gosavi, and Smita Zinjarde. "Removal of hexavalent chromium ions by Yarrowia lipolytica cells modified with phyto-inspired Fe0/Fe3O4 nanoparticles." *Journal of contaminant hydrology* 146 (2013): 63-73.
71. Khatami, Mehrdad, Hajar Q. Alijani, Baratali Fakheri, Mohammad M. Mobasseri, Masoomeh Heydarpour, Zeinab K. Farahani, and Arif Ullah Khan. "Super-paramagnetic iron oxide nanoparticles (SPIONs): Greener synthesis using Stevia plant and evaluation of its antioxidant properties." *Journal of cleaner production* 208 (2019): 1171-1177.
72. Bibi, Ismat, Nosheen Nazar, Sadia Ata, Misbah Sultan, Abid Ali, Ansar Abbas, Kashif Jilani et al. "Green synthesis of iron oxide nanoparticles using pomegranate seeds extract and photocatalytic activity evaluation for the degradation of textile dye." *Journal of Materials Research and Technology* 8, no. 6 (2019): 6115-6124.
73. de Jesús Ruíz-Baltazar, Álvaro, Simón Yobanny Reyes-López, María de Lourdes Mondragón-Sánchez, Anel Ivonne Robles-Cortés, and Ramiro Pérez. "Eco-friendly synthesis of Fe3O4 nanoparticles: evaluation of their catalytic activity in methylene blue degradation by kinetic adsorption models." *Results in Physics* 12 (2019): 989-995.
74. Ahmad, Waqas, Arif Ullah Khan, Saira Shams, Lei Qin, Qipeng Yuan, Aftab Ahmad, Yun Wei, Zia Ul Haq Khan, Sadeeq Ullah, and Aziz Ur Rahman. "Eco-benign approach to synthesize spherical iron oxide nanoparticles: A new insight in photocatalytic and biomedical applications." *Journal of Photochemistry and Photobiology B: Biology* 205 (2020): 111821.
75. Arularasu, M. V., J. Devakumar, and T. V. Rajendran. "An innovative approach for green synthesis of iron oxide nanoparticles: Characterization and its photocatalytic activity." *Polyhedron* 156 (2018): 279-290.
76. Aisida, Samson O., Kenneth Ugwu, Paul A. Akpa, Assumpta C. Nwanya, U. Nwankwo, A. K. H. Bashir, I. G. Madiba, Ishaq Ahmed, and Fabian I. Ezema. "Synthesis and characterization of iron oxide nanoparticles capped with Moringa Oleifera: the mechanisms of formation effects on the optical, structural, magnetic and morphological properties." *Materials Today: Proceedings* 36 (2021): 214-218.
77. Harshiny, Muthukumar, Chandrasekaran Nivedhini Iswarya, and Manickam Matheswaran. "Biogenic synthesis of iron nanoparticles using Amaranthus dubius leaf extract as a reducing agent." *Powder technology* 286 (2015): 744-749.
78. Iqbal, Javed, Banzeer Ahsan Abbasi, Riaz Ahmad, Amir Shahbaz, Syeda Anber Zahra, Sobia Kanwal, Akhtar Munir, Atiya Rabbani, and Tariq Mahmood. "Biogenic synthesis of green and cost effective iron nanoparticles and evaluation of their potential biomedical properties." *Journal of Molecular Structure* 1199 (2020): 126979.

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40 Synthesis, Characterisation And Antibacterial Screeningof Gadolinium2-Hydroxy 4-N- Butoxy 5-Bromo Acetophenone Thiosemicarbazone Complex

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Abstract

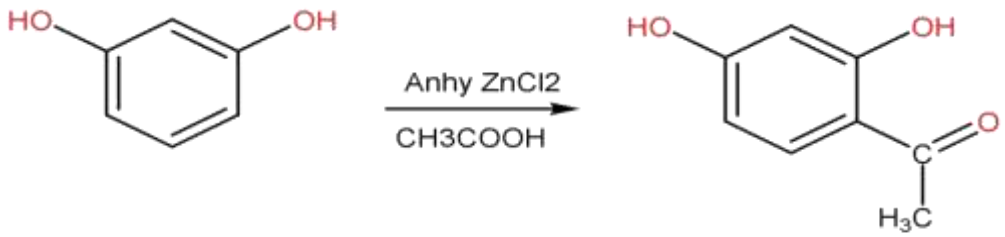
Complexes of La(III), Pr(III), Gd(III), Nd(III), Sm(III), Tb(III) and Dy(III)have been prepared by reacting metal Nitrate with 2-hydroxy 4-n-butoxy 5-bromo acetophenoneThiosemicarbazone as a ligand.The Compounds were characterized by elemental analysis (CHNS),Fourier Transform Infrared (FT-IR), Nuclear Magnetic Resonance (NMR), Ultraviolet-Visible (UV-Vis). Metal complexes were screened antibacterial activity against various bacteria like Escherichia coli,BacillusSubtilis. The Result indicated that the complexes exhibited good antimicrobial activity.
Keywords: bromoacetophenone; thiosemicarbazone,spectralstudy,antimicrobial screening.

Introduction

Thiosemicarbazones have been the subject of several studies because of their variable bonding modes, encouraging biological implications andstructural variety [1,2]. The preparation of thetransition metal complexes with thiosemicarbazone ligands has been gainingsubstantial attention due to the potentially chemotherapeutic properties of both ligands and complexes as antitumor and antibacterial agents [3]. It was reported that their dibasic tridentate fashion with ONS donors are of enormous importance as they own a wide spectrum of medicinal properties [4]. Thiosemicarbazone derivatives are of specific chemical and medicinal importance for the possession of a number of different biological activities: antiviral,anticancer, antibacterial, antifungal activity, etc.[5,6]. Pharmacological activity studies of thiosemicarbazone as antitumor agent is one of the most favorable areas of its research [7].A predominantly significant feature is the reduction of the cytotoxic action of thiosemicarbazone, which may be reduced by complexing to the metal cation [8].Preparation of transition metal complexes with thiosemicarbazone ligands received considerable attention of researchers because of the potentially valuable chemotherapeutic properties of thiosemicarbazone itself, which may be possible by complexing in terms of anticancer and antibacterial activity[1]

Material And Method

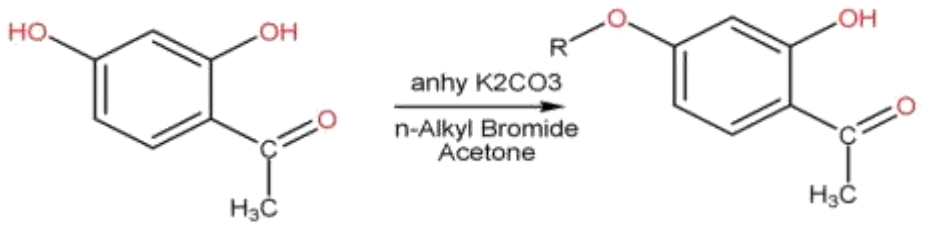
Resacetophenone was prepared from resorcinol, glacial acetic acid and anhydrous zinc chloride according to the method of Robinson and Shah [9]



Resorcinol

Reacetophenone

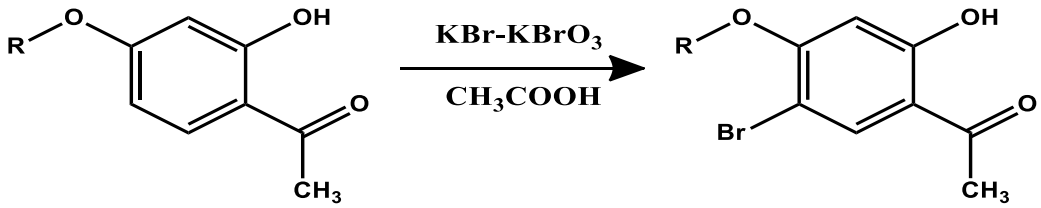
. 2-Hydroxy-4-*n*-butoxyacetophenone (HBA) was prepared by using resacetophenone, *n*-butyl bromide and anhydrous potassium carbonate in acetone [10, 11].



Resacetophenone

Res(Alkoxy/Phenoxy)Phenone

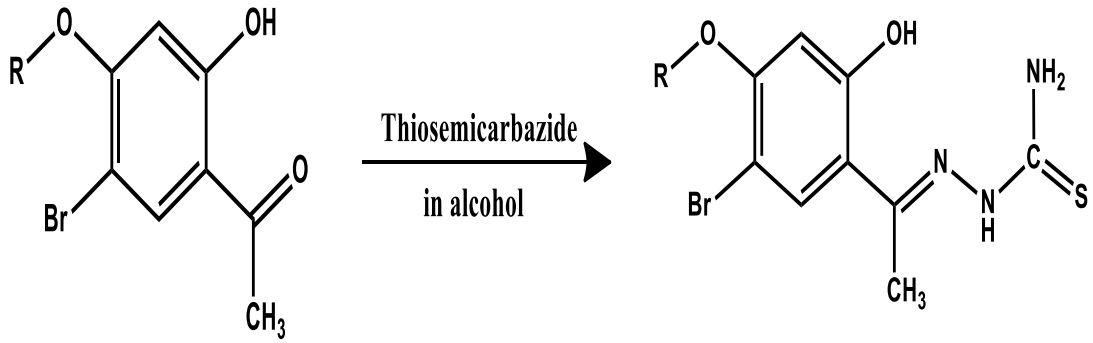
Its 2-hydroxy-4-*n*-butoxy-5- bromoacetophenone was prepared by bromination of HBA.



Res(Alkoxy/Phenoxy)Phenone

5-bromo Res(Alkoxy/Phenoxy)Phenone

Its thiosemicarbazone was prepared by refluxing its alcoholic solution with thiosemicarbazide for about 4 hour [12]**.** It was crystallized from ethanol pale yellow crystals were obtained [M.P. 129 0C]. The molecular weight determination was carried out by Rast's camphor method. The reagent HBBrAT is easily soluble in ethanol, methanol, chloroform, carbon tetrachloride, benzene etc.



5-Bromo Res(Alkox/Phenoxy)Phenone

5-Bromo Res(Alkoxy/Phenoxy) PhenoneThiosemicarbazone derivative

SYNTHESIS OF COMPLEX

A hot ethanolic (20 ml) solution of the HBBrAT (0.003 mol) and a hot ethanolic (20 ml) solution of the corresponding containingGadolinium(III) nitrate (0.001 mol) was mixed together with constant stirring. The reaction mixture was refluxed for 4 h at 80-90ºC.On cooling, a coloured precipitate was formed. It was filtered, washed with cold EtOH and dried under vacuum over P4O10 [13].m.p>400 ^{0}C

IR DATA OF LIGAND AND COMPLEX

The IR spectrum of the ligand is usually compared with that of the complex to know changes during complexation is shown in Fig. 3. The spectrum of the ligand shows a band at 3451 cm_1 indicating the presence of OH group, but it is absent in the spectra of complex which confirms the deprotonation of ligand towards the central metal atom.the ligand also shows strong band at 1200cm^{-1} which may be attributedto the the phenolic C-O vibration.A shift of this band to higher frequency 1260cm^{-1} in the coplex indicate chelation of the ligand to metal ion through phenlic oxygen.

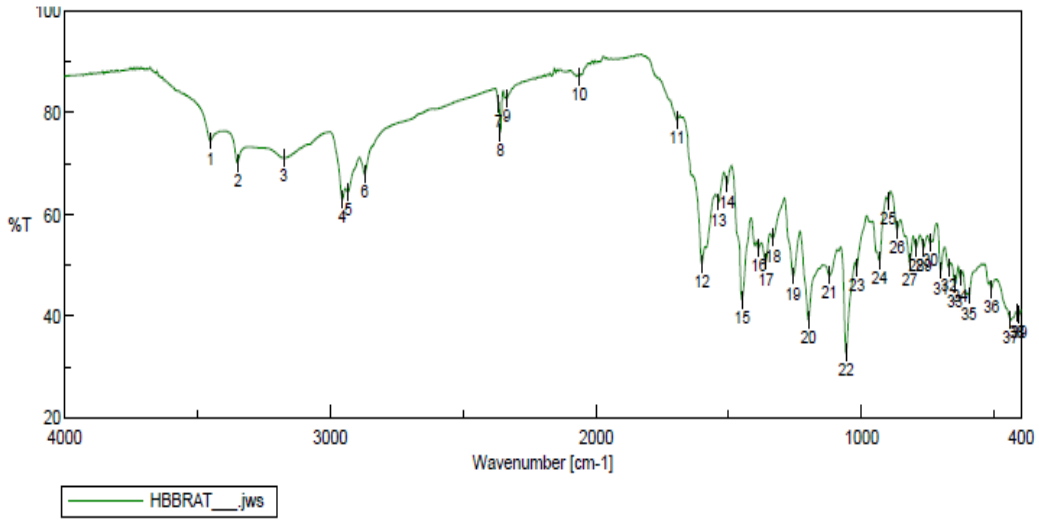
. The strong band observed at 1695 cm^{-1} in the spectra of the ligand is a characteristic of the azomethine stretching vibrations. Upon complexation, this vibration shift to lower frequency 1536 cm^{-1}indicating the bonding in unsaturated nitrogen of the azomethane group towards the metal ion.

The thiosemicarbazone ligand showed an intense band at 1257 cm_1 may be assigned to C = S group and shows 1199cm^{-1} downward shifting in metal complexs indicating participation of this group in co-ordination and no peak was observed at 2500 cm_1 indicating that the ligand is present in thione form in the solid state

The absortion band at 3331 cm^{-}1 due to N-H group in the free ligand remains unaltrered in the complexes ,indicating non participating of this group in co-ordination.

A band present at 1360 cm^{-}1 assigned to C-N group due to nitro group and this band remain unchanged in all complexes suggesting non-participating of nitro group.

some new bands have been observed at 535 due to M-N bond ,430cm^{-1} due to M-O band 327 due to M-S band, cm^{-1} and[14,15]



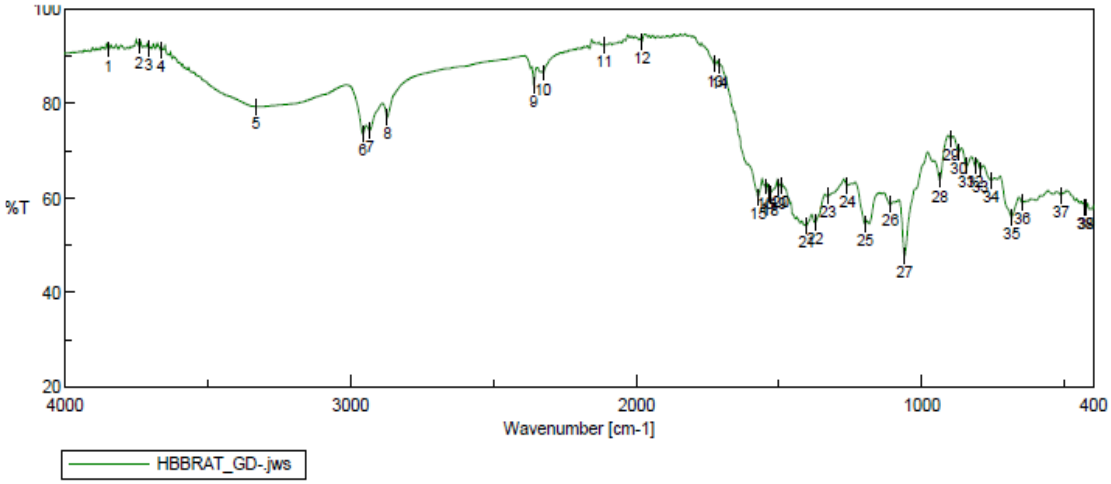


Fig. 2. IRspectra of ligand and its Gd complex.

Group	OH	**NH2**	Ar-H	C=N	C-O	**υ(C-N)**	**La-N**	La-O	La-S
Ligand	3451	3338	2954	1695	1200	1257	-----	------	-----
complex	----	3331	2957	1536	1260	1199	535	430	327

ANTI –MICROBIAL SCREENING

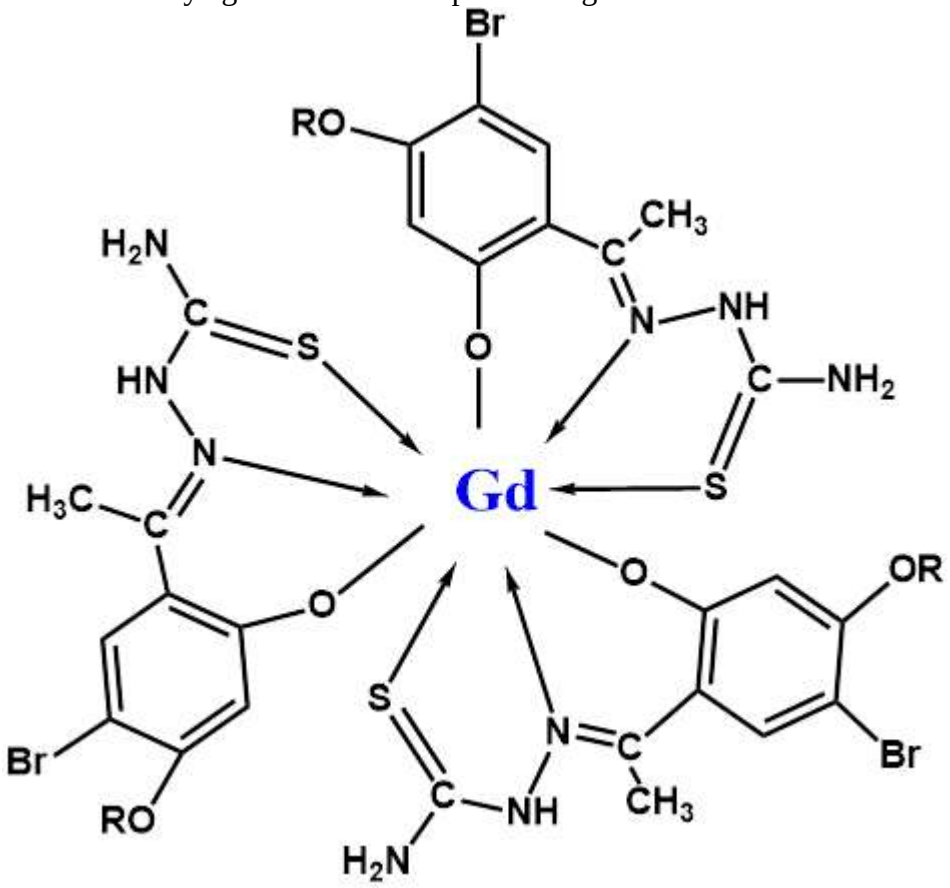
The data listed in Table 1 clearly illustrate that the free ligand and its complexes have antibacterial potency against all tested organisms, whereas the antibacterial activity of the complexes is higher than that of the ligand. It is thought that the increase in activity may be due to the chelation of lanthanide(III) ions and the presence of sulfur atoms

Activity of ligand HBBrAT Activity Of Complex HBBrAT-Gd

Compound	Concentration µg /disc area	Diameter of the inhibition zone in mm E.Coli.
HBBrAT	2	1
HBBrAT-Gd	2	4

Result And Discussion

The ligand complexes with Thiosemicarbazone formed by the condensation of HBBrAT ligand and their lanthanide metal complex displayed significant antimicrobial activity against E.Coli.compared to ligand.



Suggested Structure of the HBBrAT-Gdcomplex

References:

1. Al-Amiery AA, et al. Synthesis,characterization, theoretical crystalstructure and antibacterial activities ofsome transition metal complexes of thethiosemicarbazone (Z)-2-(pyrrolidin-2-
ylidene) hydrazinecarbothioamide.Bioinorganic Chemistry and Applications.2011;1-6.

2. Vojinović-Ješić LS, et al. Transition metalcomplexes with thiosemicarbazide-basedligands, Part 58. Synthesis, spectral andstructural characterization ofdioxovanadium (V) complexes withsalicylaldehydethiosemicarbazone.Journal of the Serbian Chemical Society.2011;76(6):865-877.

3. Sampath K, Jayabalakrishnan C.Ruthenium (III) thiosemicarbazonecomplexes: Synthesis, characterization,DNA binding, antibacterial, *in vitro*anticancer and antioxidant studies. DJ J.
Engineering Chemistry and Fuel. 2016;1:40-53.
4. El-Bahnasawy RM, et al. Electricalconductivity of salicyaldehydethiosemicarbazone and Pd (II), Cu (II) andRu (III) complexe. European ChemicalBulletin. 2014;3(5):441-446.
5. Shivhare S, Gautam MD. Synthesis characterization and antimicrobial studieson some transition metal complexes ofthiosemicarbazones. Journal of CurrentPharmaceutical Research. 2011;6(1):16-20.
6. Sarker D, et al. Synthesis,characterization, antibacterial and thermalstudies of Cu (II) complex of thiophene-2-aldehyde semicarbazone. Asian Journal ofApplied Chemistry Research. 2019;1-10.
7. Sathiyaraj S, JayabalakrishnanC.Synthesis, characterization, DNA bindingand cleavage activity of ruthenium (II)complexes with heterocyclic substitutedthiosemicarbazones. Journal of theChilean Chemical Society. 2013;58(1):1637-1642.
8. Parekh J, et al. Synthesis and antibacterialactivity of some Schiff bases derived from4-aminobenzoic acid. Journal of SerbianChemical Society. 2005;70(10):1155.
9. Desai, N. C.; Dipika, S. M. D.; Vyas, G. D. *Indian J. Chem*. **2000**, 398.
10. Mehta, D. S.; Shah, V. H. *Indian J. Het. Chem*. **2001**, *11*, 139.
11. Trivedi, P. B.; Undavia, N.K.; Dave, A. M. *Indian J. Chem*. **1993**, *32B*, 760.
12.Desai, S.; B.; Desai, P. B.; Desai, K. R. *Asian J. Chem*. **1990**, *1*, 1431.
13.Eman Fares Mohamed1*and Safaa Said Hassan2*Egyptian Journal of Chemistry*Egypt. J. Chem.* Vol. 65, No. 12 pp. 11 - 19 (2022)
14.Nur Nadia Dzulkifli1, 2*, Yang Farina1, Bohari M.Yamin1, Nazlina Ibrahim3Synthesis, Structural, Chemical Properties, And Anti-Bacterial Screening Of Sm(Iii) Thiosemicarbazone Complexes *Malaysian Journal of Analytical Sciences, Vol 21 No 3 (2017): 560 - 570 DOI: https://doi.org/10.17576/mjas-2017-2103-06*
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21 A Facile And Approach For The Synthesis of 4-Thiazolidinones Derivatives with Microbial Activity

Viralkumar Modi
Department of Chemistry, Shri U. P. Arts, Smt. M. G. Panchal Science & Shri V. L. Shah Commerce College, Pilvai(India)

ABSTRACT

reaction of Amino-aryl 1,3,4-oxadiazole derivatives into heterocyclic ring like 4-Thiazolidinone.All have studied the anti-inflammatory activity of 5-(6-methyl-2-substituted-4-pyrimidinyloxymethyl)-2,3-dihydro-1,3,4-oxadiazole-2thiones relative to that of acetylsalicylic acid. The highest activity compounds appeared to be 5-(6-methyl-2morpholino-4-pyrimidinyloxymethyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione and 5-(6-methyl-2-methylthio-4pyrimidinyloxymethyl)-3-morpholinomethyl-2,3-dihydro-1,3,4-oxadiazole-2-thione.

Keywords: -1,3,4-thiadiazole ring, Thiodiazole , thio azetidinone, cyclic ketone, Schiff base, thiazolidinone, 4Thiazolidinones, Benzaldehyde derivates etc

INTRODUCTION

The compounds containing 1,3,4-oxadiazole ring have been known for about a hundred years. Their synthesis was extensively studied at the beginning of the 20th century by stoll et al [1-5]. Since that time a vast amount of work devoted to these compounds has been done [6-14]. An increase in number of publications devoted to their biological activities observed recently is worth noting and their brief review is given here.

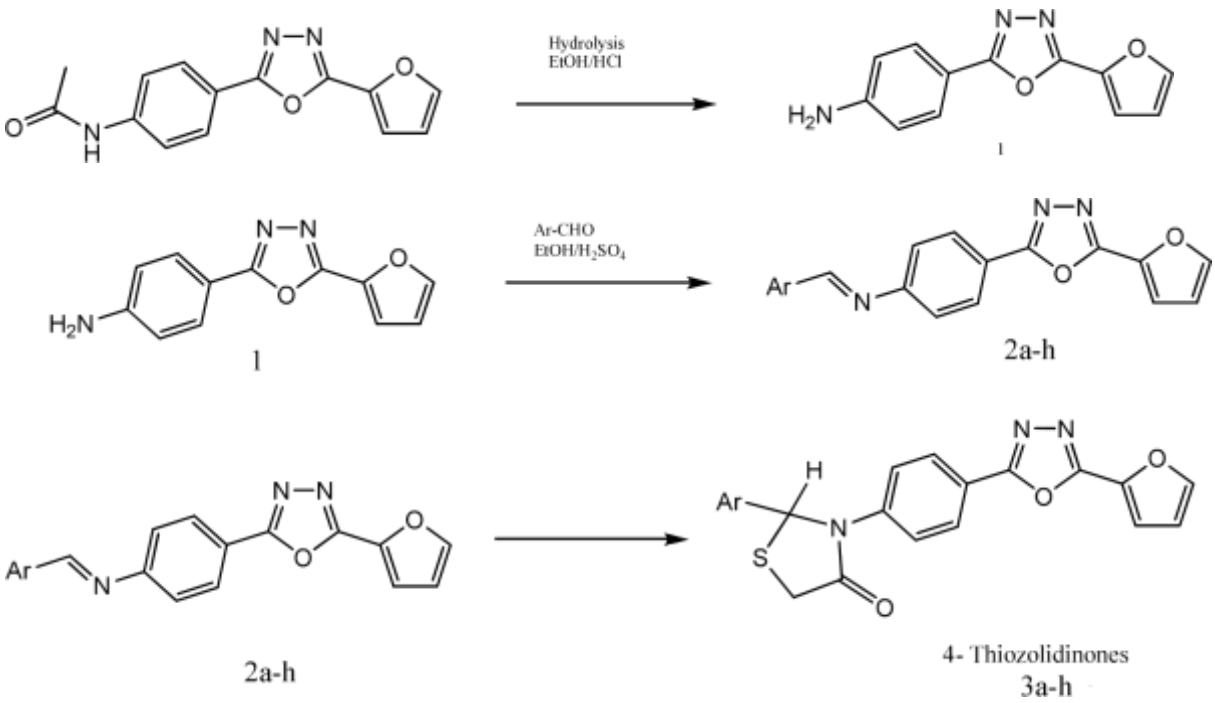
Antimicrobial activities like, antibacterial activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and antifungal activity against Candida albicans were studied by Mohan and Kataria [15] for two imidazol[1,2-d]-s-triazolo[3,4-b][1,3,4]oxadiazoles and by Rollas et al [16] for four 3-acetyl-5-(4-flourophenyl)-2substituted-2,3-dihydro-1,3,4-oxadiazoles.2-amino-5-(1/2-naphthyloxymethyl)-1,3,4-oxadiazole have been screened for their activity against the above microbes and for other two fungi(C.krusei and C.parapsilosis) by Sachin et al [17]., the compounds studied showed moderate activities.

Antiinflamatory activities of the series of 2-substituted-5-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,3,4oxadiazoles have been studied by Mullican et all [25]. Jakubkiene et all [26] have studied the anti-inflammatory activity of 5-(6-methyl-2-substituted-4-pyrimidinyloxymethyl)-2,3-dihydro-1,3,4-oxadiazole-2-thiones relative to that of acetylsalicylic acid. The highest activity compounds appeared to be 5-(6-methyl-2-morpholino-4pyrimidinyloxymethyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione and 5-(6-methyl-2-methylthio-4-pyrimidinyloxy methyl)-3-morpholinomethyl-2,3-dihydro-1,3,4-oxadiazole-2-thione.

MATERIALS AND MATHODS

The literature review pertaining to the Amino-aryl functionalized 1,3,4-oxadiazole derivatives reveals that there are no reports have been found. While few reports about Aryl-amino derivatives have been reported [27,28].

Hence, it was worthwhile to undertake the post reaction of Amino-aryl 1,3,4-oxadiazole derivatives into heterocyclic ring like 4-Thiazolidinone. The work carried is described in (Scheme 1).



MATERIALS

Furan-2-carbohydrazine and4-Acetamidobenzoicacid were purchased from local market. N-(4-(5-(furan-2-yl)-1,3,4oxadiazol-2-yl)phenyl)acetamide(1)was prepared by reported method[27,28]. The various benzaldehyde derivatives were used to prepare Schiff bases. They were obtained from local market. All the other chemicals used were of analytical grade.

(a)Synthesized compounds

Synthesis of 4-(5-furan-2-yl)-1,3,4-oxadiazol-2-yl)aniline (1):

4-(5-furan-2-yl)-1,3,4-oxadiazol-2-yl)anilinewas prepared by reportedmethod[27,28]. The resultant Schiff bases are designated as 1 and their details are shown as follows.

(1): 4-(5-furan-2-yl)-1,3,4-oxadiazol-2-yl)aniline Yield: 78% M.p.: 140°C Mol.Wt.: 227

Anal.Calcd.for $C_{12}H_9N_3O_2$(%): C, 63.43; H, 3.99; N, 18.49. Found: C,63.3;H,3.9; N, 18.4.1H NMR (DMSO-d_6 400 MHz):6.60–7.58 (7H, m, aromatic protons), 6.30 (2H, s,aromatic C-NH protons).13C NMR (DMSO-d_6 100 MHz): 114-152(Ar-10C,), 158.5, 165.4 (N=CH). IR (KBr. cm^{-1}):1655 cm^{-1}(C=N), 3030 cm^{-1} (C-H, of Ar.), 1095 (C-O-C).

Table:-1 Analytical Data and Elemental Analysis of Compounds (2a-h)

Compd.	Mol.form (Mol.wt.)	Yield	M.P. °C	Elemental Analysis % C		% H		% N	
				Found	Calcd.	Found	Calcd.	Found	Calcd.
2a	$C_{19}H_{13}N_3O_2$ (315)	72	205-207	72.3	72.37	4.1	4.16	13.2	13.33
2b	$C_{20}H_{15}N_3O_3$ (345)	75	211-212	69.5	69.56	4.3	4.38	12.1	12.17
2c	$C_{19}H_{13}N_3O_3$ (331)	78	216-217	68.8	68.88	3.9	3.95	12.6	12.68
2d	$C_{19}H_{13}N_3O_3$ (331)	74	204-206	68.8	68.88	3.9	3.95	12.6	12.68
2e	$C_{20}H_{15}N_3O_2$ (329)	73	218-220	72.9	72.94	4.5	4.59	12.7	12.76
2f	$C_{20}H_{13}N_3O_4$ (359)	72	221-222	66.8	66.85	3.6	3.65	11.6	11.69
2g	$C_{20}H_{15}N_3O_4$ (361)	75	218-219	66.4	66.48	4.1	4.18	11.5	11.63
2h	$C_{23}H_{21}N_3O_4$ (403)	71	222-225	68.4	68.47	5.2	5.25	10.3	10.42

Table:-2Spectral Data of Compounds (2a-h)

Compd.	IR cm⁻¹	NMR ppm
2a	3040(Ar-H),1655(C=N),1110(C-O-C)	6.6–7.9(12H,m,Ar-H), 8.4 (1H, s, -N=CH)
2b	3045(Ar-H),1650(C=N),1095,1200(C-O-C)	6.6–7.9(11H,m,Ar-H), 8.4 (1H, s, -N=CH) 3.7 (3H, s, $-OCH_3$)
2c	3450(OH),3030(Ar-H),1652(C=N),1112(C-O-C)	6.6–7.9(11H,m,Ar-H), 8.4 (1H, s, -N=CH) 4.3 (1H, s, -OH)
2d	3430(OH),3030(Ar-H),1652(C=N),1114(C-O-C)	6.6–7.9(11H,m,Ar-H), 8.4 (1H, s, -N=CH) 4.2 (1H, s, -OH)
2e	3032(Ar-H),1655(C=N),1110(C-O-C)	6.6–7.9(11H,m,Ar-H), 8.4 (1H, s, -N=CH) 2.3 (3H, s, $-CH_3$)
2f	3035(Ar-H),1655(C=N),1098(C-O-C)	6.3–7.9(10H,m,Ar-H), 8.4 (1H, s, -N=CH) 5.5 (2H, s, O-CH_2-O)
2g	3444(OH),3030(Ar-H),1650(C=N), 1095,1205 (C-O-C)	6.6–7.9(10H,m,Ar-H), 8.4 (1H, s, -N=CH) 3.7 (3H, s, $-OCH_3$) 4.3 (1H, s, -OH)
2h	3036(Ar-H),1655(C=N),1098,1202(C-O-C)	6.6–7.9(10H,m,Ar-H), 8.4 (1H, s, -N=CH) 2.1 (6H, s, $-2CH_3$) 3.7 (4H, s, $-2CH_2$)

Synthesis of Schiff bases of 4-(5-furan-2-yl)-1,3,4-oxadiazol-2-yl)aniline (1):formationof (2a-h).

The Schiff bases of 4-(5-furan-2-yl)-1,3,4-oxadiazol-2-yl)aniline(1)were prepared by reportedmethod [29]. Benzaldehyde derivative (a-h) (0.01mole), oxadiazol (1) (0.01mole) and ethanol (20ml) were taken in a beaker

[100ml]. The mixture was heated until a clear solution was obtained. The clear solution was kept overnight when respective Schiff base fall out which was filtered, washed by petroleum ether and air dried. The resultant Schiff bases are designated as 2a-h and their details are shown in table 1, table 2.

Table:-3 Analytical Data and Elemental Analysis of Compounds (3a-h)

C.	Mol.formula (Mol.wt.)	Yield	M.P.°C	Elemental Analysis							
				% C		% H		% N		% S	
				Found	Cal.	Found	Cal.	Found	Cal.	Found	Cal.
3a	C21H15N3O3S (389)	70	221-223	64.7	64.77	3.8	3.88	10.7	10.79	8.1	8.23
3b	C22H17N3O4S (419)	72	228-230	62.9	63.00	4.0	4.09	9.9	10.02	7.6	7.64
3c	C21H15N3O4S (405)	74	226-227	62.1	62.21	3.7	3.73	10.3	10.36	7.8	7.91
3d	C21H15N3O4S (405)	75	222-224	62.1	62.21	3.7	3.73	10.3	10.36	7.8	7.91
3e	C22H17N3O3S (403)	72	232-233	65.4	65.49	4.2	4.25	10.3	10.42	7.9	7.95
3f	C22H15N3O5S (433)	68	235-237	60.9	60.96	3.4	3.49	9.6	9.69	7.3	7.40
3g	C22H17N3O5S (435)	70	230-231	60.6	60.68	3.9	3.93	9.6	9.65	7.3	7.36
3h	C25H23N3O5S (477)	68	237-238	62.8	62.88	4.8	4.85	8.7	8.80	6.6	6.71

Table:-4 Spectral Data of Compounds (3a-h)

Compd.	IR cm⁻¹	NMR ppm
3a	3080 (CH_2 of thiazolidinone), 3032 (Ar-H), 1690 (C=O), 1654 (C=N), 1112 (C-O-C), 718 (C-S-C)	6.5 – 8.2 (12 H, m, Ar-H), 3.8 (2H, s, -CH_2), 5.8 (1H, s, C_2H)
3b	3085 (CH_2 of thiazolidinone), 3040 (Ar-H), 1695 (C=O), 1650 (C=N), 1105,1205 (C-O-C), 720 (C-S-C)	6.5 – 8.2 (11 H, m, Ar-H), 3.8 (2H, s, -CH_2), 5.8 (1H, s, C_2H) 3.9 (3H, s, -OCH_3)
3c	3080 (CH2 of thiazolidinone), 3439 (OH), 3036 (Ar-H), 1692 (C=O), 1648 (C=N), 1111 (C-O-C), 718 (C-S-C)	6.5 – 8.2 (11 H, m, Ar-H), 3.8 (2H, s, -CH_2), 5.9 (1H, s, C_2H) 4.4 (1H, s, -OH)
3d	3080 (CH_2 of thiazolidinone), 3428 (OH), 3035 (Ar-H), 1694 (C=O), 1652 (C=N), 1113 (C-O-C), 718 (C-S-C)	6.5 – 8.2 (11 H, m, Ar-H), 3.8 (2H, s, -CH_2), 5.9 (1H, s, C_2H) 4.2 (1H, s, -OH)

3e	3090 (CH_2 of thiazolidinone), 3040 (Ar-H), 1690 (C=O), 1651 (C=N), 1115 (C-O-C), 718 (C-S-C)	6.5–8.2 (11 H, m, Ar-H), 3.9 (2H, s, -CH_2), 5.8 (1H, s, C_2H) 2.1 (3H, s, -CH_3)
3f	3080 (CH_2 of thiazolidinone), 3035 (Ar-H), 1688 (C=O), 1654 (C=N), 1110 (C-O-C), 720 (C-S-C)	6.5–8.2 (10 H, m, Ar-H), 3.8 (2H, s, -CH_2), 5.9 (1H, s, C_2H), 5.4 (2H, s, O-CH_2-O)
3g	3085 (CH_2 of thiazolidinone), 3450 (OH), 3040 (Ar-H), 1680 (C=O), 1650 (C=N), 1107,1212 (C-O-C), 718 (C-S-C)	6.5–8.2 (10 H, m, Ar-H), 3.8 (2H, s, -CH_2), 5.9 (1H, s, C_2H) 3.9(3H, s, -OCH_3) 4.3 (1H, s, -OH)
3h	3085 (CH_2 of thiazolidinone), 3038 (Ar-H), 1690 (C=O),1655 (C=N), 1106,1207 (C-O-C), 720 (C-S-C)	6.5–8.2 (10 H, m, Ar-H), 3.8 (2H, s, -CH_2), 5.8 (1H, s, C_2H) 2.2 (6H, s, -$2CH_3$) 3.4 (4H, s, -$2CH_2$)

Synthesis of 4-Thiazolidinones derivatives(3a-h).

The Schiff bases of 4-(5-furan-2-yl)-1,3,4-oxadiazol-2-yl)aniline (1)were prepared by reportedmethod [30-32]. A mixture of Schiff bases (2a-h) (0.01 mole) in THF (30ml) and mercapto acetic acid (thioglycolic acid) (0.01 mole) with a pinch of anhydrous $ZnCl_2$ was refluxed for 12 hours. The solvent was then removed to get a residue, which was dissolved in benzene and passed through column of silica gel using benzene: chloroform (8:2; v/v) mixture as eluent. The eluate was concentrated and the product crystallized from alcohol to give 4-thiazolidinones (3a-h).The analytical and spectra data of compounds (3a-h) are described in Table-3

ANTIMICROBIAL ACTIVITY

The culture medium preparation

Nutrient agar medium was used. Chemical composition of the medium was,

Peptone	1.0 gm
NaCl	0.5 gm
Meat extract	0.3gm
Distilled water	100ml
pH	7.6
Agar	2.0 gm

The ingredient were weighed and dissolved in distilled water, pH was adjusted to 7.6 and then agar power was added to it. The medium was dispensed in 25 ml quantity in different test-tubes. The test-tubes were plugged by cotton-wool and sterilized at 121.5^{O}C and 15 pounds per square inch (psi) pressure for 15 minutes.

Antibacterial susceptibility testing

The study has been conducted according to the method adopted by Cruickshank et al [33]. Nutrient agar broth was melted in a water bath and cooked to 45^{O}C with gentle shaking to bring about uniform cooling. It was inoculated with 0.5-0.6 ml of 24 hour old culture especially and mixed well by gentle shaking before pouring on the sterilized Petri dish (25 ml each). The poured material was allowed to set (1.5 hour) and there after the "cups" were made by punching into the agar surface with a sterile cork borer and sooping out the punched part of agar. Into this "cups" 0.1 ml of test solution (prepared by dissolving 10gm of sample in 10ml DMF) was added by sterile micropipette. The plates were noted.

Table:-5 Biological evaluation of Compounds

Comps	Zone of inhibition (in mm			
	Gram positive		Gram negative	
	B.Subtilis	S.Aureus	E.Coli	Ps.Aeruginosa
DMF	5	5	5	5
Ampicillin	19	15	20	21
Tetracyclin	21	19	15	24
Gentamycin	20	18	19	22
2a	15	14	13	14
2b	15	16	14	12
2c	14	14	15	13
2d	12	13	13	12
2e	14	11	10	10
2f	21	20	18	22
2g	20	21	17	19
2h	18	15	19	18
3a	10	13	09	08
3b	19	17	17	20
3c	12	11	08	07
3d	11	13	15	10
3e	10	12	11	14
3f	10	12	11	13
3g	18	15	16	15
3h	18	17	19	21

CONCLUSION

schiff bases (2a-h) on cyclo-condensation reaction with thioglycolic acid (mercapto acetic acid) in the presence of anhydrous $ZnCl_2$ yields 4-thiazolidinones (3a-h). Their structures were confirmed by analytical and spectral data. The C, H, N and S contents of the prepared compounds were consistent with their predicted structures as shown in Scheme-1. The analytical and spectral data of the compounds (3a-h) are shown in Table-4. The infrared spectra show the band in the region ~1690 cm^{-1} for carbonyl group of 4-thiazolidinone ring.
The NMR spectra (Table-4)show a signal at ~5.9 ppm for CH_2 protons at position-5 in the 4-thiazolidinone ring and a signal at ~3.8 ppm for CH protons at position-2 of the 4-thiazolidinone ring. All other signals are at their respective positions for the respective protons in the NMR spectra.
The Schiff bases (3a-h) on cyclocondensation reaction with chloroacetyl chloride in the presence of triethylamine (TEA) affords the biologically active 4-thiazolidinones.

Activity of standards and inhibition due to DMF (solvent) are given in Table-5. The results (Table-5)shown by compounds and standards are corrected for DMF.

Comparison of antimicrobial activity of produced compounds with that ofstandard antimicrobial drugs reveals that the produce compounds (Schiff Bases, 4-Thiazolidinones) shows moderate to good activity against all four bacterial strains.

Among3-(4-(5-(furan-2-yl)-1,3,4-oxidiazol-2-yl)phenyl)-2-phenylthiazolidin-4-one(Table-5, 3a-h) compound 4c,4dand 4g show good antimicrobial activity.

ACKNOWLEDGEMENTS

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REFERENCES

[1] R.stolle, *J.Prakt.Chem*.,69,145(1904).
[2] R.stolle and W.kind, *J.Prakt.Chem*.,70,423(1904).
[3] R.stolle, W.Munch and W.Kind, *J.Prakt.Chem*.,70,433(1904).
[4] R.stolle and K.Thoma, *J.Prakt.Chem*.,73,288(1906).
[5] R.stolle and K.Fehrenbach, *J.Prakt.Chem*.,122,289(1929).
[6] H.N.Dogan, S.Rollas and H.Erdeniz, *IL Farmaco*,53,462(1998).
[7] H.N.Dogan, S.Rollas and M.K.Uysal, *Bioorg.Med.Chem*.,10,2893(2002).
[8] E.Palaska, G.Sahin, P.Kelicen and G.Altinok, *ILfarmaco*,57,101(2002).
[9] E.Palaska, G.Sahin, P.Kelicen and G.Altinok, *Arzneim.Forsch/ Drug Res*.,51,478(2001). [10]S.Karakus and S.Rollas, *IL Farmaco*,57,577(2002).

[11] S.G.Kucukguzel, E.E.Coruc, S.Rollas and A.ozbek, *Eur.J.Med.Chem.*,37,197(2002).
[12] N.Terziglu and A.Gursoy, *Eur.J.Med.Chem*.,38,781(2003).
[13] B.S.Holla, K.N.Poojari and B.S.Rao, *Eur.J.Med.Chem*.,37,511(2002).
[14] S.K.Srivastava, S.Srivastava and S.D.Srivastava, *Ind.J.Chem*.,38B,183(1999).
[15] J.Mohan and S.Kataria, *Indian J. Chem.,* 37B, 713 (1998).
[16] S.Rollas, N.Gulerman and H.Erdeniz, *IL Farmaco*, 57, 171 (2002).
[17] G.Sahin, E.Palaska, M.Ekizoglu and M.Ozalp, *IL Farmaco*, 57, 539, (2002). [18]O.Ates, A.Kocabalkanli, N.Cesur and G.Otuk, *IL Farmaco*, 53, 541, (1998).
[19] O.Ates, A.Kocabalkanli, N.Cesur and G.Otuk, *IL Farmaco*, 56, 975, (2001).
[20] H.P.Shah, B.R.Shah, J.J.Bhatt, N.C.Desai, P.B.Trivedi and N.K.Undavia, *Indian J. Chem*., 37B, 180 (1998).
[21] K.Ladva, P.Patel, P.Upadhyay and H.Parekh, *Indian J.Chem.*, 35B,1062 (1996).
[22] E.Palaska, G.Sahin, P.Kelicen, N.T.Turlu and G.Altinok, *IL Farmaco*, 57, 101 (2002).
[23] G.Sahin, E.Palaska, P.Kelicen, R.Demirdamar and G.Altinok, *Arzneim.-Forsch./Drug Res*., 51, 478 (2001).
[24] F.A.Omar, N.M.Mahfouz and M.A.Rahman, *Eur.J.Med.Chem*., 31, 819 (1996).
[25] M.D.Mullican, M.W.Wilson and R.D.Dyer, *J. Med. Chem*., 36, 1090 (1993).
[26] V.Jakubkiene, M.M.Burbuliene and P.Vainilavieius, *IL Farmaco*, 58, 323 (2003).
[27] X. J. Zou, L. H. Lai, G. H. Jin and Z. X. Zhang, *J. Agri. Food. Chem*., 50, 3757 (2002).
[28] K.Ladva, P.Patel and P.Upadyay, *Ind.J.Chem*.,35B,1062(1996).
[29] R.Gudipati, R.N.R.Anreddyand S.Manda, *Saudi Pharm. J.*,19,153(2011).
[30] M.KKidwai, P.KumarY.Goel and K.Kumar, *Ind.J.Chem*.,36B,175(1997).
[31] N.C.Desai, H.K.Shukla and K.A.Thaker, *J. Ind.Chem. Soc*.,61,239(1984).
[32] S.K.Srivastva, R.B.Pathakand S.C.Bhel, *J. Ind. Chem. Soc*.,68,113(1991).
[33] R.Cruickshank, J.P.Dugid, D.P.Marmion and R.H.A.Swain, "Medical Microbiology", Churchil-Livingstone, Edinburgh, London, Vol.2, 12th edition (1975).

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12 Photochemical and photocatalytic study of Quinalphose

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Abstract
Pesticides are a mixture of substances and widely used for destroying or controlling pests. Different technologies used to control pesticide concentration in nature include photolysis, semiconductor-based photocatalysis and photoelectrocatalysis, etc. The present work mainly focuses to discuss and summarize numerous characteristics of photocatalytic elimination of pesticides. The discussion includes the mechanism of semiconductor photocatalytic degradation of Quinalphose modifications over semiconductors that are available in the literature to increase the efficiency of the degradation process, and finally optimization of different parameters of a process for enhancement in the degradation course. In this study various parameters like pH of the system, amount of semiconductor catalyst, initial concentration of pesticides, and wavelength as well as the intensity of light have been reported intensively.

Keywords: Photochemical, Photocatalytic, Quinalphose, Pesticides.

Introduction

Photocatalysis is a phenomenon, in which an electron-hole pair is generated on exposing a semiconducting material. This electron can be used for reducing a substrate whereas the hole may be utilized for oxidation. Thus, the chemical reactions that occur in the presence of a semiconductor and light are collectively termed as photocatalytic reactions. Although the term "Photocatalysis" has been debated for a long, but it is still reserved for such reactions only.
Calvert et al.[1] reported that ZnO can be used as photocatalyst for photochemical synthesis of hydrogen peroxide. Photoreduction of methylene blue on ZnO surface has been investigated by Barschchevski [2]. Morrison and Freund[3] studied some. photocatalysed chemical reactions in the presence of ZnO suspensions. Dixon and Healy[4] investigated some photochemical redox reactions at the ZnO-water interface in additive free systems. Gerischer and Cammann[5] also observed some photocatalytic and photoelectrochemical processes on ZnO surfaces. Micka[6] observed photo effects in ZnO suspensions. Richard and Lemaire[7]. Investigated the analytical and kinetic study of the photo transformation of furfural alcohol in aqueous ZnO suspension. Ciping et a1.[8] observed the formation of free radical intermediates in photoreaction of ZnO dispersion. Claire et al.[9] reported the oxidising species involved in photocatalytic transformation of ZnO. Wada et a1.[10] carried out selective photooxidation of methane and ethane with oxygen over ZnO and molybdenum loaded ZnO photocatalyst. Sharma et a1.[11,12] reported photocatalytic degradation of xylidine ponceau and

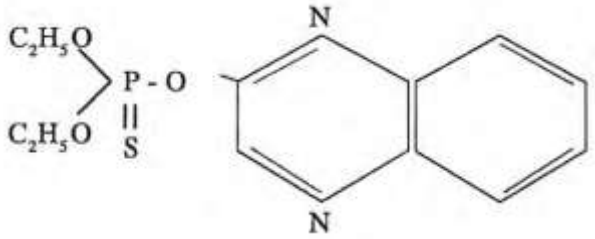
orange-G whereas Chen and Chou[13] observed photocatalytic bleaching of methyl orange using ZnO.

Experimental:

MATHODS AND MATERIAL

Name And Properties Of Quinalphos:

1. Chemical Name Quinalphos
2. IUPAC Name O,O-Quinoxaline - 2 - yl phosphorothioate
3. Empirical Formulae
4. Structural Formulae: $C_{12}H_{15}O_3N_2PS$



5. Molecular Wt. 298.3
6. max 237 and 320 nm.
7. Density 1.04
8. Solubility Insoluble in acetone. soluble in water, methanol etc ....

Materials used:

All the reagents used were purified thoroughly by distillation. The reagents used for the preparation of solutions were dried cautiously. Methanol (BDH) was dried first by keeping over sodium wire and then it was distilled over fractionating column. The fraction, distilling at $65^{O}C$, was collected and used.

The rest of the reagents used for the preparation of the solutions were purified by distillation.

O,O - Quinoxaline - 2 - yl phosphorothioate [Quinalphos], DDVP (2,2' - Dichloro dimethyl vinyl - Phosphate), Dichlorvos, Methanol, Distilled Water, NaOH (Sodium hydroxide solution), Con. HcI (Hydrochloric acid), Perchloric Acid, Zink Oxide (ZnO), Titanium dioxide ($Ti0_2$), Cadmium Sulphide (CdS), Zink Sulphide (ZnS), Methyl parathione, Chloropyrifos

Preparation of the solution:

10^{-1} M standard solution of Quinalphos was prepared in water/methanol solvent as a stock solution. 50 ml 10^{-1} M solution was taken in measuring flask for each sample. Sample was kept in visible light and UV chamber. All the samples were divided into four/five different samples as under:

Sample-l : 50 ml 10^{-1} M Solution of Quinalphos was kept in dark.

Sample-2 : 50 ml 10^{-1} M Quinalphos was kept in visible light

Sample-3 : 50 ml 10^{-1} M Quinalphos + 0.200 gm TiO_2 was kept in visible light

Sample-4 : 50 ml 10^{-1} M Quinalphos was kept in UV light

Sample-5 : 50 ml 10^{-1} M Quinalphos + 0.200 gm TiO_2 was kept in UV light

It was observed that there is no reaction in case (1). The reaction was much slow in (2) and (3), whereas it proceeds with a reasonable rate in (4) and (5). Observations of photochemical and photocatalytic reactions are reported in Table 5.1 and 5.2 respectively. A typical run for photocatalytic reaction is graphically depicted in Figure 5.1 and 5.2.

Results And Discussion

Table:1Photochemical Reaction Of Quinalphos In Visible Light.

Concentration of Quinalphos = 0.1 M pH = 3.0
Temperature = 305 K Wavelength =

Time (hour)	Abs	2 + Log A
0	0.159	1.2014
1	0.153	1.1847
2	0.148	1.1703
3	0.142	1.1523
4	0.137	1.1355
5	0.132	1.1195
6	0.128	1.1072
7	0.123	1.0900
8	0.118	1.0730
9	0.114	1.0569
10	0.110	1.0414
11	0.104	1.0170
12	0.100	1.0000
13	0.096	0.9823
14	0.092	0.9641
15	0.089	0.9494
16	0.084	0.9260
17	0.082	0.9138
18	0.078	0.8920
19	0.074	0.8692
20	0.073	0.8633

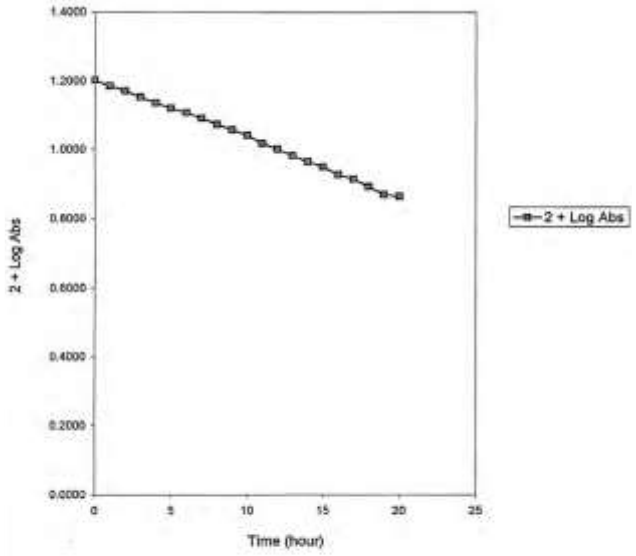


FIGURE-1
PHOTOCHEMICAL REACTION OF QUINALPHOS IN VISIBLE LIGHT(at 625 nm)

Table:2

PHOTOCHEMICAL REACTION OF QUINALPHOS IN UV LI

Concentration of Quinalphos = 0.1 M pH = 3.0
Temperature = 305 K Wavelength = 27

Time (hour)	Abs	2 + Log Abs
0	0.207	1.3160
1	0.197	1.2945
2	0.187	1.2718
3	0.178	1.2504
4	0.169	1.2279
5	0.160	1.2041
6	0.151	1.1790
7	0.143	1.1553
8	0.133	1.1239
9	0.125	1.0969
10	0.115	1.0607
11	0.106	1.0253
12	0.099	0.9956
13	0.093	0.9685
14	0.086	0.9345
15	0.079	0.8976
16	0.074	0.8692
17	0.073	0.8633

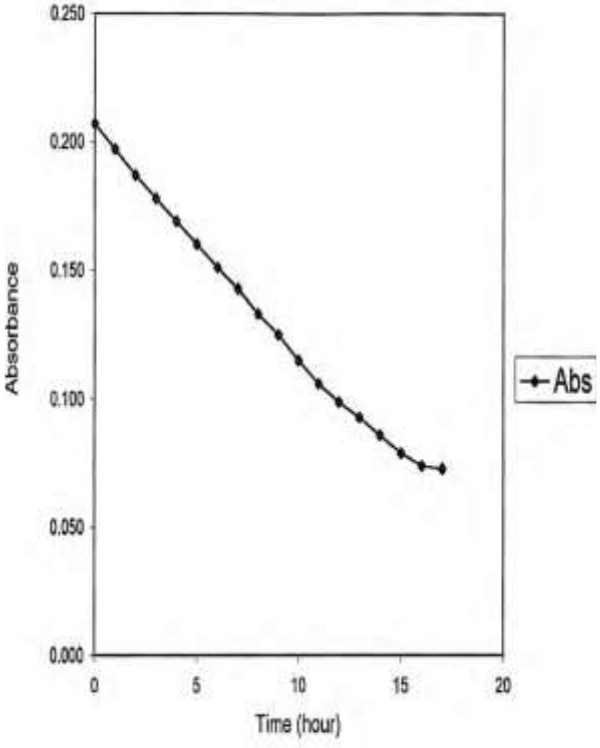


FIGURE-2
PHOTOCHEMICAL REACTION OF QUINALPHOS IN UV LIGHT(at 275 nm)

CHANGE OF ABSORPTION WITH THE CHANGE OF pH OF THE SOLUTION OF QUINALPHOS.

The effect of variation of pH of the medium was investigated by maintaining different pH in acidic range only as the solution became turbid in basic medium. The results are reported in table - 5.3 and graphically presented in Figure - 5.3. It has been observed that the rate of photocatalytic reaction of Quinalphos decreases on increasing pH. This behaviour can be explained on the basis that at higher pH values, more OH radicals will be produced.

Table:3:

CHANGE OF ABSORPTION IN THE SOLUTION OF QUINALPHOS WITH DIFFERENT pH IN VISIBLE LIGHT

Concentration of Quinalphos = 0.1 M
Temperature = 305 K

CdS =0.200 gm
Wavelength = 625 nm

Time In hour	pH = 1.0		pH = 2.0		pH = 3.0	
	Abs	2 + log Abs	Abs	2 + logAbs	Abs	2 + logAbs
0	0.150	1.1761	0.155	1.1903	0.159	1.2014
1	0.143	1.1553	0.147	1.1673	0.152	1.1818
2	0.136	1.1335	0.140	1.1474	0.147	1.1670
3	0.128	1.1072	0.133	1.1239	0.138	1.1386
4	0.122	1.0853	0.127	1.1038	0.132	1.1206
5	0.115	1.0607	0.120	1.0792	0.126	1.1017
6	0.109	1.0378	0.114	1.0569	0.120	1.0792
7	0.102	1.0090	0.108	1.0322	0.114	1.0561
8	0.096	0.9805	0.102	1.0086	0.107	1.0306
9	0.090	0.9542	0.097	0.9868	0.103	1.0111
10	0.085	0.9294	0.091	0.9590	0.097	0.9868
11	0.079	0.8976	0.085	0.9294	0.091	0.9586
12	0.075	0.8751	0.080	0.9031	0.086	0.9360
13	0.074	0.8692	0.074	0.8692	0.080	0.9015
14	0.074	0.8692	0.074	0.8692	0.074	0.8675
15	0.074	0.8692	0.074	0.8692	0.074	0.8698
16	0.074	0.8692	0.074	0.8692	0.074	0.8698
17	0.074	0.8692	0.073	0.8633	0.074	0.8675
18	0.073	0.8633	0.073	0.8633	0.074	0.8698
19	0.073	0.8633	0.072	0.8573	0.074	0.8692

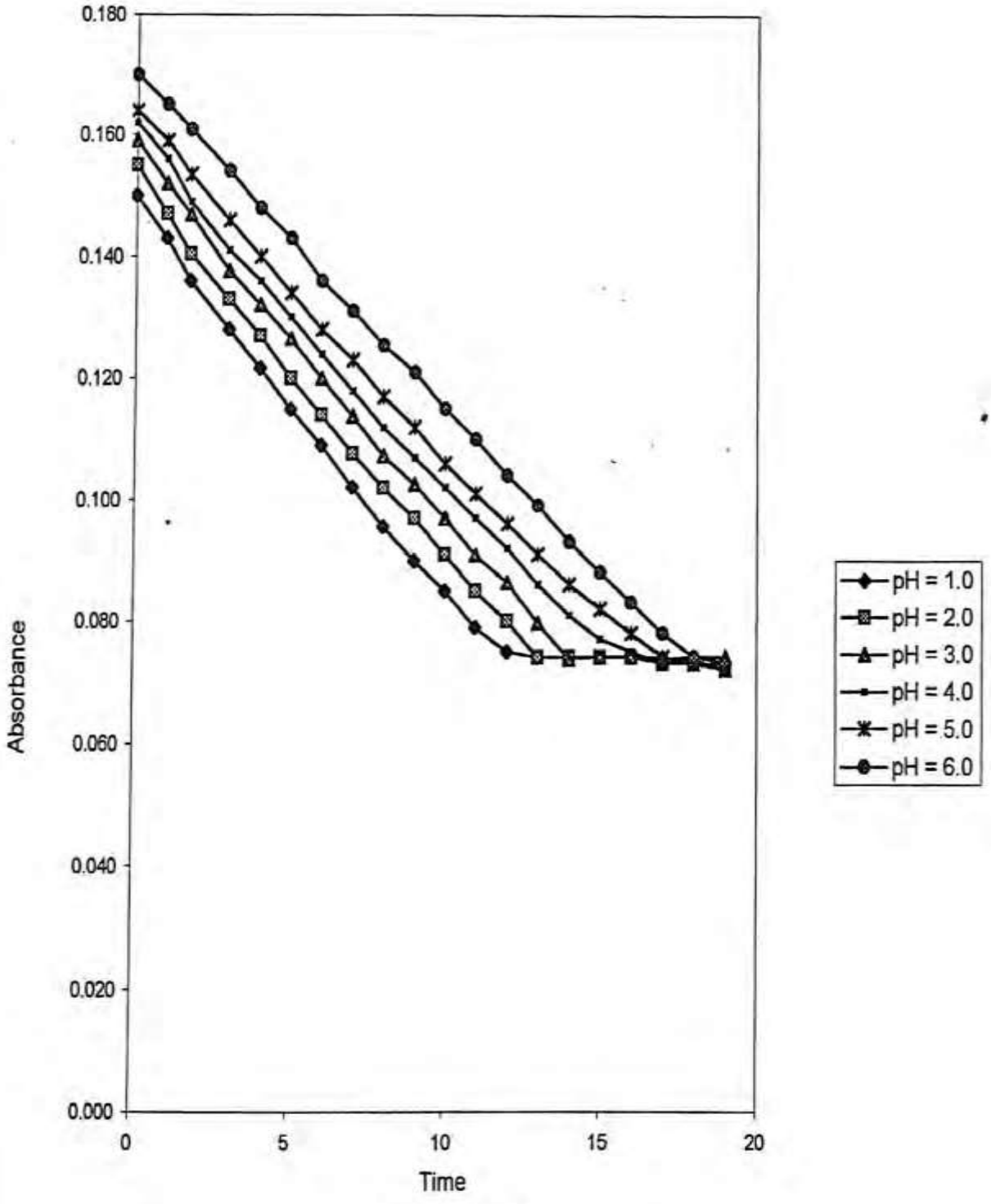


FIGURE - 3
CHANGE OF ABSORPTION IN THE SOLUTION OF QUINALPHOS WITH DIFFERENT pH IN VISIBLE LIGHT

Table:3

CHANGE OF ABSORPTION IN THE SOLUTION OF QUINALPHOS WITH VARIOUS SEMICONDUCTOR IN VISIBLE LIGHT

Concentration of Quinalphos = 0.1 M
Wt. of semiconductor = 0.200 gm.

Temp.= 303 K
pH = 3.0

Time (hour)	CdS		TiO_2		ZnO		ZnS	
	Abs	2+LogAbs	Abs	2+LogAbs	Abs	2+LogAbs	Abs	2+LogAbs
0	0.159	1.2014	0.159	1.2014	0.159	1.2014	0.159	1.2014
1	0.152	1.1818	0.154	1.1875	0.155	1.1903	0.155	1.1903
2	0.147	1.1670	0.150	1.1761	0.150	1.1761	0.152	1.1818
3	0.138	1.1386	0.142	1.1523	0.143	1.1553	0.145	1.1614
4	0.132	1.1206	0.136	1.1335	0.136	1.1335	0.139	1.1430
5	0.126	1.1017	0.129	1.1106	0.130	1.1139	0.134	1.1271
6	0.120	1.0792	0.123	1.0899	0.124	1.0934	0.129	1.1106
7	0.114	1.0561	0.116	1.0645	0.119	1.0755	0.124	1.0934
8	0.107	1.0306	0.110	1.0414	0.115	1.0607	0.119	1.0755
9	0.103	1.0111	0.106	1.0269	0.110	1.0414	0.114	1.0569
10	0.097	0.9868	0.101	1.0049	0.104	1.0170	0.109	1.0374
11	0.091	0.9586	0.096	0.9820	0.099	0.9956	0.104	1.0170
12	0.086	0.9360	0.090	0.9542	0.095	0.9777	0.099	0.9956
13	0.080	0.9015	0.085	0.9294	0.090	0.9542	0.095	0.9777
14	0.074	0.8692	0.079	0.8976	0.085	0.9294	0.090	0.9542
15	0.074	0.8692	0.074	0.8692	0.080	0.9031	0.085	0.9294
16	0.074	0.8692	0.074	0.8692	0.077	0.8865	0.081	0.9085
17	0.074	0.8692	0.074	0.8692	0.074	0.8692	0.076	0.8808
18	0.074	0.8692	0.073	0.8633	0.073	0.8633	0.074	0.8692
19	0.074	0.8692	0.073	0.8633	0.073	0.8633	0.073	0.8633

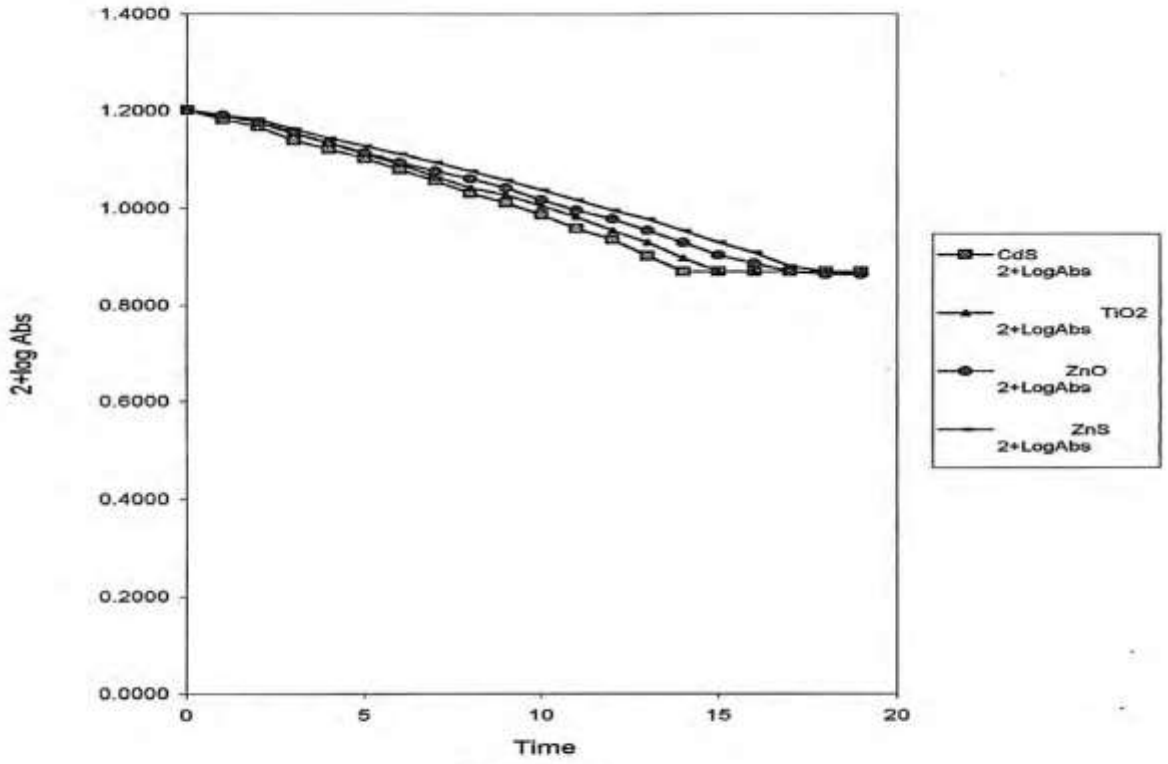


FIGURE : 4
CHANGE OF ABSORPTION IN THE SOLUTION OF QUINALPHOS WITH VARIOUS SEMICONDUCTOR IN VISIBLE LIGHT(at 630 nm)

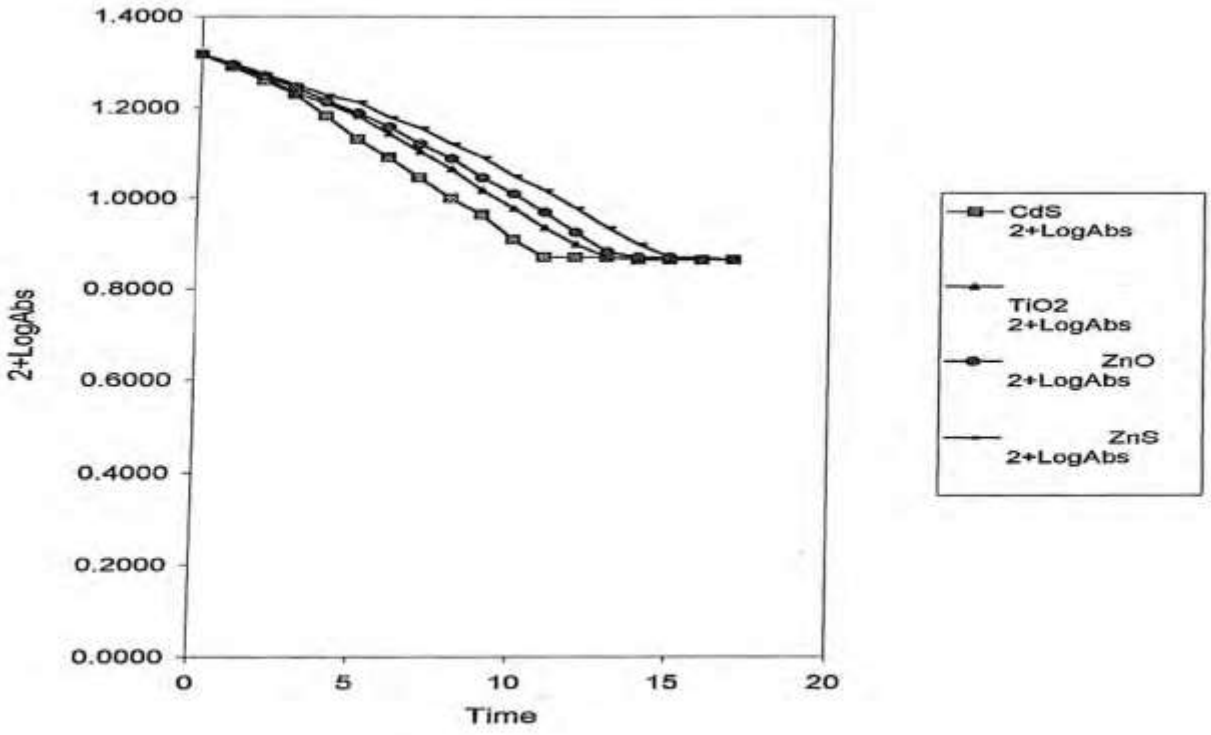


FIGURE-5
CHANGE OF ABSORPTION IN THE SOLUTION OF QUINALPHOS WITH VARIOUS SEMICONDUCTOR IN UV LIGHT(at 275 nm)

Table:4

CHANGE OF ABSORPTION IN THE SOLUTION OF QUINALPHOS WITH VARIOUS SEMICONDUCTOR IN UV LIGHT

Concentration of Quinalphos = 0.1 M
Wt. of semiconductor = 0.200 gm.

Temp.= 303 K
pH = 3.0

Time (hour)	CdS		TiO_2		ZnO		ZnS	
	Abs	2+LogAbs	Abs	2+LogAbs	Abs	2+LogAbs	Abs	2+LogAbs
0	0.207	1.3160	0.207	1.3160	0.207	1.3160	0.207	1.3160
1	0.195	1.2900	0.196	1.2923	0.197	1.2945	0.198	1.2967
2	0.182	1.2601	0.185	1.2672	0.186	1.2695	0.188	1.2742
3	0.170	1.2304	0.171	1.2330	0.176	1.2455	0.178	1.2504
4	0.152	1.1818	0.162	1.2095	0.164	1.2148	0.169	1.2279
5	0.135	1.1303	0.152	1.1818	0.154	1.1875	0.163	1.2128
6	0.123	1.0899	0.139	1.1430	0.144	1.1584	0.151	1.1790
7	0.111	1.0453	0.127	1.1038	0.132	1.1206	0.143	1.1553
8	0.100	1.0000	0.116	1.0645	0.122	1.0880	0.132	1.1206
9	0.092	0.9638	0.104	1.0170	0.111	1.0453	0.123	1.0899
10	0.081	0.9085	0.095	0.9777	0.102	1.0090	0.112	1.0492
11	0.074	0.8692	0.086	0.9345	0.093	0.9685	0.104	1.0170
12	0.074	0.8692	0.079	0.8976	0.084	0.9243	0.095	0.9777
13	0.074	0.8692	0.074	0.8692	0.076	0.8808	0.086	0.9345
14	0.073	0.8633	0.074	0.8692	0.074	0.8692	0.079	0.8976
15	0.073	0.8633	0.073	0.8633	0.074	0.8692	0.074	0.8692
16	0.073	0.8633	0.073	0.8633	0.073	0.8633	0.074	0.8692
17	0.073	0.8633	0.073	0.8633	0.073	0.8633	0.073	0.8633

CHANGE OF ABSORPTION IN THE SOLUTION OF QUINALPHOS WITH THE CHANGE OF INTENSITY OF LIGHT.

The effect of light intensity on the. rate of photocatalytic. Reactions Quinalphos was also observed. The light intensity was varied by changing the distance between the light source and the exposed surface of semiconductor. The results are reported in Table -5;7 and figure - 5.7.

It was observed that there is an increase in the rate of reaction as the intensity of incident light was increased. This may be explained on the basis that with the increase in light intensity, more photons will be available for excitation and therefore, more electron-hole paris will be generated in the semiconductor resulting into enhanced rate of reaction.

Table:5

EFFECT OF INTENSITY OF LIGHT IN THE SOLUTION OF QUINALPHOS

Concentration of Quinalphos = 0.1 M

Temp.= 303 K
pH = 3.0

Time (hour)	Absorbance		
	Distance		
	18 Inches	14 Inches	10 Inches
0	0.159	0.159	0.159
1	0.153	0.152	0.150
2	0.148	0.148	0.139
3	0.142	0.138	0.130
4	0.138	0.132	0.123
5	0.132	0.126	0.113
6	0.127	0.120	0.105
7	0.122	0.114	0.096
8	0.119	0.108	0.089
9	0.114	0.102	0.081
10	0.110	0.095	0.078
11	0.104	0.089	0.077
12	0.100	0.084	0.075
13	0.096	0.079	0.075
14	0.093	0.076	0.075
15	0.089	0.075	0.075
16	0.086	0.075	0.075
17	0.082	0.075	0.075
18	0.079	0.075	0.075
19	0.074	0.075	0.075
20	0.073	0.074	0.074

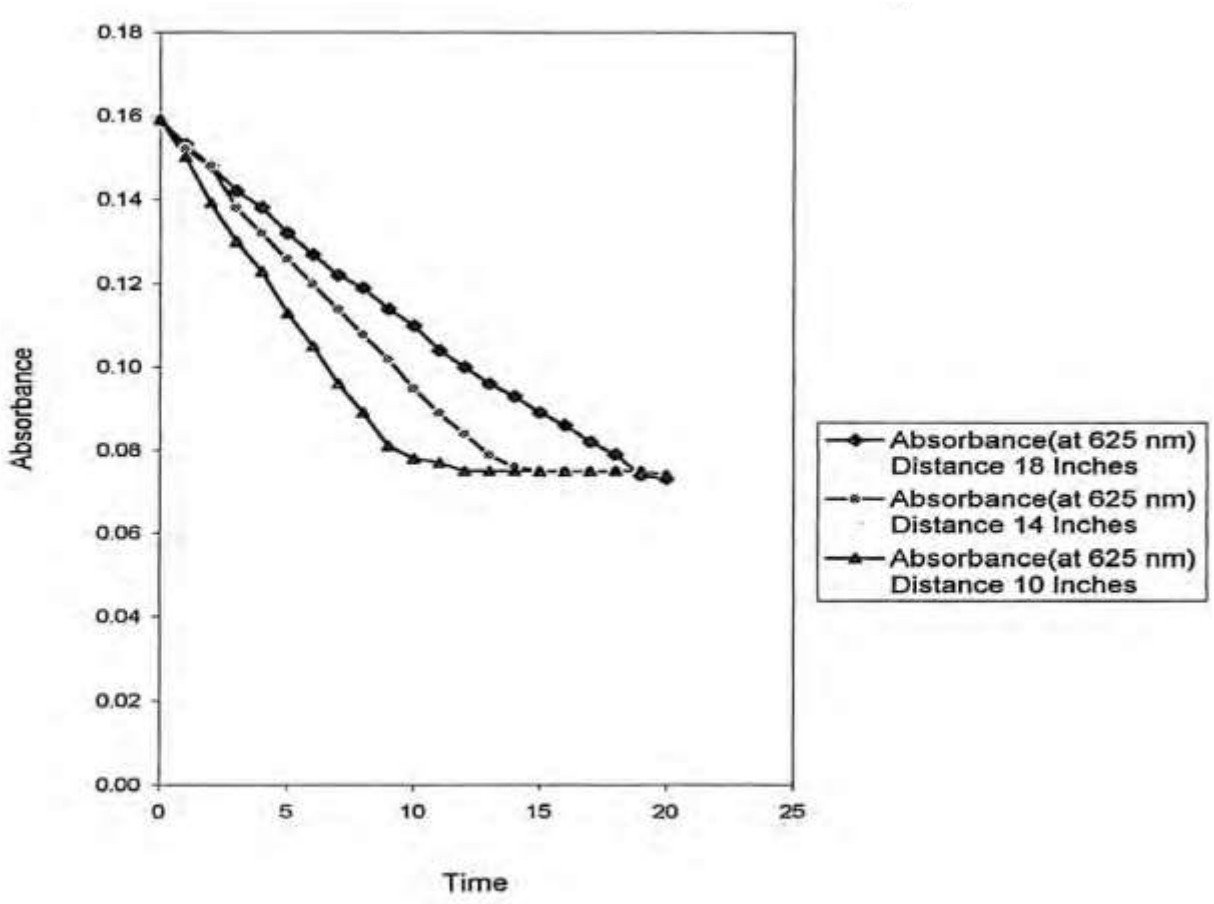


FIGURE - 7
EFFECT OF INTENSITY OF LIGHT IN THE SOLUTION OF QUINALPHOS (at 625 nm)

REFERENCES:

1. Calvert, K. Theurer, · G. T. Rankm and W.M. Macnervin, Z. H. Fiz. Khim, 76, 2575 (1954)
2. N. Barschchevski, Zh. Fiz. Khim, 36., 249 (1962)
3. S. R. Morrison and T. Freund, J. Chem. Phys., 47, 1543 (1967)
4. D. R. Dixon and T.W. Healy, Aust. J. Chem., 24, 1193 (1971)
5. H. Gerischer and K. Cammann, Ber. Bunsenges Phys. Chem., 76, 383 (1972)
6. K. Micka, Electroanal Chern., Interfacial Electrochern., 42, 447 (1973)
7. C. Richard and Z. Lemaire, J. Photochem. Photobiol., 55A, 127 (1990).
8. C. Ciping, R. Xinmin, L. Daohui and Z. Yong Kang, Ganguang Kexue Yu Kuang Huaxue, 9., 137 (1991).
9. R. Claire, B. Pierre and J. M. Aubry, J. Photochem. PhototbioL, 60A, 235 (1991).
10. K. Wada, K. Yashida, Y. Watanake and T. Suzuki, J. Chem. Soc. Chem. Commun., 10 (1991).
11. Ajay Sharma, Praibha Rao, R. P. Mathur and Suresh C. Ameta, J. Photo chem. Photobiol, 86A, 197 (1995)
12. Ajay Sharma, Rameshwer Amely, R. P. Mathur and Scamedli, Hung J. Industr. Chem., 23, 31 (1995).
13. L. C. Chen. and T. C. Chou, J. Mol. Catal., 85, 201 (1993).

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29 Green Sysnthesis Of Plasmonic Silver Nano Particles As Sensor To Toxic Metals

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Abstract

In the present work we summarize the recent advances of green synthesised plasmonic AgNPs with Curry leave extract and their applications, particularly regarding the fundamentals and applications of surface plasmon resonance (SPR) in Agnanoparticles. AgNPs have been characterized by SEM and TEM. The spectroscopic data was reported by UV- Vis spectrquant pharo 300.The produced AgNPs were mixed with the aqueous solution of toxic metal ions. It is reported that these nano particles show good sensing with toxic metal ions.The research results reveal an excellent sensing property that has important application value in medical detection.

Keywords: Plasmonic nanoparticles, AgNPs, Green synthesis, Bio synthesis,Curry leave extract.

Introduction

Many physical and chemical techniques, including chemical reduction and milling, were used to create nanoparticles (NPs) and increase their efficiency at the start of the 20th century.[1] However, these traditional methods cannot be regarded as environmentally friendly because they require expensive and hazardous chemicals.[2] Considering this, researchers are currently very interested in the bio-genic production of metal and metal oxide nanoparticles (NPs), which use microorganisms and aqueous plant extract and are environmentally safe, stable, therapeutically adaptive, and economical.[1,3] As a result, the production of nanoparticles using bioinspired technology has grown in importance within the discipline of nanoscience and nanotechnology.[4,5]Up till now, a variety of plant extracts, microorganisms, and metal oxide nanoparticles have been used in their synthesis.[6,7] In addition to their extensive use in the synthesis of nanoparticles, plant biomass is widely available, renewable, and environmentally friendly. As such, our group and others have focused on using it as a catalyst for chemical synthesis[8,9] as well as the generation of biodiesel.[10,11]
The research community is becoming increasingly interested in silver nanoparticles (silver NPs) among metal NPs because of their extensive applications in cell biology, chemistry, microbiology, food technology, pharmacology, and parasitology.[12,13] The physical and chemical properties of the silver nanoparticles are determined by their shape.28 In general, a number of methods have been used to synthesize silver nanoparticles, including the sol–gel method, the hydrothermal method, chemical vapor deposition, thermal decomposition, the microwave-assisted combustion method, etc.[14-16] The antibacterial activity of silver nanoparticles (AgNPs) produced

recently through biogenic synthesis using biomaterials like plant extract and microorganisms as reducing agents has been extensively studied.[17,18] AgNPs are created when various biomolecules, including flavonoids, ketones, aldehydes, tannins, carboxylic acids, phenolic acids, and plant proteins, oxidize Ag+ to Ag0.

Using UV-visible spectroscopy is an easy and popular analytical method to track the creation of AgNPs. Surface plasmon resonance (SPR) is the phenomenon whereby conducting electrons in the outermost orbital of metal nanoparticles collectively vibrate in resonance with specific wavelengths upon interaction with an electromagnetic field. AgNPs dissolved in a colloidal solution take on color and absorbance due to SPR excitation. Typically, the SPR peaks at 435 nm are used to verify that silver nitrate has been reduced to AgNPs.[19] In absorbance spectra, spherical NPs typically show only one SPR band, but depending on their form, anisotropic particles might show two or more SPR bands.35 Sometimes, the lack of a peak in the UV-Vis spectra in the 335–560 nm area is taken as a sign that NP aggregation is absent.[17,21]

We have covered in detail the use of several plants in the biogenic synthesis of silver nanoparticles and their application in antibacterial activity. We also talked about how the size and structure of the produced silver nanoparticles affected their antibacterial efficacy against different harmful microorganisms. When trying to create metal nanoparticles (NPs), it's important to keep in mind that while NPs' size, shape, and stability are important factors, their stability is even more important since NPs can form huge aggregates that reduce their efficiency by causing precipitation.

Method And Material

Preparation Of Plant Extract:

Curry tree (Fig. 1) (Murraya koenigii) leaf extract was used to create silver nanoparticles due to its affordability, accessibility, and therapeutic qualities. In January and February, fresh leaves were picked from college campuses. To get rid of trash and other polluted substances, they were first surface cleaned with running tap water and subsequently cleaned with distilled water. Next, the mixture was crushed and air-dried at room temperature. A beaker with 500 mL of double-distilled water and about 30 g of finely crushed leaves was brought to a boil for 30 minutes. After cooling down, the extract was filtered using Whatman filter paper No. 42 and kept at 4.0^0 C for further use.

Fig. 1 Image of Curry (Murraya koenigii)leaves

Preparation of AgNPs from Curry leaf extract:

As such, silver nitrate GR was utilized (bought from Merck, India). A 100 ml beaker containing a 1 mM solution of silver nitrate was made. Then, 10 mL of AgNO3 solution with the con 1 mM was mixed with 1, 2, 3, 4, and 5 mL of extract added one at a time. Additionally, different concentrations of AgNO3 (1–5 mM) were used to create silver nanoparticles while maintaining a consistent extract content (5 mL). To reduce the amount of silver nitrate photoreduction at room temperature, this setup was stored in a dark cabinet. The solution's color shift indicated that the amount of Ag+ had decreased.

Binding of metal ions (Cd^{+2}) with CLE-AgNPs :

The NLE-prepared silver nanoparticles are combined with one milliliter of each of the solutions containing 0.1 millimeters of Cd+2. After keeping the solutions above in the dark for an hour, AgNPs bound to the metal ions in them. The CLE-AgNPs' brown color and the solution's reddish brown color now show a robust metal ion binding to the CLE-AgNPs. TEM examination and UV-Vis spectrographs provide additional confirmation of the production of CLE-AgNPs-Metal ions.

CHARACTERIZATION OF SILVER NANOPARTICLES:

UV-Vis Analysis:

The Department of Chemistry at R. R. Mehta College of Science, Palanpur, B. K., Gujarat, India, used a UV-Vis spectrophotometer (SpectroquantR Pharo 300: Merck) to measure the optical properties of AgNPs. Following the addition of AgNO3 to the plant extract, spectra were obtained between 350 and 500 nm at various intervals up to a maximum of 24 hours. After adding the aforementioned metal ion solutions to the previously generated CLE-AgNPs, respectively, the spectra of the CLE-AgNPs-Metal ion (Cd+2) and CLE-AgNPs-Metal ion (Cd+2) were also obtained.

Fig. 2 Image of UV-Vis Spectrophotometer

SEM Analysis:
The Sophisticated Instrumentation Centre for Applied Research and Testing (SICART), Vallabh Vidyanagar – 388120, Dist.: Anand, Gujarat, India, used a scanning electron microscope (ESEM EDAX XL-30, Philips) to examine the morphological characteristics of the produced silver nanoparticles from NLE. SEM slides were produced by spreading the solutions onto them 24 hours after $AgNO_3$ was added. The samples were coated with a tiny layer of platinum to make them conductive. After that, the samples underwent 30 KV of accelerating voltage for SEM characterization.
TEM Analysis:
The Sophisticated Instrumentation Centre for Applied Research and Testing SICART, Vallabh Vidyanagar – 388120, Dist: Anand, Gujarat, India, used a Transmission Electron Microscope (Tecnai 20, PHILIPS) to study the morphological characteristics of synthesized silver nanoparticles from extract (NLE and CLE), CLE-AgNPs-Metal ion (Cd+2), and CLE-AgNPs-Metal ion (Cd+2). AgNPs TEM slides were generated by smearing the solutions on slides 24 hours after AgNO3 was added to the extract (NLE and CLE) separately. CLE-AgNPs-Metal ion (Cd+2) and CLE-AgNPs-Metal ion (Cd+2) were the results. The samples were coated with a tiny layer of platinum to make them conductive. After that, the samples underwent 200 KV of accelerating voltage characterization in the TEM.

3.1 Uv-Vis Spectrophotometer Analysis:

10 ml of 0.1 M AgNO3 solution was taken in 5 distinct 100 ml beakers for the λmax determination. Next, 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml of NLE were gradually added to each beaker to provide 5 sets of samples. The samples were then all held in the dark for 30 minutes. Subsequently, it was seen that the solution's color changed from yellow to brown. The plant extract of neem (Mirraya koenigii) was added to beakers containing an aqueous solution of silver nitrate for each experiment. This caused the solution's color to change from yellowish to reddish brown over the course of the reaction because the silver nanoparticles' surface plasmon vibrations were excited244.The color of the aqueous silver nitrate solution changed from pale yellow to brown upon the addition of various volumes (1–5 mL) of leaf extracts while maintaining a constant volume of 5 mL (1 mM). This shift indicated the synthesis of silver nanoparticles. Numerous parameters that had been found to affect the yields of silver nanoparticles were optimized, including time, concentrations of silver nitrate and Neem (Mirraya koenigii) leaf extract, and both. Utilizing 1 mM of silver nitrate, silver nanoparticles were generated at varying leaf extract concentrations, ranging from 1 to 5 mL. The Plasmon resonance band, which is visible at 436-446 nm, was analysed using UV spectra, in accordance with previous research findings (245). There is an increase in wavelength up to 448 nm if we increase the leaf extract content to 3 mL. The small fluctuations in absorbance readings indicate that the changes are related to particle size246. The intensity of absorption increases as extract concentration rises. Examining the size and form of nanoparticles in aqueous solutions is done by UV-Vis spectroscopy. When varying the concentration of AgNO3, while maintaining a constant amount of Neem extract (1 mL), consistent color variations were noted. The Surface Plasmon Resonance's activation caused the brown color to develop, which is typical of AgNPs with absorbance values that have previously been reported in the visible range of 446-448 nm247.The intensity of the

absorption peaks increases at regular intervals, and the incubation period lengthens the intensity of the color. Additionally, showed that when the concentration of the silver nitrate salt increases, so does the intensity of the absorption peaks. The literature has previously reported extremely similar results for the absorbance at 445 nm of silver nanoparticles generated by extracts from Cochlospermum religiosum (248) and Pithophoraoe dogonia (249). The recorded UV-vis spectra suggested that the most rapid bioreduction was accomplished with a reducing agent consisting of Neem (Mirraya koenigii) leaf extract. Visual observation along with the UV-vis spectra showed that the creation of silver nanoparticles happened quickly in less than 15 minutes. The produced Cd-AgNPs' plasmonic absorption bands are shown in Figure 2. At 440 nm, the absorption bands overlap at the same intensity. This validates our created probe's synthetic repeatability. TEM pictures of Cd-AgNPs provided additional evidence of the synthetic reproducibility (Fig. 6). Verify the produced NPs' high selectivity for the Cd(II) ions in the aqueous solutions that cause the Cd-Ag NPs to aggregate.

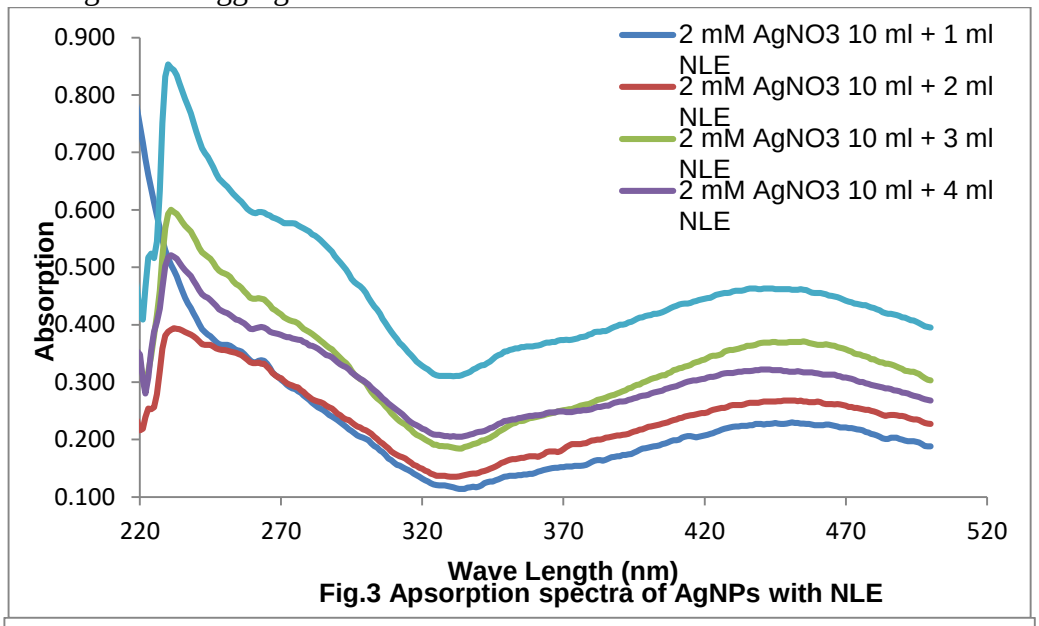

Fig.3 Apsorption spectra of AgNPs with NLE

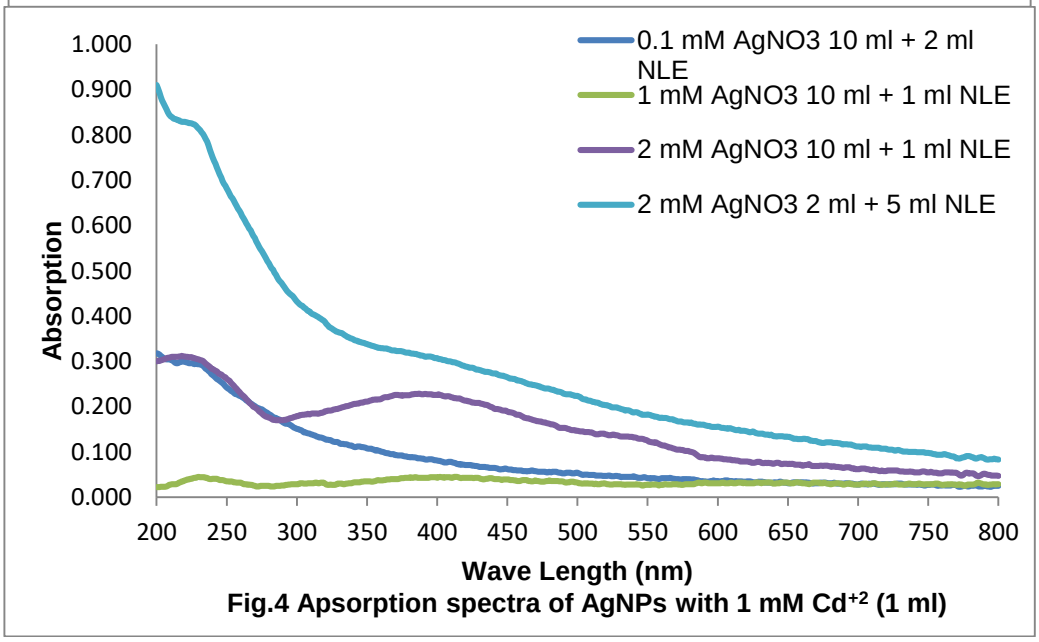

Fig.4 Apsorption spectra of AgNPs with 1 mM Cd^{+2} (1 ml)

3.2 SEM and TEM ANALYSIS:

SEM shed more light on the silver nanoparticles' shape and size characteristics. The produced nanoparticles were larger than the ideal range of sizes, which is between 1 and 100 nm. According to the results, the particles had a spherical shape when the ratio was 30:1, 60:1, and 120:1, but a sheet shape when the ratio was 240:1. Because of the concentration rise in a 240:1 ratio, the form changes.

It has become possible to determine the size, shape, and morphology of nanoparticles using transmission electron microscopy (TEM). The results indicate that the silver nanoparticles are primarily spherical and evenly distributed, however, some of the NPs had irregularly shaped structures, as depicted in Figure 5. The uniform and spherical nature of the nanoparticles complies with the UV-visible spectrum's SPR band shape. These suggest that these silver nanoparticles range in size from 20 to 42 nm. A certain distribution within the smaller particle size range suggests that the produced particles are likewise within that lower range.

The addition of Cd (II) resulted in a redshift of 404 to 432 nm and a decrease in the plasmonic peak intensity of CLE-AgNPs, although no change of this kind was detected with the addition of the other metal ions. The remarkable selectivity of the produced NPs for Cd (II) ions over the other metal ions is confirmed by these results. Since Cd(II) is a hard acid, it prefers to bond with hard bases250 in accordance with the HSAB principle. Because the Pilvai neem extract contains polyphenols, Cd (II) binds to an oxygen atom in the extract.Consequently, the NPs aggregate due to the metal-ligand interaction between the oxygen atom and Cd (II). TEM analysis was used to validate this interaction. Following the addition of Cd (II) ions to the produced NPs solution, Figure 6 displays the TEM images. In the presence of additional reactive cations, the produced CLE-AgNPs' improved selectivity was further investigated.

According to TEMstudies, when metal nanoparticles aggregate with metal ions, the size of the particles increases. Therefore, heavy metals in water, like chromium, are easily removed by nanoparticles. Given that the majority of heavy metals are known to be dangerous for both humans and animals, it is possible to reduce pollution of water and soil and remove heavy metals from it in order to protect both humans and animals from fatal diseases. Silver nanoparticles can be used to do this.

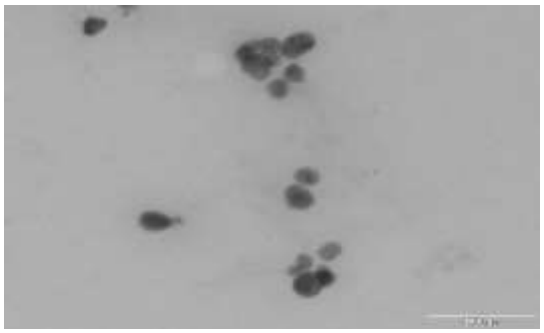

Fig. 5 SEM image of CLE-AgNPs

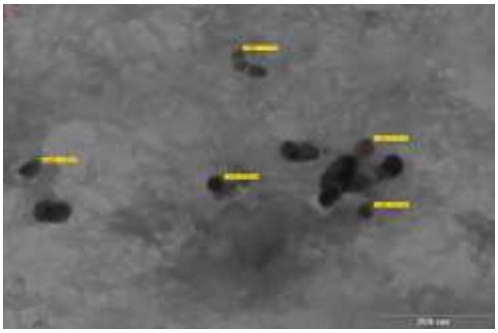

Fig. 6 TEM image of CLE-AgNPs-Cd^{+2}

References

1. S. Ahmed , M. Ahmad , B. L. Swami and S. Ikram , *J. Adv. Res.*, 2016, 7 , 17 —28 CrossRef CAS .
2. S. R. Vijayan , P. Santhiyagu , R. Ramasamy , P. Arivalagan , G. Kumar , K. Ethiraj and B. R. Ramaswamy , *Enzyme Microb. Technol.*, 2016, 95 , 45 —57 CrossRef CAS .
3. S. Ahmed , Annu , S. Ikram and S. Yudha , *J. Photochem. Photobiol., B*, 2016, 161 , 141 —153 CrossRef CAS .
4. P. Mohanpuria , N. K. Rana and S. K. Yadav , *J. Nanopart. Res.*, 2008, 10 , 507 —517 CrossRef CAS .
5. G. Pathak , K. Rajkumari and L. Rokhum , *Nanoscale Adv.*, 2019, 1 , 1013 —1020 RSC .
6. S. A. Saiqa Ikram *J. Nanomed. Nanotechnol.*, 2015, 6 , 1000309 CrossRef .
7. S. Ahmed and S. Ikram , *Nano Res. Appl.*, 2015, 1 , 1 —6 Search PubMed .
8. K. Rajkumari , D. Das , G. Pathak and L. Rokhum , *New J. Chem.*, 2019, 43 , 2134 —2140 RSC .
9. B. Changmai , I. B. Laskar and L. Rokhum , *J. Taiwan Inst. Chem. Eng.*, 2019, 102 , 276 —282 CrossRef CAS .
10. B. Changmai , P. Sudarsanam and L. Rokhum , *Ind. Crops Prod.*, 2020, 145 , 111911 CrossRef CAS .
11. B. Nath , B. Das , P. Kalita and S. Basumatary , *J. Cleaner Prod.*, 2019, 239 , 118112 CrossRef CAS .
12. S. Nour , N. Baheiraei , R. Imani , M. Khodaei , A. Alizadeh , N. Rabiee and S. M. Moazzeni , *J. Mater. Sci.: Mater. Med.*, 2019, 30 , 120 CrossRef .
13. O. Bondarenko , K. Juganson , A. Ivask , K. Kasemets , M. Mortimer and A. Kahru , *Arch. Toxicol.*, 2013, 87 , 1181 —1200 CrossRef CAS .
14. A. Pal , S. Shah and S. Devi , *Mater. Chem. Phys.*, 2009, 114 , 530 —532 CrossRef CAS .
15. T. Wu , H. Shen , L. Sun , B. Cheng , B. Liu and J. Shen , *ACS Appl. Mater. Interfaces*, 2012, 4 , 2041 —2047 CrossRef CAS .
16. X. Zhang , H. Sun , S. Tan , J. Gao , Y. Fu and Z. Liu , *Inorg. Chem. Commun.*, 2019, 100 , 44 —50 CrossRef CAS .
17. V. R. Remya , V. K. Abitha , P. S. Rajput , A. V. Rane and A. Dutta , *Chem. Int.*, 2019, 3 , 165 —171 Search PubMed .
18. M. Rafique , I. Sadaf , M. S. Rafique and M. B. Tahir , *Artif. Cells, Nanomed., Biotechnol.*, 2017, 45 , 1272 —1291 CrossRef CAS .
19. C. Vanlalveni , K. Rajkumari , A. Biswas , P. P. Adhikari , R. Lalfakzuala and L. Rokhum , *Bionanoscience*, 2018, 8 , 624 —631 CrossRef .
20. N. Durán , P. D. Marcato , G. I. H. De Souza , O. L. Alves and E. Esposito , *J. Biomed. Nanotechnol.*, 2007, 3 , 203 —208 CrossRef .
21. A. J. Kora , R. B. Sashidhar and J. Arunachalam , *Carbohydr. Polym.*, 2010, 82 , 670 —679 CrossRef CAS .

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37 Green Synthesis Of Plasmonic Silver Nano Particles And Its Sructural Characterization

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Abstract

Surface plasmon resonance in noble metallic nanoparticles has been an important research subject since last many years. Recent advances in the synthesis, assembly, characterization, and theories of traditional and non-traditional metal nanostructures open a new pathway to the many different applications of plasmonics. In the present investigation we summarize the recent advances of bio synthesised plasmonic AgNPs and their applications, particularly regarding the fundamentals and applications of surface plasmon resonance (SPR) in Agnanoparticles, AgNPs have been characterized by SEM and TEM. The spectroscopic data was reported by UV- Vis spectrquant pharo 300.

Keywords: Plasmonic nanoparticles, AgNPs, Green synthesis, Bio synthesis,Azadirachtaindica leave extract.

1. Introduction

Nanotechnology is gaining enormous attention as a new area of research dealing with the development of nanomaterials and nanoparticles (NPs) for their utilization in diverse fields such as catalysis, electrochemistry, biomedicines, pharmaceuticals, sensors, food technology, cosmetics, *etc.*[1–3] Nanoparticles (NPs) are nanometer-sized (<100 nm) atomic or molecular scale solid particles having some excellent physical properties compared to the bulk molecules depending on their size and morphology.[4,5] Among all types of NPs, metal and metal oxide nanoparticles have been thoroughly examined using science and technology due to their excellent properties such as high surface to volume ratio, high dispersion in solution, *etc.*[6,7] Owing to these, metal and metal oxide nanoparticles display enhanced antimicrobial properties.[8,9]

Owing to their wide availability, renewability and environment-friendly nature, in addition to their vast applications in the synthesis of NPs, plant biomass are also largely targeted by our group and others as a catalyst for chemical synthesis[10.,11] and biodiesel productions.[12,13]

Among metal NPs, silver NPs is gaining enormous interest in the research community due to their wide scope of application in microbiology, chemistry, food technology, cell biology, pharmacology and parasitology.[14,15] The morphology of the silver NPs is the deciding factor of their physical and chemical properties.[16] Recently, bio-genic synthesis of silver NPs (AgNPs) using biomaterials such as plant extract and microbes as reducing agent and their antimicrobial activity is widely investigated.[17]

UV-visible spectroscopy is a simple and widely used analytical technique to monitor the formation of AgNPs. Upon interaction with an electromagnetic field, the conducting electrons present in the outermost orbital of metal NPs collectively oscillate in resonance with certain wavelengths to exhibit a phenomenon called surface plasmon resonance (SPR). The excitation of SPR is responsible for the formation of color and absorbance in a colloidal solution of AgNPs. The SPR peaks at around 435 nm are usually taken to confirm the reduction of silver nitrate into AgNPs.[18] In general, spherical NPs exhibit only a single SPR band in the absorbance spectra, whereas two or more SPR bands were observed for anisotropic particles depending on the shape.[19] The absence of peak in the region 335 and 560 nm in UV-Vis spectra are sometime used as an indication of the absence of aggregation in NPs.[20] We have comprehensively discussed the bio-genic synthesis and silver nanoparticles using various plants and their application in antimicrobial activity.

PREPARATION OF PLANT EXTRACT:

Preparation of Neem leaf extract:
Silver nanoparticles were prepared by using Neem (Fig. 2) (A. Indica) leaf extract on the basis of cost effectiveness, ease of availability and its medicinal property. Fresh leaves were collected from college campus. They were surface cleaned with running tap water and then washed with distilled water to remove debris and other contaminated contents. Then air dried at room temperature crushed in Mixure. About 20 gm of finely crushed leaves were kept in a beaker containing 300 mL double distilled water and boiled for 30 min. Then cooled down it and filtered with Whatman filter paper no.42 and then extract was stored at 4^0 C for further use.

Fig. 1 Image of Neem (*Azadirachta indica)* leaves

Preparation of AgNPs from Neem leaf extract:
Silver nitrate GR used as such (purchased from Merck, India). 100 mL, 1 mM solution of silver nitrate was prepared in 100 ml beaker. Then 1, 2, 3, 4 and 5 mL of extract was added separately to 10 mL of $AgNO_3$ solution of containing the con 1 mM. Silver nanoparticles were also synthesized by varying concentration of $AgNO_3$ (1-5 mM) keeping extract concentration constant (5 mL). This setup was kept in a dark cupboard to minimize photoreduction of silver nitrate at room temperature. Reduction of Ag^+ was confirmed by the colour change of solution.
Binding of metal ions (Cr^{+3}) with NLE-AgNPs :
The silver nano-particles prepared from NLE is aggregated with 1 ml solutions of 0.1 mM Cr^{+3} separately. For binding of AgNPs with Metal ions above solutions kept it in dark for one hour. The brown colour of the NLE-AgNPs solution now turns reddish brown indicate strong binding of metal ion with NLE-AgNPs. The formation of NLE-AgNPs-Metal ion is further confirmed by UV-Vis spectrographs and TEM analysis.

CHARACTERIZATION OF SILVER NANOPARTICLES:

UV-Vis Analysis:
The optical property of AgNPs was determined by UV-Vis spectrophotometer (Spectroquant^R Pharo 300: Merck) at Dept. of Chemistry, R. R. Mehta College of Science, Palanpur, B. K., Gujarat, India. After the addition of $AgNO_3$ to the plant extract, the spectras were taken in different time intervals up to 24Hrs. between 350 nm to 500 nm. The spectras of NLE-AgNPs-Metal ion (Cr^{+3}) was taken afteraddition of above mentioned metal ions solutions into the above prepared NLE-AgNPs and respectively.

Fig. 2 Image of UV-Vis Spectrophotometer

SEM Analysis:

The morphological features of synthesized silver nanoparticles from NLE was studied by Scanning Electron Microscope (ESEM EDAX XL-30, PHILIPS) at Sophisticated Instrumentation Centre for Applied Research and Testing SICART, Vallabh Vidyanagar – 388120, Dist: Anand, Gujarat, India. After 24Hrs. of the addition of $AgNO_3$ the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 30 KV.

TEM Analysis:

The morphological features of synthesized silver nanoparticles from extract (NLE) and NLE-AgNPs-Metal ion (Cr^{+3}) was studied by Transmission Electron Microscope (Tecnai 20, PHILIPS) at Sophisticated Instrumentation Centre for Applied Research and Testing SICART, Vallabh Vidyanagar – 388120, Dist: Anand, Gujarat, India. The TEM slides of AgNPs after 24Hrs. of the addition of $AgNO_3$ into extract (NLE and) separately and NLE-AgNPs-Metal ion (Cr^{+3}) was prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the TEM at an accelerating voltage of 200 KV.

3.1 UV-Vis SPECTROPHOTOMETER ANALYSIS:

For the determination of λ_{max} 10 ml of 0.1 M $AgNO_3$ solution were taken in 5 different 100 ml beaker then 1 ml, 2 ml, 3 ml, 4 ml and 5 ml of NLE was added in each beaker gradually to make 5 sets of samples. Then kept all the samples in dark for half hour. After that it was seen that the colour of the solution was turns yellow to brown. All experiments were carried out by addition of plant extract of Neem (Azadirachta indica) into the beakers containing aqueous solution of silver nitrate resulting to the change in the colour of the solution to yellowish to reddish brown within reaction duration due to excitation of surface plasmon vibrations in silver nanoparticles[21]. On addition of different volume (1-5 mL) of leaf extracts to aqueous silver nitrate solution keeping its volume 5 mL (1 mM) constant, the colour of the solution changed from light yellow to brown indicating formation of silver nanoparticles. Different parameters were optimized including concentration of silver nitrate and Neem (A. indica) leaf extract, and time which had been identified as factors affecting the yields of silver nanoparticles. Silver nanoparticles were synthesized at different concentrations of leaf extract such as 1-5 mL using 1 mM of silver nitrate were analysed by UV spectra of Plasmon resonance band observed at 436-446 nm similar to those reported in literature[22]. If we increase the leaf extract concentration to 3 mL, there is increase in wavelength up to 448 nm as presented in Fig. 6. The slight variations in the values of absorbance signifies that the changes are the particle size[23]. On increasing concentration of extract there is increase in intensity of absorption. UV-Vis spectroscopy is used for examination of size and shape of nanoparticles in aqueous solutions. Regular changes in colour was observed when different concentrations of $AgNO_3$ was used by keeping Neem extract (1 mL) constant. The brown colour appeared due to the excitation of the Surface Plasmon Resonance, typical of AgNPs having absorbance values which were reported earlier in the visible range of 446-448 nm[24]. There is increase in intensity of absorption peaks after regular intervals of time and the colour intensity increased with the duration of incubation. It was also observed from Fig. 9 that the intensity of absorption peaks increases with increase in the concentration of the silver nitrate salt. All the results are very close already reported in literature showing absorbance at 445 nm of silver

nanoparticles synthesized by Cochlospermum religiosum extract[25]. The UV-vis spectra recorded, implied that most rapid bio reduction was achieved using Neem (A. indica) leaf extract as reducing agent. The UV-vis spectra and visual observation revealed that formation of silver nanoparticles occurred rapidly within 15 min. Fig.5 exhibits the plasmonic absorption bands of Cr-AgNPs synthesized. The absorption bands overlap at 440 nm with the same intensities. This confirms the synthetic reproducibility of our prepared probe. The synthetic reproducibility was further confirmed by TEM images of Cr-AgNPs (Fig.8,9). Confirm the high selectivity of the prepared NPs for the Cr(III) ions in the aqueous systems that lead to the aggregation of Cr-Ag NPs.

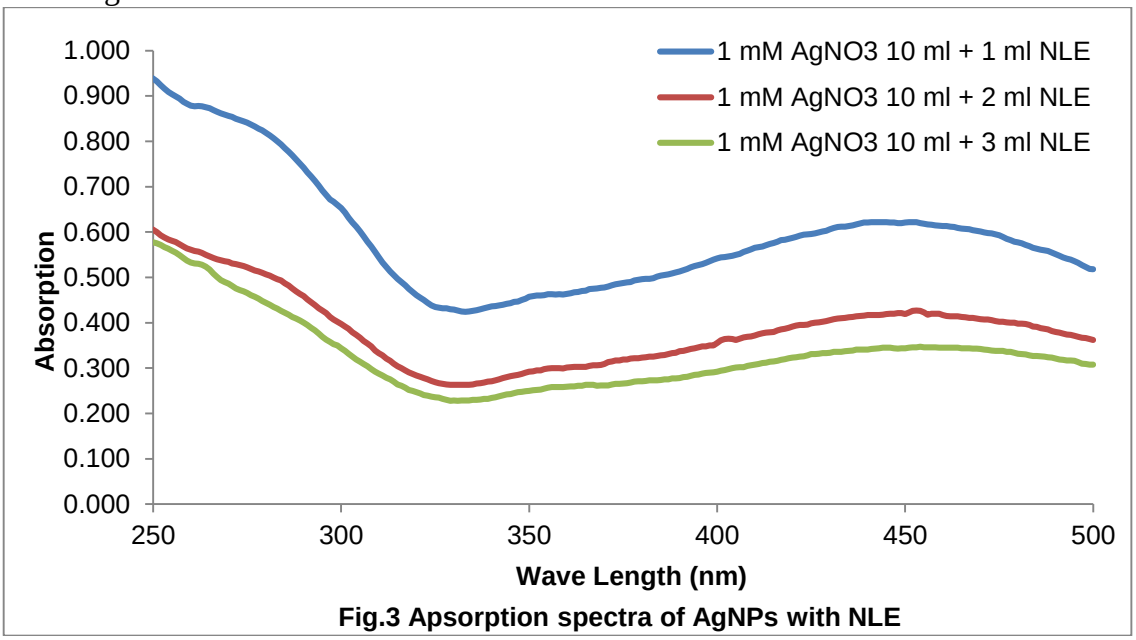

Fig.3 Apsorption spectra of AgNPs with NLE

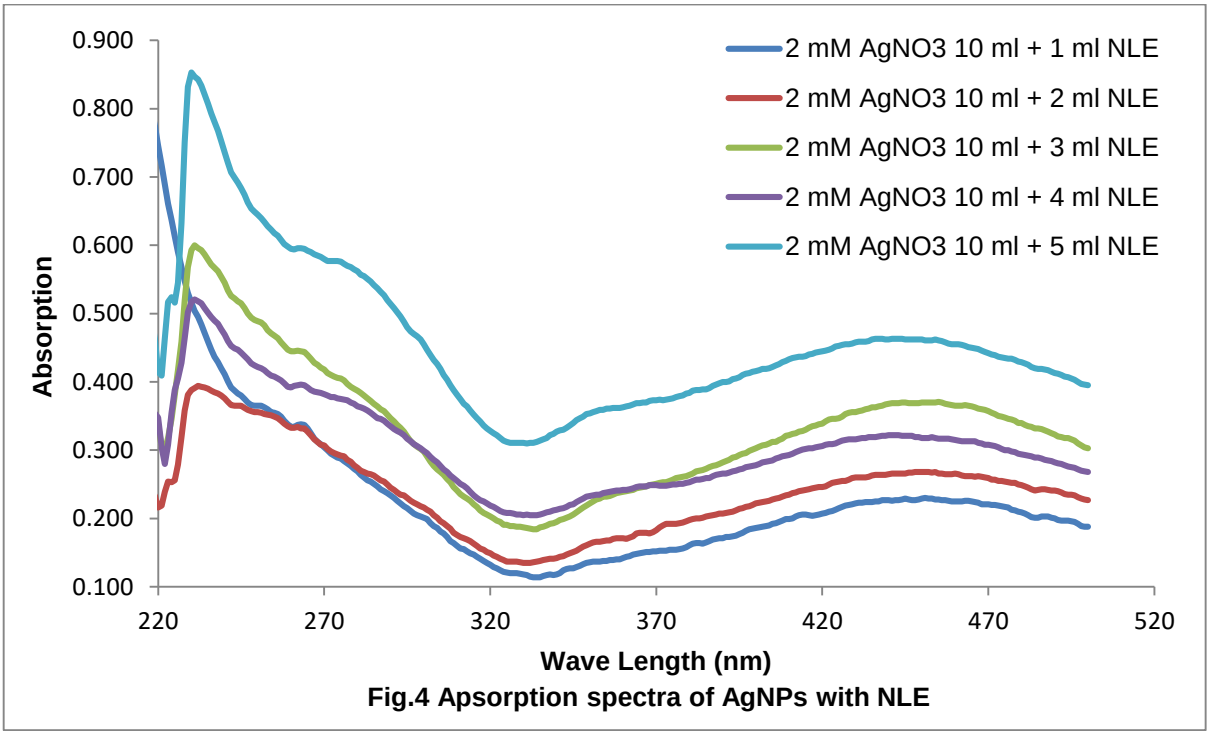

Fig.4 Apsorption spectra of AgNPs with NLE

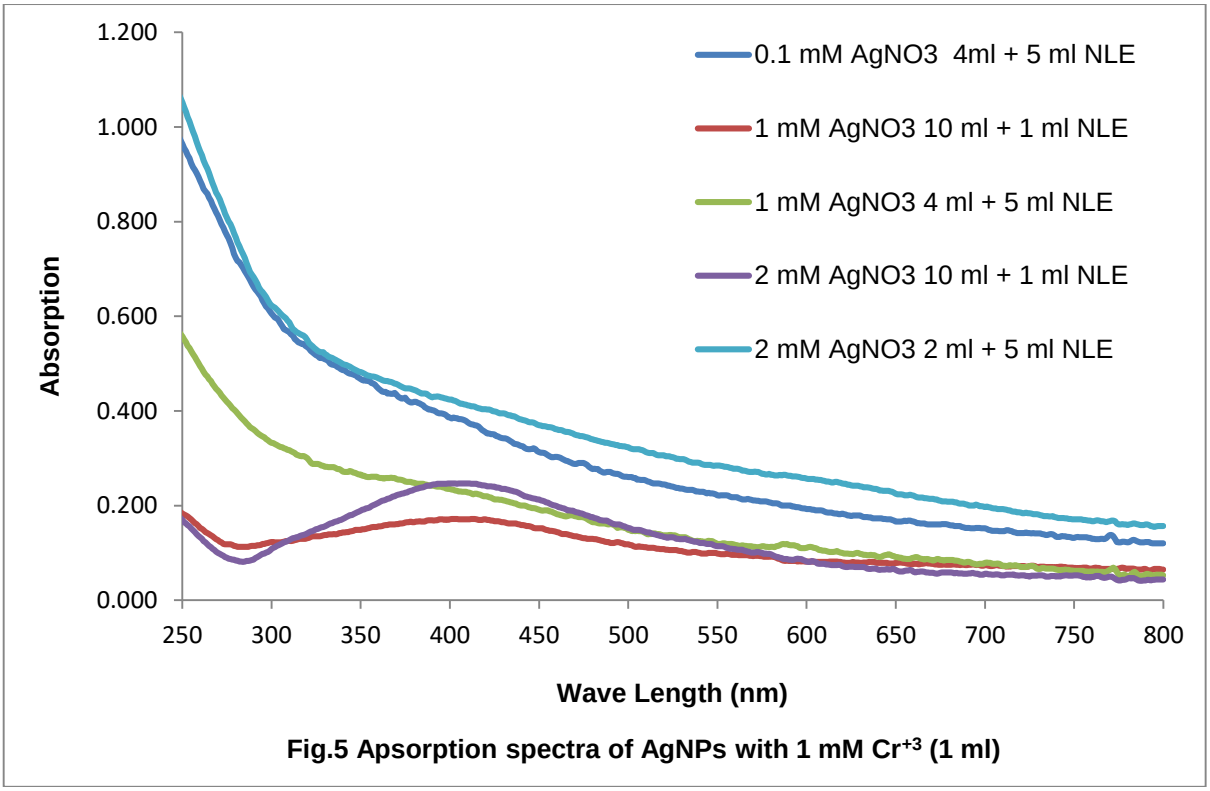

Fig.5 Apsorption spectra of AgNPs with 1 mM Cr^{+3} (1 ml)

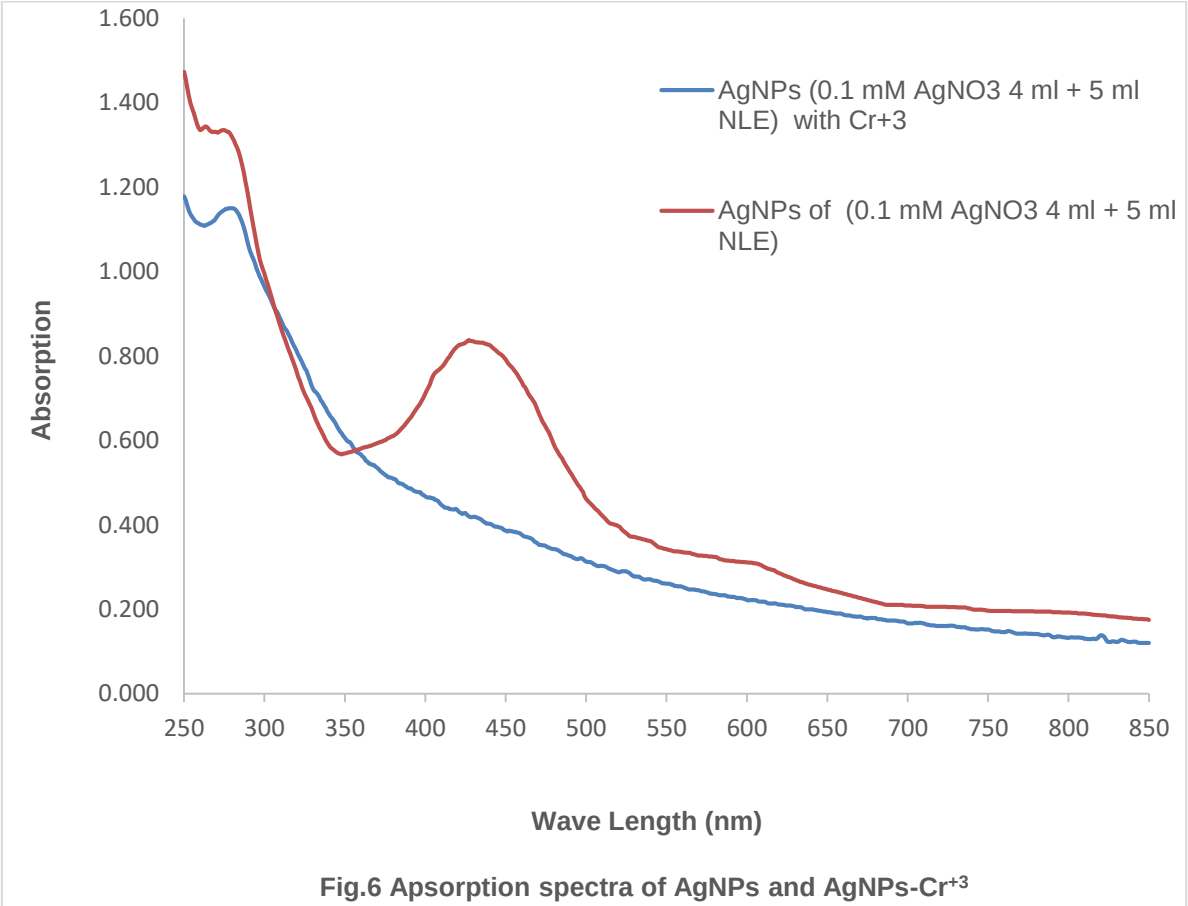

Fig.6 Apsorption spectra of AgNPs and AgNPs-Cr^{+3}

3.2 SEM and TEM ANALYSIS:

SEM provided further insight into the morphology and size details of the silver nanoparticles. The size of the prepared nanoparticles was more than the size of nanoparticle which should be; i.e.; between 1-100 nm. The result showed that the particles were of spherical shape in case of 30:1, 60:1, and 120:1 ratios, but sheet shape in case of 240:1 ratio. The shape varies due to the concentration increased in 240:1 ratio.

Transmission electron microscopy (TEM) has been used to identify the size, shape and morphology of nanoparticles. It reveals that the silver nanoparticles are well dispersed and predominantly spherical in shape, while some of the NPs were found to be having structures of irregular shape as shown in Fig. 5. The nanoparticles are homogeneous and spherical which conforms to the shape of SPR band in the UV-visible spectrum. These indicates that the size of these silver nanoparticles is 20 to 42 nm. Some distribution at lower range of particle size indicates that the synthesized particles are also in lower range of particle size.

After the addition of the Cr (III), a redshift from 404 to 432 nm along with a decrease in the plasmonic peak intensity of NLE−AgNPs was observed while the rest of the metal ions did not induce any such change after the addition of NLE−AgNPs. These results confirm the excellent selectivity of the prepared NPs for Cr (III) ions over the rest of the metal ions. Cr(III) is a hard acid; therefore, according to HSAB principle, it prefers to bind with hard base[26]. The Neem extract of Pilvai has polyphenolic contents, thus Cr (III) forms a bond with the oxygen atom present in the extract. Therefore, metal-ligand interaction between the Cr (III) and oxygen atom causes the aggregation of NPs. This interaction was confirmed with TEM, analysis. Figure 6 shows the TEM images after the addition of the Cr (III) ions onto prepared NPs solution. The enhanced selectivity of the prepared NLE-AgNPs was further determined in the presence of other reactive cations.

TEM analysis shows that the particles size of nanoparticles are increased when metal nanoparticle aggregation with metal ions. Thus heavy metals such as chromium in water can be easily removed by nanoparticles. As we know that most of heavy metals are harmful for human beings and animals also, it can be removed from water and soil by using silver nanoparticles and pollution of water and soil can be reduced to save mankind and animals from deadly diseases.

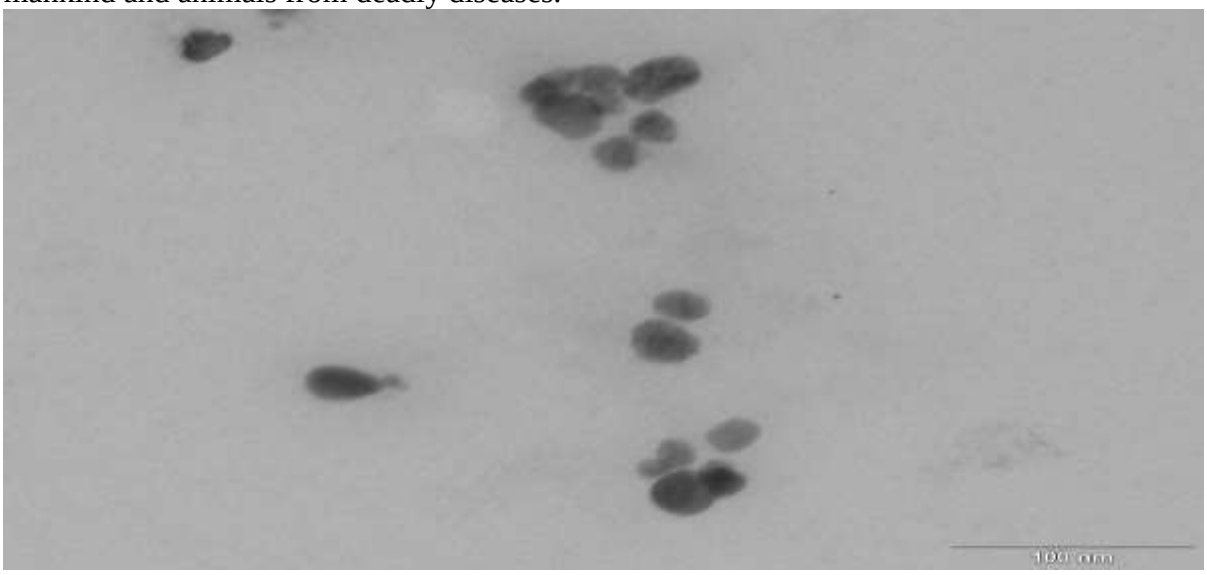

Fig. 7 SEM image of NLE-AgNPs

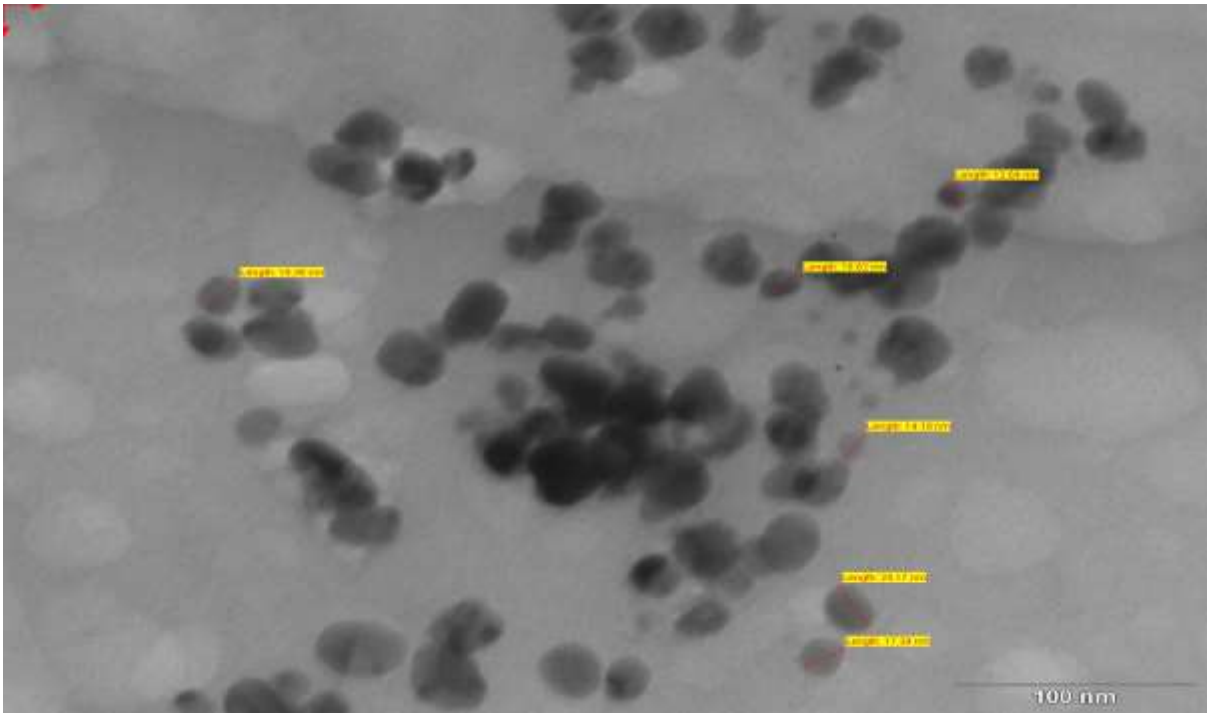

Fig. 8 TEM image of NLE-AgNPs

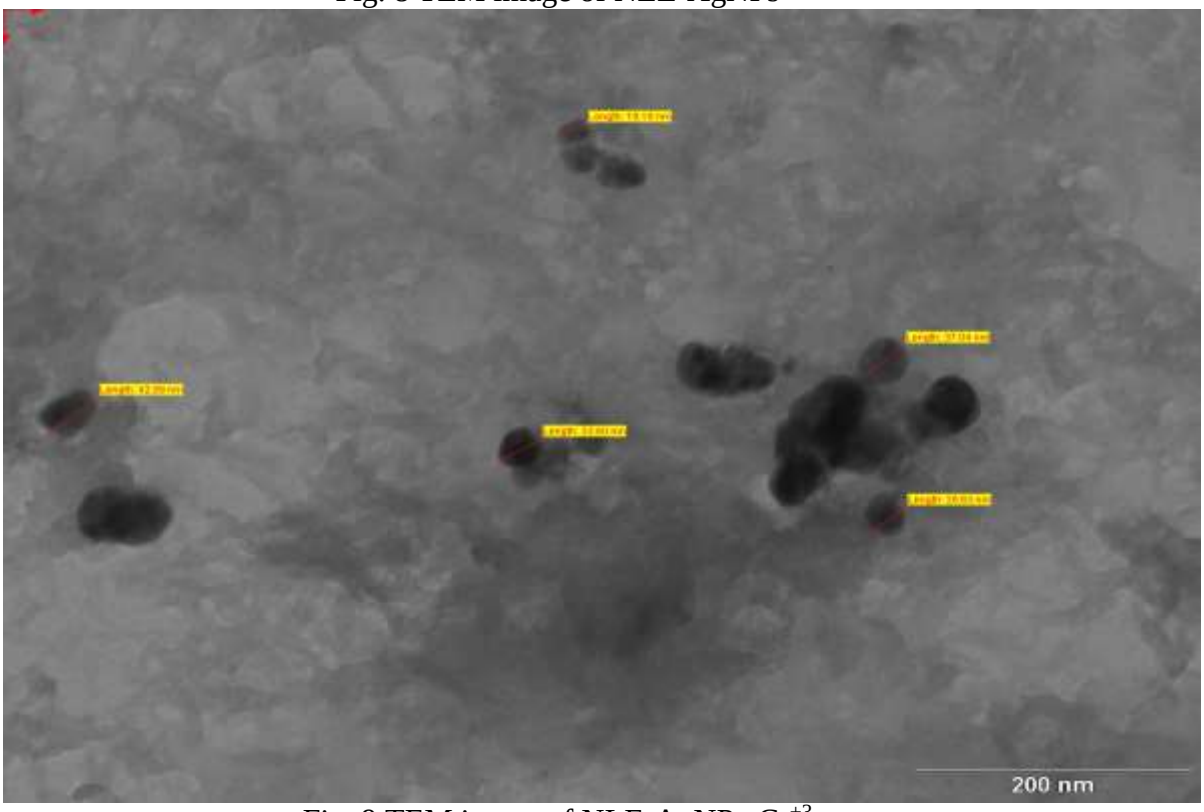

Fig. 9 TEM image of NLE-AgNPs-Cr^{+3}

REFERENCES

22. M. Ayelén Vélez , M. Cristina Perotti , L. Santiago , A. María Gennaro and E. Hynes , *Bioactive compounds delivery using nanotechnology: design and applications in dairy food* , Elsevier Inc., 2017, Search PubMed .
23. A. Bera and H. Belhaj , *J. Nat. Gas Sci. Eng.*, 2016, 34 , 1284 —1309 CrossRef CAS .
24. L. J. Frewer , N. Gupta , S. George , A. R. H. Fischer , E. L. Giles and D. Coles , *Trends Food Sci. Technol.*, 2014, 40 , 211 —225 CrossRef CAS .
25. V. J. Mohanraj and Y. Chen , *Trop. J. Pharm. Res.*, 2007, 5 , 561 —573 Search PubMed .
26. L. Stadler , M. Homafar , A. Hartl , S. Najafishirtari , R. Zbo , M. Petr , M. B. Gawande , J. Zhi and O. Reiser , *ACS Sustainable Chem. Eng.*, 2019, 7 , 2388 —2399 CrossRef CAS .
27. M. D. Purkayastha and A. K. Manhar , *Nanosci. Food Agri.*, 2016, 2 , 59 —128 Search PubMed .
28. S. Chatterjee , Dhanurdhar and L. Rokhum , *Renewable Sustainable Energy Rev.*, 2017, 72 , 560 —564 CrossRef CAS .
29. S. Bagheri and N. M. Julkapli , *J. Magn. Magn. Mater.*, 2016, 416 , 117 —133 CrossRef CAS .
30. N. Krithiga , A. Rajalakshmi and A. Jayachitra , *J. Nanosci.*, 2015, 1 , 128204 Search PubMed .
31. S. S. Sana , V. R. Badineni , S. K. Arla and V. K. Naidu Boya , *Mater. Lett.*, 2015, 145 , 347 —350 CrossRef CAS .
32. P. Velmurugan , M. Cho , S. S. Lim , S. K. Seo , H. Myung , K. S. Bang , S. Sivakumar , K. M. Cho and B. T. Oh , *Mater. Lett.*, 2015, 138 , 272 —275 CrossRef CAS .
33. D. Bose and S. Chatterjee , *Indian J. Microbiol.*, 2015, 55 , 163 —167 CrossRef CAS .
34. G. Marslin , R. K. Selvakesavan , G. Franklin , B. Sarmento and A. C. P. Dias , *Int. J. Nanomed.*, 2015, 10 , 5955 —5963 CrossRef CAS .
35. B. Sadeghi , A. Rostami and S. S. Momeni , *Spectrochim. Acta, Part A*, 2015, 134 , 326 —332 CrossRef CAS .
36. A. Devadiga , K. V. Shetty and M. B. Saidutta , *Int. Nano Lett.*, 2015, 5 , 205 —214 CrossRef CAS .
37. R. K. Salar , P. Sharma and N. Kumar , *Resour.-Effic. Technol.*, 2015, 1 , 106 —115 Search PubMed .
38. A. Verma and M. S. Mehata , *J. Radiat. Res. Appl. Sci.*, 2016, 9 , 109 —115 CrossRef CAS .
39. V. Ravichandran , S. Vasanthi , S. Shalini , S. Adnan and A. Shah , *Mater. Lett.*, 2016, 180 , 264 —267 CrossRef CAS .

40. S. Ahmed , A. K. Manzoor and S. Ikram , *J. Bionanosci.*, 2016, 10 , 282 —287 CrossRef CAS .
41. B. Sundararajan , G. Mahendran , R. Thamaraiselvi and B. D. Ranjitha Kumari , *Bull. Mater. Sci.*, 2016, 39 , 423 —431 CrossRef CAS .
42. D. Bose and S. Chatterjee , *Appl. Nanosci.*, 2016, 6 , 895 —901 CrossRef CAS .
43. Y. K. Mohanta , S. K. Panda , K. Biswas , A. Tamang , J. Bandyopadhyay , D. De , D. Mohanta and A. K. Bastia , *IET Nanobiotechnol.*, 2016, 10 , 438 —444 CrossRef .
44. C. S. Espenti , K. S. V. K. Rao and K. M. Rao , *Mater. Lett.*, 2016, 147 , 129 —133 CrossRef .
45. K. Anandalakshmi , J. Venugobal and V. Ramasamy , *Appl. Nanosci.*, 2016, 6 , 399 —408 CrossRef CAS .
46. S. Ahmed , Saifullah , M. Ahmad , B. L. Swami and S. Ikram , *J. Radiat. Res. Appl. Sci.*, 2016, 9 , 1 —7 CrossRef .
47. K. Khanra , S. Panja , I. Choudhuri , A. Chakraborty and N. Bhattacharyya , *Nanomed. J.*, 2015, 7 , 128 —133 CrossRef CAS .

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25 Synthesis And Characterization Of Novel 2-Aminopyrimidine Compounds From Chalcones

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Abstract
Aromatic heteroatom bearing cyclic compound such as pyrimidine, benzimidazole, benzoxazole etc. Triazoles is the heterocyclic compounds having five membered rings with three nitrogen atoms in it. Chemistry of heterocyclic compounds plays an important role in our daily lives. Food and drink have been made safe to consume; the whole area of pharmaceuticals has allowed the development and synthesis of new medicines for illnesses and diseases; In this context, a series of novel chalcones from 1-(4-aminophenyl)ethenone and 2-chloro-7H-pyrrolo[2,3-d]pyrimidine by condensation reaction. Prepared chalcones on condensation reaction with guanidine to produced various triazoles B1-B15. All prepared compounds were characterized by ^{1}HNMR, ^{13}CNMR, IR and Mass spectroscopy.

Keywords: Heterocyclic compounds, Guanidine, Chalcones, 2-aminopyrimidine and Spectral Characterization.

1. Introduction
Chalcones are one of the most important classes of natural products existing in many plant species. In nature, they serve as precursors for flavonoids and isoflavonoids biosynthesis. They are 1,3-diphenyl-2-propen- 1-ones (two aromatic rings connected with a carbonyl moiety). According to Harborne and Mabry (1982), chalcones are crucial flavonoid and isoflavonoid precursors. The preparation of many chalcones by Claisen- Schmidt condensation of methyl ketones with aldehydes in a basic environment Claisen and others, 1881. These substances exhibit antimalarial action in vitro. against variants of Plasmodium that are both chloroquine-sensitive and chloroquine-resistant falciparum[1]. Authors recently reported on the synthesis of chalcones. utilizing acetic acid and perchloric acid as well under acidic conditions [2]. Numerous chalcones have been identified as potent tyrosinases as new depigmenting agents since they act as antioxidants and inhibitors [3].

A heterocyclic compound is one in which the ring structure contains at least one atom other than carbon [4, 5]. Due to their enormous potential for processing and synthesizing a broad range of pharmacological actions, heterocyclic compounds have a significant role to play in medicinal chemistry and are currently the focus of a lot of research. Heterocyclic compounds are often found in natural products and play a significant role in the synthesis of biological molecules including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). In fact, DNA is the most important

macromolecule in existence, and nucleotides, the units that make up our genes, are derivatives of the purine and pyrimidine bases. Both heme, the non-protein component of haemoglobin, which is generated from enormous porphyrin rings, and chlorophyll, a green pigment that helps plants create oxygen by absorbing carbon dioxide, are responsible for carrying oxygen in animals. Contrarily, synthetic heterocyclic compounds have a variety of therapeutic uses, such as antibacterial [6], antifungal [7], analgesic [8], anti-inflammatory [9], antimycobacterial [10], antitubercular [11], antimalarial [12], trypanocidal [13], anti-HIV activity [14], anticonvulsant [15], antitumoural [16], antileishmanial agents, and genotoxic [17]. They are also a key structural component of many synthetic drugs and compounds derived from agriculture. For example, fluorescent sensors and dyes, brighteners, polymers, information storage, and analytical reagents are only a few of the several crucial material science uses of most heterocyclic compounds [18]. The creation of organic conductors, semiconductors, photovoltaic cells, molecular wires, organic light-emitting diodes (LED), light harvesting systems, optical data carriers, chemically programmable switches, and liquid crystalline compounds can also make use of a variety of heterocyclic compounds [19-22].

Pyrimidine was first isolated by Gabriel and Colman in 1899. The chemistry of pyrimidine and its derivatives have been studied since the past century due to their diverse pharmacological properties. Pyrimidine and purine, the two-nitrogen containing heterocyclic aromatic compounds are the parents of the "bases" that constitute a key structural unit of nucleic acids, even though pyrimidine itself does not exist in nature. Both pyrimidine and purine are planar and this flat shape is very important when we consider the structure of nucleic acids. In the presence paper, we have synthesized novel pyrimidines B1-B15 from novel chalcones and guanidine.

2. Methods and Materials

2.1 Chemicals and Reagents

All chemicals used were of laboratory reagent grade and used without further purification. Various aldehydes, 1-(4-aminophenyl)ethenone, 2-chloro-7H-pyrrolo[2,3-d]pyrimidine, guanidine, KOH and ethanolwere used as received from Merck, Mumbai, India.

2.2 Experimental

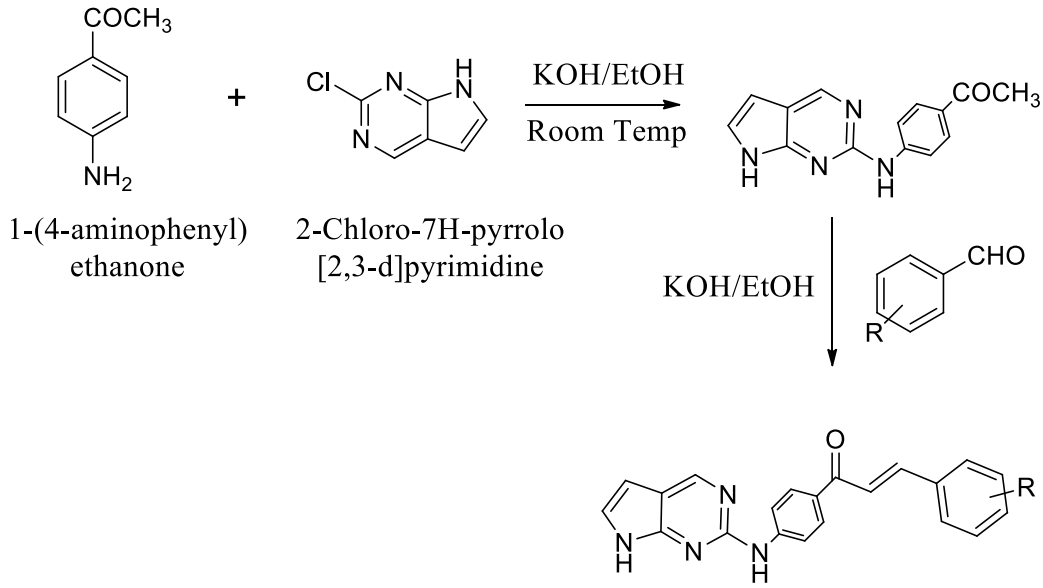
Bruker Avance-400 instrument was used for Proton NMR study and 100MHZ frequency instrument was used for ^{13}C NMR. Parts per million unit was used to expressed chemical shift value. ABB Bomem Inc. FT-IR 3000 Spectrophotometer was used for Infrared Spectral study. Data obtained was expressed in cm^{-1} unit. Shimadzu LCMS-2010 was used for MASS spectral analysis. Perkin Elmer-2400 Series II CHNS/O Elemental Analyzer was used for Composition measurement.

2.3 Method of Synthesis

2.3.1 Synthesis of various chalcones A1-A15

In a 250 ml round bottom flask, 2-chloro-7H-pyrrolo[2,3-d]pyrimidine (0.1 mol) and1-(4-aminophenyl)ethenone (0.1 mol) dissolved in ethannol (50 ml) with constant shaking maintaining the temperature below 25°C. After the completion of dissolution, the mixture was refluxed for 1.5 hr. then it was cooled and poured into crushed ice.

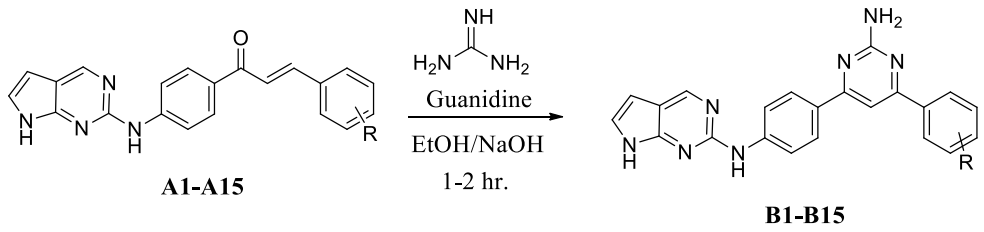
Solid was separated by filtration and crystalline from ethanol. To a well stirred solution, add40% potassium hydroxide (40 ml) followed by addition of aromatic aldehyde (0.01 mol) drop wise at 0^{o}C). After the completion of addition, the mixture was stirred for further 1-1.5 hours and left overnight. The contents were poured into ice water and crystallized from ethanol (Scheme 1).



Scheme-1 Synthesis of Pyrimidine based Chalcones

2.3.2 Synthesis of 2-aminopyrimidines

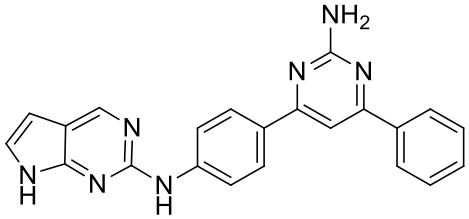
Take chalcones (0.01 mol)in 250 ml RBF, add 0.01 molguanidine, 40 ml ethanol and 40 ml 40% KOH to this mixture solution. Reflux the entire mixture for 1-2 hr to produced pyrimidine. Completion of reaction was monitored by TLC (Scheme 2).



Scheme-2 Synthesis of Pyrimidines B1-B15

3. CHARACTERIZATION

B1 compound of the series is taken as the representative compound. In the ^{1}HNMR spectrum the characteristic signals due to each protons and functional groups with protons are well described on the basis of shielding and deshielding effects. The signal due to aromatic proton of compound was observed in more downfield region at chemical shift value around 6 to 8ppm. ^{1}HNMR, ^{13}CNMR, IR, MASS spectroscopic data of B1 compoundshown below.

Compound code: B1	
Molecular formula: $C_{22}H_{17}N_7$	
M. P. (°C): 232	
^{1}H NMR (400 MHz, $CDCl_3$) δ ppm:	9.2 (NH_2, s), 6.6-8.4 (13H, Ar-H, complex), 4.2 (1H, s, -NH), 3.8 (1H, s, -NH).
^{13}C NMR (100 MHz, $CDCl_3$) δ ppm:	128.2, 129.4, 130.3, 131.6, 139.2, 143.6, 151.8, 153.6, 155.1, 156.8.
IR cm^{-1} (KBr):	3510, 3442, 3320, 3029, 1660, 1592, 1569, 744.
Mass (M+1):	379.10
Elemental analysis:	Calculated (%): C: 69.64; H: 4.52; N:25.84. Found (%) : C: 69.32; H: 4.64;N: 25.72

4. Result and Discussion

Table 1.1 Data showing synthesis of Pyrimidnes B1-B15.

Sr. No.	Compounds Code	R	Reaction Time[a]hr	% Yiled[b]
1	B1	-H	3.5	75
2	B2	4-OH	4	73
3	B3	3-OH	4	70
4	B4	2-OH	4	70
5	B5	2- OCH_3	4.5	70
6	B6	4-OCH_3	4.5	69
7	B7	2-Cl	3.5	80
8	B8	4-Cl	3.5	81
9	B9	3-Cl	3.5	82
10	B10	2-NO_2	3.5	83
11	B11	4-NO_2	3.5	82
12	B12	3-NO_2	3.5	83
13	B13	3-Br	3.5	78
14	B14	2- Br	3.5	80
15	B15	4- Br	3.5	80

From the Table 1.1 show the various condensation product of condensation reaction between compounds A1-A15 and guanidine. It clearly indicates that the compounds bearing electron withdrawing group are synthesized in shorter reaction time as compared to compounds bearing electron donating group. Compounds B10-B12 bearing electron withdrawing were synthesized in 3.5 hr as shorter time as compared to compound B5 and B6 bearing electron donating group in 4.5 hr.

5. Conclusion

In conclusion the highly functionalized 2-aminopyrimidines derivatives (B1-B15) were synthesized from various chalcones and guanidine by condensation reaction. Chalcones used in the pyrimidine synthesis are synthesized from various aromatic aldehydes, 1-(4-aminophenyl)ethenone and 2-chloro-7H-pyrrolo[2,3-d]pyrimidine. All the compounds are well characterized by different spectroscopic techniques.

6. References

1. Aqil F, Ahmad I (2003) Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. World J Microbiol Biotechnol 19:653–657.
2. Bauer AW, Kirby WMM, Sherris JC, Turch M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Path 45:493–496.
3. Bruno O, Ranise A, Bonadavalli F, Schenone P, D'Amico M, Felipplli W, Filippelli A, Rossi F (1993) 3,5-Diphenyl-1H-pyrazole derivatives. XI. N-Aryl-5(3)-phenyl-4-(3,5-diphenyl-1-pyrazolyl)-3(5)- pyrazole amines, 5-substituted 1,6-dihydro-4-phenyl-5-(3,5-diphenyl-1-pyrazolyl)pyrimidines with antipyretic, antiinflammatory and other activities. Farmaco 48:949–966.
4. Rastogi, A. C., and K. L. Chopra. "Kinetics of growth of polyvinylchloride (PVC) films." Thin Solid Films 18.2 (1973): 187-200.
5. Gautam, K. S., et al. "Molecular structure of polystyrene at air/polymer and solid/polymer interfaces." Physical review letters 85.18 (2000): 3854.
6. Haussener, Sophia, and Miguel Antonio Modestino. "Solar production of nylon polymers and precursors for nylon polymer production." U.S. Patent Application No. 16/496,060.
7. Park, Jinseon, et al. "Selective Infiltration in Polymer Hybrid Thin Films as a Gas Encapsulating Layer for the Stretchable Electronics." ACS Applied Materials & Interfaces (2020).
8. Umashankar, M., et al. "Effect of Nylon-66 Interleaving on Dielectric Properties of Carbon-Epoxy Composites." Techno-Societal 2018. Springer, Cham, 2020. 285-293.
9. Singh, Maninder, et al. "Animal Hair-Based Triboelectric Nanogenerator (TENG): A Substitute for the Positive Polymer Layer in TENG." Journal of Electronic Materials (2020): 1-8.
10. Villani, Maurizio, et al. "Polyurethane-Based Composites: Effects of Antibacterial Fillers on the Physical-Mechanical Behavior of Thermoplastic Polyurethanes." Polymers 12.2 (2020): 362.
11. Yi, Chenglin, et al. "Polymer-guided assembly of inorganic nanoparticles." Chemical Society Reviews (2020).
12. Bartzsch, Matthias, et al. "Device and circuit simulation of printed polymer electronics." Organic electronics 8.4 (2007): 431-438.
13. Lotfi, Amirhossein, et al. "Natural fiber–reinforced composites: a review on material, manufacturing, and machinability." Journal of Thermoplastic Composite Materials (2019): 0892705719844546
14. Filippo, Ciampanelli. Hominem reducere ad Deum: la funzione mediatrice del Verbo incarnato nella teologia di San Bonaventura. Vol. 310. Gregorian Biblical BookShop, 2010.

15. Xu, Bin, Ailin Deng, and Song Xue. "Experimental study on filling imbalance of plastic parts with microcylinders." Polymer Engineering & Science 60.1 (2020): 22-31.
16. Kim, Hoyeon, et al. "Multilayer Structuring of Nonleaded Metal (BiSn)/Polymer/Tungsten Composites for Enhanced γ-Ray Shielding." Advanced Engineering Materials (2020): 1901448.
17. Xu, Zhenpeng, et al. "Additive manufacturing of two-phase lightweight, stiff and high damping carbon fiber reinforced polymer microlattices." Additive Manufacturing 32 (2020): 101106.
18. Kulkarni, Apoorv, Gian Domenico Sorarù, and Joshua M. Pearce. "Polymer-derived SiOC replica of material extrusion-based 3-D printed plastics." Additive Manufacturing 32 (2020): 100988.
19. Geier, Johannes, and Holger Lessmann. "Leather and shoes." Kanerva's occupational dermatology (2020): 877-889.
20. Kim, Hyun, and Hong Jeon. "Amide-modified polymer compositions and sports equipment made using the compositions." U.S. Patent Application No. 11/592,109.
21. Kröger, Martin. "Developments in Polymer Theory and Simulation." (2020): 30.
22. McBride, Michael, et al. "Toward data-enabled process optimization of deformable electronic polymer-based devices." Current Opinion in Chemical Engineering 27 (2020): 72-80.

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26 Novel Nirtogen Containing Phenazine Heterocyclic Precurssor: A Class Of Biologically Active Scaffold

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Abstract
Nitrogen containing heterocyclic compound has been extensively studied and used in certain specific biological activities. Phenazine and its derivative constitute an important class of fused heterocycles that are found in more than 200 naturally occurring alkaloids. With passage of time newer and more complex variants of phenazine structure are being discovered. Novel scaffold of phenazine was synthesized by the base catalysed cyclisation of 2-hydroxynaphthalene-1,4-dione with Naphthalene-1,2-diaminefollowed by malononitrile and aromatic aldehyde in presence of ethanol. The overall reaction was conventional multistep process. The structure of synthesized compounds was confirmed on the basis of elemental analysis, IR, NMR and mass spectra results. The pharmacological studies of title compounds were screened for two gram positive bacteria Staphylococcus aureus ATCC 9144 and Bacillus Subtilis ATCC 6633) and two gram negative bacteria(Escherichia coli ATCC 25922 and Proteus vulgaris ATCC 10271), whereas antifungal activity screened against two plant pathogens Candida albicans ATCC 10231 and Aspergillus niger ATCC 6275 *in vitro* by disc diffusion method. The strength of synthesized compounds was compared with standard drug.
KEY WORDS: Pharmacological, Heterocycle, In Vitro, Phenazine.

Inroduction
Phenazine derivatives are the emerging pharmacophore, which has drawn a growing interest in the area of drug designing. Phenazine and its nitrogen containing precursors had diversified biological properties. One of the most frequently encountered heterocycles in medicinal chemistry is phenazine with pyrazoline and its analogs have widespread applications as a potential anti-inflammatory, anticancer and analgesics agent [1, 2,3]in field of medicinal chemistry. Moreover, a large number of phenazine derivatives have been reported as potential antifungal and antimicrobial agents [4, 5, 6].
Pyrazoline derivatives of phenazine heerocycles have important therapeutic properties,
among many derivatives are biologically active scaffold and important constituent of many pharmaceutical product and used as a antibiotics [7,8] and bladder cancer cell treatment[9]. The halogenated derivatives of phenazine possess potential antihyperlipidemic activity and have no significant toxic side effect at the sub lethal drop level 2 mg/kg [10]. In addition Endophenazine G and other phenazine

derivatives reported to potential antimethicline resistant agent [11] and HIV-1 integrate inhibitors[12].

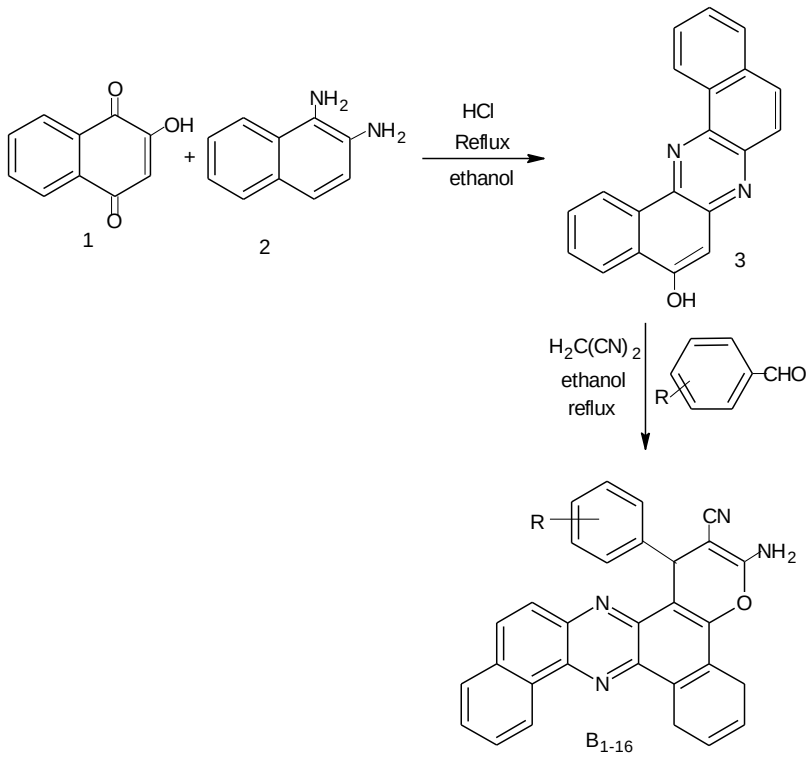
The exploration for new biologically active heterocyclic analogues and continues to be an area of intention research in medicinal chemistry. In the light of these findings, the synthesis of new chemical entities incorporating the quinoline and pyrazoles with quinazolinones may prove to be useful frame of the biological activity point of view. The targeted molecules screened for antibacterial and antifungal activities in vitro by disc diffusion method.

MATERIAL AND METHOD

General Instrumentation

The reagent grade chemicals were purchased from commercial sources and further purified before use. The melting points of all synthesized compounds were taken in open capillary tube and are uncorrected. The purities of all synthesized compounds were checked by TLC on Merck silica gel 60 F 254 using toluene: ethyl acetate (8:2) as mobile phase, and spots were visualized under UV radiation. The IR spectra of the synthesized compounds were recorded on Perkin-Elmer 1300 FTIR spectrophotometer using KBr pellets and frequencies are recorded in cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance II 400 NMR spectrometer using $CDCl_3$ as a solvent. The chemical shifts were reported in (δ ppm) downfield using tetra methyl silane as internal standard. Shimadzu LCMS-2010 was used for Mass spectral analysis. Elemental analyses of newly synthesized compounds were carried out on Carlo Ebra 1108 analyzer. Microanalysis of compounds was within ±0.4% of theoretical values and the spectral data (Elemental analysis, IR, NMR and mass spectra) were compatible with the assigned structures. The compound 2-hydroxynaphthalene-1,4-dioneandnaphthalene-1,2-diamine weresynthesized by literature procedure [13].

Scheme I Synthetic Pathway for Target Molecule



Experimental Section

B_1 : 3-amino-1-phenyl-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile

Take 2-hydroxynaphthalene-1,4-dione(0.01mol, 1.58g) in 250 ml round bottom flask, to this solution add Naphthalene-1,2-diamine (0.01 mol, 1.74 g)and 2-3 drops of conc HCl and 40 ml ethanol, reflux the reaction mixture for 30-40 minutes. After completion of reaction (cheched by TLC), addmalononitrile (0.01mol, 0.66g) and benzaldehyde (0.01mol, 1.06g) to it and add further 40ml ethanol. Reflux the content mixture for again 30-40 minutes. After completion of reaction (checked by TLC), cool the reaction mixture, solid thus obtained was novel phenazine B_1. Recrystallised the crude product from absolute ethanol.

Yeild: 80 % M.P.: 229-230 ^{0}C.IR(KBr):3317(C-N), 3062, 2860(C-H aromatic), 2127(C-N aliphatic), 1617,1591, 1568(C=C), 748 (C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.4 (s,2H, -NH_2), 3.2 (s,1H, pyrilium ring), 6.3-8.4 (m,15H,Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 61.4(CH_2-C), 122.0-145.1(Ar-28C), 160.2(CN-C).Mass spectrum: molecular ion peak found at M+ 450.0Anal; (%) $C_{30}H_{18}N_4O$ Calcd; C, 80.61; H, 4.09; N,12.55; Found; C, 79.98; H, 4.03; N, 12.44.

Remaining compounds of the series B_2-B_{17}were synthesized by similar prescribed above method.

B_2:3-amino-1-(4-hydroxyphenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile

Yeild: 79 % M.P.: 249-250 ^{0}C.IR(KBr):3425(O-H),3319(C-N), 3063, 2865(C-H aromatic), 2155(C-N aliphatic), 1617,1592, 1592(C=C), 749 (C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.6 (s,2H, -NH_2), 3.1 (s,1H, pyrilium ring), 6.3-8.4 (m,14H,Ar-H), 9.9 (s,1H,-OH). ^{13}C NMR (100 MHz, $CDCl_3$): 63.3(CH_2-C), 121.1-145.5(Ar-28C), 159.4(CN-C).Mass spectrum: molecular ion peak found at M+ 466.1Anal; (%) $C_{30}H_{18}N_4O_2$ Calcd; C, 77.22; H, 4.01; N,12.20; Found; C, 77.24; H, 3.98; N, 12.19.

B_3:3-amino-1-(3-hydroxyphenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile

Yeild: 79 % M.P.: 259-260 ^{0}C.IR(KBr):3429(O-H),3321(C-N), 3063, 2860(C-H aromatic), 2158(C-N aliphatic), 1618,1593, 1591(C=C), 747 (C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.5 (s,2H, -NH_2), 3.2 (s,1H, pyrilium ring), 6.3-8.4 (m,14H,Ar-H), 9.7 (s,1H,-OH). ^{13}C NMR (100 MHz, $CDCl_3$): 63.1(CH_2-C), 121.3-145.4(Ar-28C), 160.0(CN-C).Mass spectrum: molecular ion peak found at M+ 466.1Anal; (%) $C_{30}H_{18}N_4O_2$ Calcd; C, 77.22; H, 4.01; N,12.20; Found; C, 77.23; H, 3.98; N, 12.18.

B_4:3-amino-1-(2-hydroxyphenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile

Yeild: 81 % M.P.: 254-255^{0}C.IR(KBr):3427(O-H),3318(C-N), 3061, 2864(C-H aromatic), 2157(C-N aliphatic), 1619,1591, 1593(C=C), 750 (C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.5 (s,2H, -NH_2), 3.1 (s,1H, pyrilium ring), 6.3-8.4 (m,14H,Ar-H), 9.8 (s,1H,-OH). ^{13}C NMR (100 MHz, $CDCl_3$): 63.2(CH_2-C), 121.1-145.5(Ar-28C), 160.4(CN-C).Mass spectrum: molecular ion peak found at M+ 466.1Anal; (%) $C_{30}H_{18}N_4O_2$ Calcd; C, 77.22; H, 4.01; N,12.20; Found; C, 77.23; H, 3.99; N, 12.18.

B_5:3-amino-1-(2-methoxyphenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile

Yeild: 84 % M.P.: 233-234^{0}C.IR(KBr):3329(C-N), 3062, 2860(C-H aromatic), 2125(C-N aliphatic), 1617,1591, 1569(C=C), 1242, 1107(C-O), 746 (C-H mono and

disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.6 (s,2H, $-NH_2$), 3.2 (s,1H, pyrilium ring), 3.8(s, 3H,$-OCH_3$),6.4-8.7 (m,14H,Ar-H).^{13}C NMR (100 MHz, $CDCl_3$): 39.3(OCH_3-C), 63.7(CH_2-C), 121.6-145.6(Ar-28C), 160.4(CN-C).Mass spectrum: molecular ion peak found at M+ 480.1Anal; (%) $C_{31}H_{20}N_4O_2$ Calcd; C, 77.49; H, 4.20; N,11.66; Found; C, 77.48; H, 4.18; N, 11.64.

B_6:3-amino-1-(4-methoxyphenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile

Yeild: 78 % M.P.: 211-212^{0}C.IR(KBr):3330(C-N), 3065, 2861(C-H aromatic), 2126(C-N aliphatic), 1618,1592, 1570(C=C), 1241, 1108(C-O), 748 (C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.5 (s,2H, $-NH_2$), 3.1 (s,1H, pyrilium ring), 3.7(s, 3H,$-OCH_3$),6.4-8.7 (m,14H,Ar-H).^{13}C NMR (100 MHz, $CDCl_3$): 39.2(OCH_3-C), 63.6(CH_2-C), 121.5-145.7(Ar-28C), 160.1(CN-C).Mass spectrum: molecular ion peak found at M+ 480.1Anal; (%) $C_{31}H_{20}N_4O_2$ Calcd; C, 77.49; H, 4.20; N,11.66; Found; C, 77.47; H, 4.17; N, 11.65.

B_7:3-amino-1-(2-chlorophenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile

Yeild: 87 % M.P.: 214-215^{0}C.IR(KBr):3317(C-N), 3065, 2861(C-H aromatic), 2153(C-N aliphatic), 1617,1592, 1570(C=C), 778(C-Cl), 746(C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.5 (s,2H, $-NH_2$), 3.2 (s,1H, pyrilium ring), 6.3-8.6 (m,14H,Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 63.3(CH_2-C), 121.3-145.5(Ar-28C), 160.3(CN-C).Mass spectrum: molecular ion peak found at M+ 484.0Anal; (%) $C_{30}H_{17}ClN_4O$ Calcd; C, 74.30; H, 3.53; N, 11.55; Found; C, 74.28; H, 3.51; N, 11.52.

B_8:3-amino-1-(4-chlorophenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile

Yeild: 88 % M.P.: 223-224^{0}C.IR(KBr):3319(C-N), 3066, 2862(C-H aromatic), 2155(C-N aliphatic), 1618,1593, 1571(C=C), 780(C-Cl), 748(C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.6 (s,2H, $-NH_2$), 3.3 (s,1H, pyrilium ring), 6.4-8.7 (m,14H,Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 63.4(CH_2-C), 121.5-145.7(Ar-28C), 160.4(CN-C).Mass spectrum: molecular ion peak found at M+ 484.1Anal; (%) $C_{30}H_{17}ClN_4O$ Calcd; C, 74.30; H, 3.53 ; N,11.55; Found; C, 74.29; H, 3.52; N, 11.53.

B_9:3-amino-1-(3-chlorophenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile

Yeild: 80 % M.P.: 234-235^{0}C.IR(KBr):3320(C-N), 3064, 2862(C-H aromatic), 2151(C-N aliphatic), 1617,1591, 1571(C=C), 776(C-Cl), 747(C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.4 (s,2H, $-NH_2$), 3.3 (s,1H, pyrilium ring), 6.4-8.7 (m,14H,Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 63.2(CH_2-C), 121.1-145.3(Ar-28C), 160.1(CN-C).Mass spectrum: molecular ion peak found at M+ 484.0Anal; (%) $C_{30}H_{17}ClN_4O$ Calcd; C, 74.30; H, 3.53; N, 11.55; Found; C, 74.27; H, 3.52; N, 11.54.

B_{10}:3-amino-1-(2-nitrophenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile

Yeild: 88 % M.P.: 239-240 ^{0}C.IR(KBr):3317(C-N), 3065, 2861(C-H aromatic), 2163(C-N aliphatic), 1617,1591, 1572(C=C), 1551, 1363(C-N), 749 (C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.5 (s,2H, $-NH_2$), 3.3 (s,1H, pyrilium ring), 6.4-8.6 (m,14H,Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 63.7(CH_2-C), 121.3-145.7(Ar-28C), 160.4(CN-C).Mass spectrum: molecular ion peak found at M+ 495.1Anal; (%) $C_{30}H_{17}N_5O_3$ Calcd; C, 72.72; H, 3.46; N, 14.93; Found; C, 72.70; H, 3.44; N, 14.91.

B_{11}:3-amino-1-(4-nitrophenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile
Yeild: 84 % M.P.: 244-245^{0}C.IR(KBr):3319(C-N), 3063, 2860(C-H aromatic), 2161(C-N aliphatic), 1618,1592, 1572(C=C), 1552, 1362(C-N), 747 (C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.6 (s,2H, -NH_2), 3.4 (s,1H, pyrilium ring), 6.5-8.7 (m,14H,Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 63.6(CH_2-C), 121.5-145.9(Ar-28C), 160.1(CN-C).Mass spectrum: molecular ion peak found at M+ 495.1Anal; (%) $C_{30}H_{17}N_5O_3$ Calcd; C, 72.72; H, 3.46; N, 14.93; Found; C, 72.71; H, 3.45; N, 14.92.

B_{12}:3-amino-1-(3-nitrophenyl)-1H-dibenzo[a,i]pyrano[2,3-a]phenazine-2-carbonitrile
Yeild: 84 % M.P.: 244-245^{0}C.IR(KBr):3317(C-N), 3062, 2860(C-H aromatic), 2127(C-N aliphatic), 1617,1591, 1568(C=C), 748 (C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.4 (s,2H, -NH_2), 3.2 (s,1H, pyrilium ring), 6.3-8.4 (m,15H,Ar-H), 9.15 (d,1H,-NH). ^{13}C NMR (100 MHz, $CDCl_3$): 61.4(CH_2-C), 122.0-145.1(Ar-28C), 160.2(CN-C).Mass spectrum: molecular ion peak found at M+ 450.1Anal; (%) $C_{30}H_{18}N_4O$ Calcd; C, 80.61; H, 4.09; N,12.55; Found; C, 79.98; H, 4.03; N, 12.44.

B_{13}:3-amino-1-(3-bromophenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile
Yeild: 86 % M.P.: 242-243^{0}C.IR(KBr):3323(C-N), 3063, 2862(C-H aromatic), 2165(C-N aliphatic), 1617,1592, 1572(C=C), 1553, 1361(C-N), 748 (C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.4 (s,2H, -NH_2), 3.4 (s,1H, pyrilium ring), 6.4-8.6 (m,14H,Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 63.5(CH_2-C), 121.1-145.4(Ar-28C), 160.1(CN-C).Mass spectrum: molecular ion peak found at M+ 495.0Anal; (%) $C_{30}H_{17}N_5O_3$ Calcd; C, 72.72; H, 3.46; N, 14.93; Found; C, 72.70; H, 3.45; N, 14.92.

B_{14}:3-amino-1-(2-bromophenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile
Yeild: 86 % M.P.: 247-248^{0}C.IR(KBr):3318(C-N), 3064, 2862(C-H aromatic), 2151(C-N aliphatic), 1617,1591, 1571(C=C), 746(C-H mono and disubstituted benzene), 642(C-Br). ^{1}HNMR (400 MHz, $CDCl_3$): 2.5 (s,2H, -NH_2), 3.3 (s,1H, pyrilium ring), 6.3-8.6 (m,14H,Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 63.1(CH_2-C), 121.5-145.7(Ar-28C), 160.1(CN-C).Mass spectrum: molecular ion peak found at M+ 528.1Anal; (%) $C_{30}H_{17}BrN_4O$ Calcd; C, 68.06; H, 3.24; N, 10.58; Found; C, 68.04; H, 3.22; N, 10.56.

B_{15}:3-amino-1-(4-bromophenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile
Yeild: 82 % M.P.: 235-236^{0}C.IR(KBr):3321(C-N), 3065, 2861(C-H aromatic), 2155(C-N aliphatic), 1617,1593, 1570(C=C), 748(C-H mono and disubstituted benzene), 645(C-Br). ^{1}HNMR (400 MHz, $CDCl_3$): 2.6 (s,2H, -NH_2), 3.2 (s,1H, pyrilium ring), 6.4-8.7 (m,14H,Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 63.4(CH_2-C), 121.4-145.6(Ar-28C), 160.4(CN-C).Mass spectrum: molecular ion peak found at M+ 528.0Anal; (%) $C_{30}H_{17}BrN_4O$ Calcd; C, 68.06; H, 3.24 ; N,10.58; Found; C, 68.05; H, 3.23; N, 10.55.

B_{16}:3-amino-1-(3,4-dimethoxyphenyl)-1H-dibenzo[c,h]pyrano[3,2-a]phenazine-2-carbonitrile
Yeild: 78 % M.P.: 224-225^{0}C.IR(KBr):3327(C-N), 3063, 2860(C-H aromatic), 2127(C-N aliphatic), 1617,1591, 1570(C=C), 1243, 1107(C-O), 748 (C-H mono and disubstituted benzene). ^{1}HNMR (400 MHz, $CDCl_3$): 2.5 (s,2H, -NH_2), 3.1 (s,1H, pyrilium ring), 3.7(s, 6H,-OCH_3),6.4-8.7 (m,13H,Ar-H).^{13}C NMR (100 MHz, $CDCl_3$):

39.3(OCH_3-C), 63.4(CH_2-C), 121.6-145.9(Ar-28C), 160.2(CN-C).Mass spectrum: molecular ion peak found at M+ 510.1Anal; (%) $C_{32}H_{22}N_4O_3$ Calcd; C, 75.28; H, 4.34; N,10.97; Found; C, 75.28; H, 4.33; N, 10.95.

Determination of Antimicrobial Activity

Disc diffusion method

The *in vitro* antimicrobial activity of synthesized compounds was carried out by disc diffusion method[14,15]. The cup was bore in to the inoculated Petri dish. The cups were made (equidistance) by punching in to the agar surface with sterile cup borer and scooping out the punch part of the agar. After punching a bore, in to these cups were added 0. 01 ml portion of the test compound (0. 01 g dissolved in 10 ml DMF solvent) in solvent with the help of sterile syringe. The solution was allowed to defuse for about an hour in to the medium.

Bacterial and Plant Pathogenic Stains Used

The *in vitro* antimicrobial studies of target molecule was screened against two gram positive bacteria(Staphylococcus aureus ATCC 9144 and Bacillus Subtilis ATCC 6633) and two gram negative bacteria(Escherichia coli ATCC 25922 and Proteus vulgaris ATCC 10271), whereas antifungal activity screened against two plant pathogens Candida albicans ATCC 10231 and Aspergillus niger ATCC 6275.

Measurement of the zone of Inhibition

After 2 h, for the diffusion of the substance in the agar medium and the plates were incubated at 37 ^{0}C for 24 h. After incubation period observed the plate for zone of inhibition around the cups. Measure the diameter of each zone in mm.

A solvent control was also run to know the activity of the blank . This was carried out in DMF at concentration of 0. 05 ml in similar manner and the zone of the inhibition of the bacterial growth were measured in diameter and it was 0.0 mm. The standard drugs were also screened under similar condition.

The zone of inhibition measured for antibacterial activity at concentrations 1 x 10^4μg/ml,Penicillin-G and Streptomycin were used as standard and zone of inhibition measured for anti fungal activity also at concentrations 1 x 10^4 μg/ml and Fluconazole was used as a standard.

Result And Discussion

The target molecule phenazine heterocycles incorporating with heterocyclic moieties $B_{1\text{-}16}$ were synthesized and structure was confirmed by the spectral results.The IR spectrum frequency found at 3317 cm^{-1} indicates the N-H stretching. The frequency found at 3128 cm^{-1} indicates thearomatic C-H stretching. The frequency found at 2153 cm^{-1} indicates aliphatic C-N stretching. The frequency found at 1617, 1592 and 1570 cm^{-1} indicates the C=C stretching. The frequency found at 746 cm^{-1} indicates mono and disubstituted benzene C-H stretching.It was further confirming by ^{1}H NMR spectra which showed signal of two singlet proton of the NH_2 found at δ 2.5 ppm in downfield region. The signal of one singlet proton of CH of pyrilium ring found at δ 3.2 ppm. The signal of multiplatate 14 aromatic protons was found in region between δ 6.3 ppm to 8.6 ppm. In ^{13}C NMRspectrum the signal of methine carbon found at 63.5. The signal of aromatic carbons found at 121.3, 128.9, 129.4, 130.5, 131.6,

140.2, 140.8, 142.1, 143.4, 145.5 The signal of CN carbon found in most downfield region at 160.3.In the mass spectrum were molecular ion peak found at M+ 484.0 indicates the molecular weight of the compoundB_1.

Antimicrobial Assay

The *in vitro* antimicrobial screening results of synthesized compounds were recorded in the table 1. The strength of synthesized compounds was compared with standard drug.

Table: 1 Antimicrobial activity of new synthesized compounds against the bacterial strains and plant pathogens tested based on disc diffusion techniques

Microorganism ATCC code	Sample(Zone of inhibition in mm)															
	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_9	B_{10}	B_{11}	B_{12}	B_{13}	B_{14}	B_{15}	B_{16}
S.aureus (9144)	10	12	13	12	11	11	19	18	17	21	24	23	16	17	16	10
B.subtilis(6633)	10	11	12	11	10	11	18	17	18	20	24	22	16	18	16	10
E.coli(25922)	19	27	24	26	22	21	13	14	14	11	12	11	16	17	16	19
P.vulgaris(13315)	19	28	25	25	23	22	13	15	15	12	12	11	17	17	16	18
A.niger(6275)	22	17	16	16	21	19	11	12	13	11	12	12	13	12	12	21
C.albicans(10271)	21	15	14	14	20	18	11	13	12	12	13	11	13	12	11	22
Standard used for Gram positive bacteria: Penicillin G (Zone of inhibition in mm) : 25±0.58 Standard used for Gram negative bacteria: Streptomycin (Zone of inhibition in mm) : 29±1.10 Standard used for antifungal activity: Fluconazole (Zone of inhibition in mm) : 23±0.58 Amount of sample: 80µl Concentration: 1 x 10^4 µg/ml																

Conclusion

The title phenazine precursor$B_{1\text{-}16}$ were synthesized by well-organized method. The active pharmacophore Phenazine heterocycle present in a newly synthesized compounds possessed good antibacterial and antifungal activity *in vitro*. The compound B_{10},B_{11},B_{12} (R_1= 2-NO_2,4-NO_2, 3-NO_2) showed very good activity against gram positive bacteria while compound B_2,B_3,B_4(R_1= 4-OH,3-OH,2-OH) displayed very good activity against gram negative bacteria compared to standard. More over compound B_1 ((R= -H) and compound B_5, B_{16}(R=2-OCH_3,2,4-OCH_3) showed very good antifungal activities compared to standard. From these work, we were able to identify a few active molecules which are capable to inhibiting the growth of some bacteria and fungus species in vitro. Future investigation of quinazolinone structure could give some hopeful results in the field of medicinal chemistry.

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References

(1) Tupe, S. G., Kulkarni, R. R., Sirazi, F., Sant, D. G., Joshi, S. P., Deshpande, M. V.,(2015), "Possible mechanism of antifungle phenazine 1-carboxamide Pseudomanas sp. Against dimorphic fungi Benjaminiella pointrosil and human pathogen candida albicans", *J. Appl. Microbiol.*,118:39-48.

(2) Varsha, K. K., Nishant, G., Sneha, S., Shilpa, G., Devendra, L., Priya, S., Nampoothiri, K., (2016),"Antifungal, anticancer and aminopeptidase inhibitory potential of a phenazine compound produced by Lactococcus BSN307", *Indian J. Microbial.*, 56:411-416.

(3) Bonev, B., Hooper, J., Parisot, J., (2008), " Principles of assessing bacterial susceptibility to antibiotics using agar diffusion method", J. Antimicrob Chemother, 61(6):1295-1301.

(4) Lu, X., Zhu, X., Zhang, M., Wu, Q., Zhou, X., Li, J., (2019), "Synthesis and fungicidal activity of 1,3,4-oxidizol-2-ylthioether derivatives containing a phenazine 1-carboxylic acid scaffold",*Nat. Prod. Res.*,33:2145-2150.

(5) Hyden, S. C., Bryant, J. J., Mackey, M. A., Hofer, K., Lindner, B. D.,Nguyen, V. P., Jaschke, A., Bunz, U. H. F.,(2014),"Antimicrobial activity of water soluble triazole phenazine clickamers against E.coli.", *Chem. Eur. J.*,20:719-723.

(6) Krishnaiah, M., Almeida, N. R., Udumula, V., Song, Z.H., Chhonker, Y. S., Abdelmoaty, M. M., Nascimento, V. A., Murry, D.J., Conda-Sheridan, M., (2018), "Synthesis, Biological Evaluation, and Metabolic Stability of Phenazine Derivatives as Antibacterial Agents", *European Journal of Medicinal Chemistry* 143, 936–47.

(7) Geiger, A., Keller-Schierlein, W., Brandi, M., Zaehner, H.,(1988), "Metabolites of microorganisms 247 phenazines from Streptomyces antibiotics stain Tu 2706", *J. antibiot.*, 41:1542.

(8) Whelan, P., Dietrich, L. E. P., Newman, D. K., (2006), "Rethinking secondary metabolism : Physiological roles for phenazine antibiotics",*Nat. Chem. Biol.*,2:71-78.

(9) Hernandez, P., Alem, D., Nieves, M., Cerecetto, H., Gonzalez, M.,Martinez Lopez, W., Laveggi, M. L.,(2019), "Chemo sensitizer effect of cisplatin treated bladder cancer cells by phenazine 5,10-dioxides", Environ Toxicol Pharmacol, 69, 9-15.

(10) Borrero, N. V., Bai, F., Perez, C., Duong, B. Q., Rocca, J. R., Jin, S., Huigens, R.W.,(2014), "Phenazine Antibiotic Inspired Discovery of Potent Bromophenazine Antibacterial Agents against Staphylococcus Aureus and Staphylococcus Epidermidis", *Organic and Biomolecular Chemistry*, 12(6): 881–6.

(11) V. Udumul, V., Endres, J. L., Harper, C. N., Jaramillo, L., Zhong, H.A., Bayles, K.W., Conda-Sheridan,M.,(2017), "Simple Synthesis of Endophenazine G and Other Phenazines and Their Evaluation as Anti-Methicillin-Resistant Staphylococcus Aureus Agents", *European Journal of Medicinal Chemistry*, 125: 710–21.

(12) Deng, R., Zhang, Z., Li, H., Wang, W., Hu, H., Zhang, X., (2021),"Identification of a novel bioactive phenazine derivative and regulation of phoP on its production in Streptomyces lomondensis SO15", *J. Agric. Food. Chem.*,69:974-981.

(13) Paciaroni, N. G., Borrero, N. V., Rocca, J. R., Huigens III, R. W., (2015)," Rapid Synthesis of Phenazine- 1-Carboxylic Acid Derived SmallMolecules from Diverse Anilines: Privileged Structures for Discovery",*Res. Rev. J. Med. Org. Chem.*, 2: 67-76.

(14) Bonev, B., Hooper, J., Parisot, J., (2008), " Principles of Assessing Bacterial Susceptibility to Antibiotics Using Agar Diffusion Method", *Int. J. Antimicrob Chemother,* 61(6):1295-1301.
(15)Barry, A.L., (1976) "The Microbial Susceptibility Test, Principles and Practices", Philadelphia Pa, USA Illus Le and Febiger, pp. 180.
(16) Chowdhury, G., Sarkar, U., Pullen, S.,(2012), "DNA strand cleavage by the phenazine di –N – Oxide natural product myxin under both sterobic and anaerobic conditions",*Chem. Res. Toxicol.,* 25, 197-206.
(17) Guo, S., Liu, R., Wang, W., Hu, H., Li, Z., Zang, X., (2020),"Desining and artificial pathway for biosynthesis of a novel phenazine N-oxide in Pseudomonas chlororaphis HT66", *ACS Syhtn. Biol.*,9:883-892.
(18) Gao, X., Lu, Y., Fang, L., Fang, X., Xing, Y., Gou, S., Xi, T., (2013),"Synthesis and anticancer activity of some novel 2-phenazinamine derivatives",*Eur. J. Med. Chem.*,69:1-9.

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Synthesis and Structural Elucidation of Substituted Quinazolin-4(3H) One Derivatives As a Potential Therapeutic Agents In Vitro.

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Abstract

Quinazoline shows wide range of application in the area such as antifungal, anti-tumor, anti-malaria, anticonvulsant, anti-microbial, anti-inflammatory and antihyperlipidemic activity because of these it shows great interest to study. As a result, we were able to create substituted quinazoline-4-one derivatives, which were then subjected to a further reaction with cyanuric chloride. ^{1}H NMR, ^{13}C NMR, IR, and MASS spectroscopic techniques were used to characterized all synthesized compound. The open disc method was used to test the synthetic compounds against antimicrobial properties. Gram-negative bacteria Escherichia coli and Proteus vulgaris and gram-positive bacteria Staphylococcus aureus and Bacillus megaterium were used in the investigation.
KEYWORDS: Antimicrobial activities, Chalcone, Quinazolinone, Cyanuric Chloride.

1.Introduction

Heterocyclic compound such as quinazoline derivatives shows wide range of medicinal application, these heterocycle groups are very significant [1]. Number of substituted quinazolinone derivatives exhibit a broad range of microbial activities, including acaricidal, weedicide, anticancer, antimalarial, antifungal, antimicrobial, antiviral, antiprotozoan, anti-inflammatory, diuretic, muscle relaxant, antitubercular, antidepressant, and antiviral properties. Quinazoline and quinazolinone chemicals are also included in many pharmacological molecules and are utilized to prepare a variety of functional materials for synthetic chemistry. emphasis on the diverse biological actions of quinazolines and quinazolinones, which have enormous promise [2]. Based on the ring system's substitution patterns, quinozolinones will be categorized into five groups [3]. It consists of 2,3,2,4-disubstituted-4(3H)-quinazolinones, 2,3-disubstituted-4(3H)-quinazolinones, 4substituted-quinazolines, and 5-substituted-4(3H)-quinazolinones. These compounds can be categorized into three different groups [4] based on where the keto or oxo group is located. Out of the three quinazolinone structures (2(1H)quinazolinones, 4(3H)quinazolinones, and 2,4(1H,3H)quinazolinedione), 4(3H)quinazolinones are the most common, either as natural products or as intermediates in numerous suggested biosynthetic pathways. Due to its numerous pharmacological actions, the quinazolinone nucleus has drawn considerable attention [5]. Many of them have therapeutic properties, including anticancer [6–8], antiinflammatory [9–10], antimicrobial [11],antihypertensive [12], and antifungal effects. According to reports, different substituents at the

quinazolinone nucleus' 2/3 position have a significant impact on pharmacological action [13].

In the presence research paper, we have synthesized novel substituted quinazoline 4-one compounds A1–A12 using alpha methyl ketone and a variety of aromatic aldehydes to produced chalcones which upon condensation with urea followed by treatment with cyanuric chloride. and these derivatives are then further processed using cyanuric chloride. All the synthesized compounds were characterized using spectroscopic techniques and tested for antimicrobial activity.

2. METHODS AND MATERIALS

2.1 Chemicals and Reagents

All the chemicals such as aromatic aldehyde, urea, cyanuric chloride, sodium hydroxide, and ethanol etc. are laboratory reagent-grade and were used before further purification as received from Merck, Mumbai, India

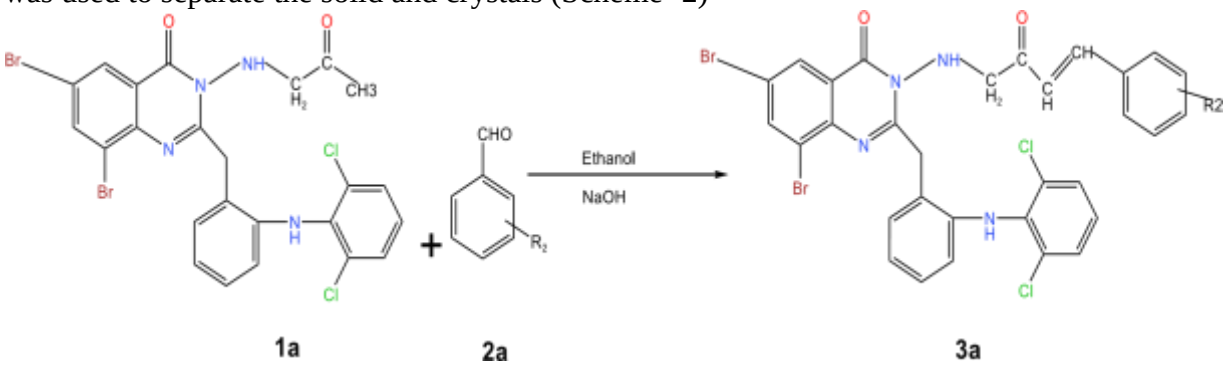
2.2 Experimental

For the ^{1}HNMR investigation, a Bruker Avance-400 instrument was used and for the ^{13}C NMR study, a 100MHZ frequency equipment. Chemical shift value was reported in parts per million. The infrared spectrum analysis was conducted using FT-IR 3000 Spectrophotometer from ABB Bomem Inc. The measured data were expressed in cm^{-1} units. For MASS spectrum analysis, a Shimadzu LCMS-2010 was used.

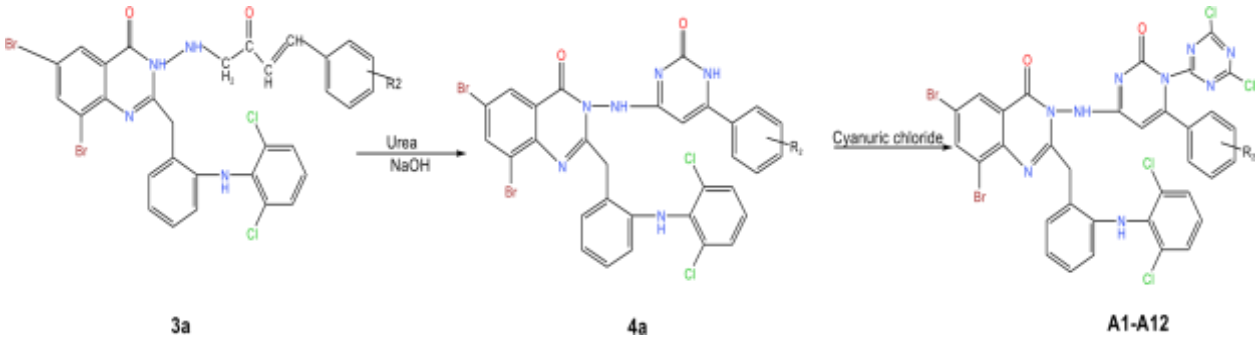
2.3 Method of Synthesis

2.3.1 Synthesis of substituted quinazoline 4--one derivatives A1-12.

To a solution of substituted aromatic compound contain heterocycle along with acetyl group (5.79g, 0.01 mol) in absolute ethanol (40 ml), add substituted aromatic aldehyde (0.01 mol) and 2% NaOH (0.01 mol) and refluxed the mixture for 10-12 hr, cooled and poured into ice cold water. The solid thus obtained was filtered and washed with water and recrystallized from ethanol. Take this prepared chalcone (6.67 gm 0.02 mol) and urea (1.0gm 0.02 mol) inethanolic sodium hydroxide solution (25ml), stirred the mixture. The reaction mixture was refluxed for 8-10 hrs. on a water bath and cooled. The separated solid was filtered, washed with water and recrystallized from ethanol and the product is substituted quinazoline 4-one derivative. Reflux the above prepared substituted quinazoline 4-one treated with 0.01 mol of cyanuric chloride using 40 ml of 40% sodium hydroxide in 40 ml of ethanol for 30- to 40-minutes. TLC is used to verify the reaction's completion. After the reaction was completed, the solution was cooled and put into crushed ice. Filtration was used to separate the solid and crystals (Scheme- 2)



Scheme 1Synthesis of substituted chalcone from Aromatic aldehyde



Scheme 2Synthesis of substituted quinazoline 4-one derivatives A1-12.

Table 1.1 Data showing synthesis of 6-substituted quinazoline 4-one derivatives A1-A12.

Sr. No.	Compounds Code	R2	% Yield[a]	Melting Point (°C)
1	A1	-H	80	210
2	A2	2-OH	78	240
3	A3	3-OH	76	250
4	A4	4- OH	78	230
5	A5	2-CL	74	235
6	A6	3-Cl	80	232
7	A7	4-Cl	85	245
8	A8	2-NO_2	85	220
9	A9	3-NO_2	85	242
10	A10	4-NO_2	86	223
11	A11	2-OCH3	80	225
12	A12	4-OCH3	78	224

[a] Isolated yield

3. Result and Discussion

3.1 Optimization of Reaction

A reaction between alpha methyl ketone 1a and benzaldehyde 2a in 40 ml ethanol using different concentration of sodium hydroxide to produced 6,8-dibromo-2-[[2-(2,6-dichloroanilino)phenyl]methyl]-3[[(~{E})-2-oxo-4-phenyl-but-3-enyl]amino]quinazolin-4-one 3a was taken as the model reaction. Data obtained are shown in the Table 1.2.

Table 1.2 Effect of different amount of catalyst on synthesis of chalcones

Entry	% of NaOH Sol in Ethanol.	Time[a] (Hours)	Yields[b] (%)
1	1	10	65
2	2	10	70
3	3	10	75
4	4	11	78
5	5	11	78
6	6	11	78
7	7	11	78
8	8	11	80
9	9	12	78
10	10	12	78

[a] Reaction was monitored by TLC, [b] Isolated yields.

It was found that best result was obtained by taking 8 mmol% amount of catalyst with 80% yield of product in 11 hours. So, this amount was taken as optimum amount and a library of quinazoline-4 -one derivatives A1-A12 were synthesized according Scheme-2.

4. ANTIMICROBIAL ACTIVITY

4.1 Preparation of Media:

Nutrient agar is used to measure bacterial activity. These steps are used to create nutrient agar: 15gm Agar-Agar, 5gm Peptone, 3gm Metal Extract, 5gm NaCl To dissolve all the components, one liter of distilled water was mixed with peptone before being boiled. The medium was stabilized in an autoclave for 20 minutes at 125°C and 15 pound pressure. A sterilized Petri plate was filled with 20 cc of the medium after it had been cooled to 45°C. The medium's pH was changed to be in the range of 7.0 and 7.5. The aforesaid organism's culture was created in nutrient broth that had been dissolved in purified water. Nutritional broth contains the following.

1) Beef extract : 10 gm,
2) Peptone : 10 gm
3) Sodium chloride : 5 gm

4.2 Experimental Data of Antimicrobial Study.

Table 1.3 Antibacterial Activities of *COMPOUND A1-A12.*

Samples	*S.aureus* (+Ve)	*B.megaterium* (+Ve)	*E.coli* (-Ve)	*P.vulgaris* (-Ve)
A1	9	11	7	9
A2	4	9	8	7
A3	7	9	6	8
A4	12	8	9	4
A5	8	6	7	6
A6	7	3	8	5
A7	4	7	4	10
A8	10	11	10	8
A9	6	5	12	9
A10	7	4	6	8
A11	4	9	8	10
A12	10	10	5	12
Ampicillin	15	14	17	16
Gentamycin	16	15	14	16

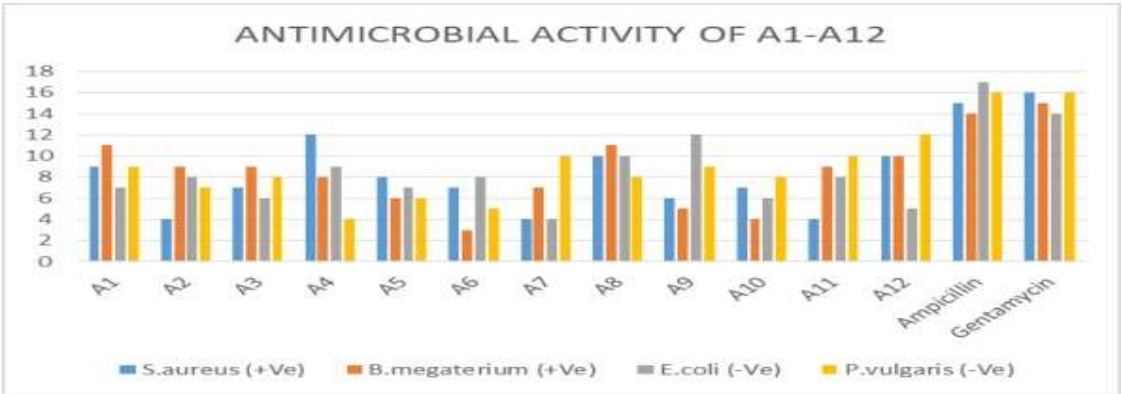


Figure 1 Antimicrobial activities of compounds A1-A11.

(I) Against *Staphylococcus aureus*:
Maximum activities were found in compound (A4, A8, A12, A1) zone of inhibition -12.0 m.m (near to standard drug) and minimum activities were found in compounds (A2, A7, A11) zone of inhibition -4.0 m.m. (II) Against *Bacillus megaterium*:
Maximum activities were found in compounds (A1, A8, A12) zone of inhibition -11.0 m.m and minimum activities were found in compounds (A6, A9, A10) zone of inhibition -4.0 m.m.

(III) Against *Escherichia coli*:
Maximum activities were found in compounds (A9, A8, and A4) zone of inhibition -11.0 m.m whereas minimum activities were found in compounds (A12,A10, A3) zone of inhibition -3.0 m.m.

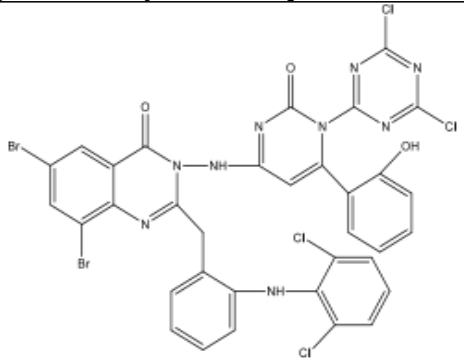
(IV) Against *Proteus vulgaris*:
Maximum activities were found in compounds (A12, A7) zone of inhibition-12.0 m.m. and minimum activities were found in compounds (A4, A6) zone of inhibition -3.0 m.m

5. CHARACTERIZATION

A2 & A11 compounds of the series is taken as the representative compound. In the ^{1}HNMR spectrum the characteristic signals due to each protons and functional groups with protons are well described on the basis of shielding and deshielding effects. The signal due to aromatic proton of compound was observed in more downfield region at chemical shift value around 6 to 8ppm. ^{1}HNMR, ^{13}CNMR, IR, MASS spectroscopic data of A2 & A11 compounds shown below.
The A2 and A11 compounds from the series are used as examples. On the basis of shielding and
deshielding actions, the characteristic signals owing to each proton and functional groups with protons are well defined in the 1HNMR spectrum. At a chemical shift value of around 6 to 8 ppm, the signal resulting from the compound's aromatic proton was detected in the region farther downfield. ^{1}HNMR, ^{13}CNMR, IR, and MASS spectroscopic data of compounds A2 and A11 are displayed below.

Compound code: A2 6,8-dibromo-2-[[2-(2,6-dichloroanilino)phenyl]methyl]-3-[[1-(4,6-dichloro-1,3,5triazin-2-yl)-6-(2-hydroxyphenyl)-2-oxo-pyrimidin-4-yl]amino] quinazolin-4-one

Molecular formula:
$C_{34}H_{19}Br_2Cl_4N_9O_3$
Molecular weight: - 903



M. P. (°C): 240

^{1}HNMR (400 MHz, $CDCl_3$) δ 1.2 (1H, -OH group) 3.69 (2H, s), 6.32 (1H,), δ ppm: 6.51 (1H,), 6.77-7.16 (4H, 6.82 (s), 7.16-7.36 (2H, 7.22 (t,), 7.46-7.67 (4H,), 7.75 (1H,), 8.14 (1H), 8.4 (2H, s, NH).

^{13}CNMR (100 MHz, CDCl3) δ ppm: δ 33.0 (1C, s), 112.8 (1C, s), 113.6 (1C, s), 116.8 (1C, s), 117.3 (1C, s), 119.6 (1C, s), 120.4 (1C, s), 123.8 (1C, s), 125.5 (1C, s), 127.3 (1C, s), 128.2 (1C, s), 128.3-128.5 (2C, 128.4 (s), 128.4 (s)), 128.9 (1C, s), 129.2 (2C, s), 129.3-129.5 (2C, 129.4 (s), 129.4 (s)), 130.2 (1C, s), 130.6 (2C, s), 134.5 (1C, s), 139.5 (1C, s), 139.6-139.8 (2C, 139.7 (s), 139.7 (s)), 144.3 (1C, s), 150.0 (1C, s), 150.8-151.0 (2C, 150.9 (s), 150.9 (s)), 153.9 (1C, s), 160.5 (1C, s), 165.2 (1C, s), 169.3 (2C, s).

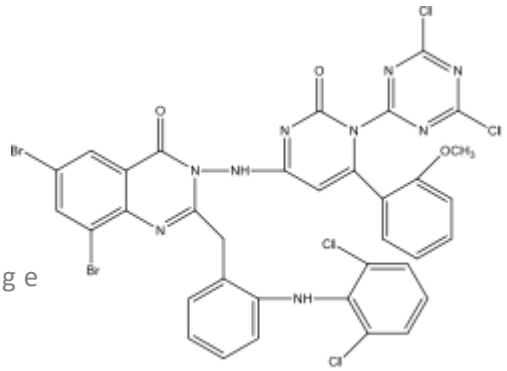
IR cm^{-1} (KBr):	3248, 1808, 1708, 1629, 1594, 1554, 1535, 1338, 1302, 1249, 1032, 1936, 539, 755, 834, 3105, 3089, 615.
Mass (M+1):	904
Elemental analysis:	Calculated (%): C, 45.21; H, 2.12; N, 13.96 Found (%) : C, 45.11; H, 2.32; N,14.24

Compound code: A11

6,8-dibromo-2-[[2-(2,6-dichloroanilino)phenyl]methyl]-3-[[1-(4,6-dichloro-1,3,5triazin-2-yl)-6-(2-hydroxyphenyl)-2-oxo-pyrimidin-4-yl]amino] quinazolin-4-one

Molecular formula: $C_{35}H_{21}Br_2Cl_4N_9O_3$

Molecular weight: - 914



M. P. (°C): 225

^{1}HNMR (400 MHz, $CDCl_3$) δ 1.2 (1H, -OH group) 3.69 (2H, s), 3.88 (3H, s), δ ppm: 6.32 (1H,), 6.51 (1H,), 6.77-7.16 (4H, 6.82 (s), 7.16-7.36 (2H), 7.46-7.67 (4H), 7.74 (1H), 8.4 (2H, s, NH).

^{13}CNMR (100 MHz, $CDCl_3$) δ ppm: δ 33.0 (1C, s), 56.0 (1C, s), 112.8 (1C, s), 113.6 (1C, s), 115.8 (1C, s), 117.3 (1C, s), 119.6 (1C, s), 120.4 (1C, s), 123.8 (1C, s), 125.5 (1C, s), 127.3 (1C, s), 128.2 (1C, s), 128.3-128.5 (2C, 128.4 (s), 128.4 (s)), 128.9 (1C, s), 129.2 (2C, s), 129.3-129.5 (2C, 129.4 (s), 129.4 (s)), 130.2 (1C, s), 130.6 (2C, s), 134.5 (1C, s), 139.5 (1C, s), 139.6-139.8 (2C, 139.7 (s), 139.7 (s)), 144.3 (1C, s), 150.8-151.0 (2C, 150.9 (s), 150.9 (s)), 153.9 (1C, s), 156.3 (1C, s), 160.5 (1C, s), 165.2 (1C, s), 169.3 (2C, s).

IR cm^{-1} (KBr): 3248, 1808, 1708, 1629, 1594, 1554, 1535, 1338, 1302, 1249, 1032, 1936, 539, 755, 834, 3105,3089,615

Mass (M+1): 915

Elemental analysis: Calculated (%): C, 45.83; H, 2.31; N, 13.74.
Found (%) : C, 45.51; H, 2.39; N, 13.93.

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6. Conclusion

In conclusion the highly functionalized substituted quinazoline 4-one derivative A1-A12 were synthesized from substituted chalcone and urea followed by further reaction with Cyanuric Chloride using ethanol as the solvent. All produced compounds were identified and tested for antimicrobial activity against gram +ve and gram -ve bacteria. With the majority of the compounds, satisfactory antibacterial activity results were attained.

7. References

1. D.J. Connolly, D. Cusack, T.P. O'Sullivan, P.J. Guiry, Synthesis of quinazolinones and quinazolines, Tetrahedron, 61 (43) (2005) 10153–10202.

2. Abida, P. Nayyar, and M. Arpanarana, An updated review: newer quinazoline derivatives under clinical trial, International Journal of Pharmaceutical & Biological Archive, 2 (6) (2011) 1651–1657.
3. S.B. Mhaske, N.P. Argade, The chemistry of recently isolated naturally occurring quinazolinone alkaloids, Tetrahedron, 62 (42) (2006) 9787–9826.
4. A.K. Mahato, B. Srivastava, S. Nithya, Chemistry structure activity relationship and biological activity of quinazoline-4(3H)-one derivatives, Inventi Rapid: Med Chem, 2 (1) (2011).
5. A.M. Alafeefy, A.E. Ashour, O. Prasad, L. Sinha, S. Pathak, F.A. Alasmari, et al., Development of certain novel N-(2-(2-(2-oxoindolin-3-ylidene)hydrazinecarbonyl)phenyl)-benzamides and 3-(2oxoindolin-3-ylideneamino)-2-substituted quinazo-lin-4(3H)-ones as CFM-1 analogs: design, synthesis, QSAR analysis and anticanceractivity, Eur. J. Med. Chem. 92 (2015) 191–201.
6. M. Mahdavi, K. Pedrood, M. Safavi, M. Saeedi, M. Pordeli, S.K. Ardestani, Synthesis and anticancer activity of N-substituted 2-arylquinazolinones bearingtrans-stilbene scaffold, Eur. J. Med. Chem. 95 (2015) 492–499.
7. S. Yin, L. Zhou, J. Lin, L. Xue, C. Zhang, Design, synthesis and biological activities of novel oxazolo[4,5-g]quinazolin-2(1H)-one derivatives as EGFR inhibitors, Eur.J. Med. Chem. 101 (2015) 462–475.
8. M.A. El-Hashash, M.E. Azab, R.A. Faty, A. Amr, Synthesis, antimicrobial and anti-inflammatory activity of some new benzoxazinone and quinazolinone candidates,Chem Pharm Bull (Tokyo). 64 (3) (2016) 263–271.
9. J.W. Chern, P.L. Tao, K.C. Wang, A. Gutcait, S.W. Liu, M.H. Yen, Studies onquinazolines and 1,2,4benzothiadiazine 1,1-dioxides. 8.1, 2 synthesis and phar-macological evaluation of tricyclic fused quinazolines and 1,2,4-benzothiadiazine1,1-dioxides as potential alpha1-adrenoceptor antagonists, J. Med. Chem. 41 (17)(1998) 3128–3141.
10. M.A. Khalil, N.S. Habib, Synthesis of thiadiazol derivatives of 4(3H)-quinazolinoneas potential antimicrobial agents, Farmaco Sci. 42 (12) (1987) 973–978.
11. N. Das, D. Garabadu, A.G. Banerjee, S. Krishnamurthy, S.K. Shrivastava, Synthesis and pharmacological evaluation of some N3-aryl/heteroaryl-substituted 2-(2-chlorostyryl)-6,7dimethoxy-quinazolin-4(3H)-ones as potential anticonvulsant agents, Med. Chem. Res. 23 (9) (2014) 4167–4176.
12. K.M. Amin, M.M. Kamel, M.M. Anwar, M. Khedr, Y.M. Syam, Synthesis, biological evaluation and molecular docking of novel series of spiro [(2H,3H) quinazoline-2,1′-cyclohexan]-4(1H)- one derivatives as anti-inflammatory and analgesic agents, Eur. J. Med. Chem. 45 (6) (2010) 2117–2131.
13. K. Kubo, T. Shimizu, S. Ohyama, H. Murooka, A. Iwai, K. Nakamura, Novelpotent orally active selective VEGFR-2 tyrosine kinase inhibitors: synthesis, structure-activity relationships, and antitumor activities of N-phenyl-N′-{4-(4-quinolyloxy)phenyl}ureas, J. Med. Chem. 48 (5) (2005) 1359–1366.

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18 Synthesis and Characterization of Some Novel heterocyclic Compounds

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Abstract

Oxazine derivatives has wide applications in Pharmaceutical and medicinal chemistry. 2-Amino-1-(4-benzyloxy-2-hydroxy-3-iodophenyl)-6-(substituted aryl)-6H-1,3-Oxazine (C_{1-10}) were synthesized by condensation and cyclization with urea in ethanolic sodium hydroxide solution. Compounds (B_{1-10}) were synthesized by coupling with aromatic substituted aldehyde. All the synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR spectra.
Keywords: Chalcones, phenone derivatives, oxazine.
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Introduction

Heterocyclic derivatives of chalcones that have been prepared have made known biological and pharmacological activities, resulting high-quality chemotherapeutics compounds[1]. Oxazine derivatives are one among the heterocyclic compounds that shows biological activities[2] similar to anti-hyperglycaemic[3], anti-leishmanial[4], anti-tubercular[5], anti-ulcer[6] and anti-cancer[7]. Oxazine are heterocyclic compounds containing one oxygen and one nitrogen with three isomeric forms[8]. Anti-inflammatory and Anti-oxidant based drugs are used for prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer[9]. The significance on 1, 3-oxazine derivatives has improved recently as molecules containing dihydro-1, 3-oxazine ring system exhibited a large spectrum of pharmacological activities such as anti-malarial[10], anti-tumor[11], anti-becterial[12-14], anti-oxidant[15-17], anti-inflammatory activity[18] and their flexibility because synthetic intermediates[19]. Particular attention has been paid to these compounds since the discovery of the non-nucleoside reverse transcriptase inhibitor triflоromethyl-1, 3-oxazine-2-one, which shows high activity against a variety of HIV-1 mutant strains[20]. This has been the prime step for the synthesis of various compounds consolidating the 1, 3-oxazine moiety[21].

Meterial And Methods

Melting point of the synthesized compounds was determined in electro thermal apparatus using fused capillary tubes. Monitoring of the reaction and the purity of the compounds was checked by thin-layer chromatography using silica gel G plates of 0.5 mm thickness as stationary phase in combination of *n*-hexane: ethyl acetate in different ratios as mobile phase.

The Infrared (IR) spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrophotometer (model Shimadzu 8700) in the range of 4000-400

cm-1 using KBr pellets and value of λmax are reported in cm-1 and the spectra were interpreted. Proton Nuclear Magnetic Resonance (^{1}NMR) and (^{13}CNMR) Nuclear Magnetic Resonance spectra were recorded on Bruker Avance II 400 NMR spectrometer using $CDCl_3$. Chemical shift (δ) are reported in parts per million downfield from internal reference, Tetramethylsilane (TMS) and the spectra were interpreted.

Synthesis of 1–(4–benzyloxy-2-hydroxy phenyl) ethanone

General Procedure: 1–(2, 4 dihydroxyphenyl) ethanone (0.10 mol), Benzyl bromide (0.1 mol) and Potassium carbonate (0.1 mol) were taken in 100ml of Acetone. Reaction mixture was shake for 7 hrs at reflux 50-60°C temperature. Reaction mixture was cooled to room temperature and quenched with 100ml cold water. The final product 1– (2-hydroxy–4-benzyloxyphenyl) ethanone was passed through pass through a filter and rinsed with water. Prepared product was recrystallized by ethanol.

Synthesis of 1–(4–benzyloxy-2-hydroxy–3-iodo phenyl) ethanone: (A)

1– (2-hydroxy–4-benzyloxyphenyl) ethanone (0.1 mol) was taken in 100ml of ethanol. Iodination method[7] has been used. Iodine granules (0.1 mol) and 300ml were taken in 250ml R.B.F and stirred them till 15 minutes. Iodic acid (0.1 mol) dilute in to 4ml of dist.water in a small beaker. Slowly add this iodic acid solution in to the reaction mixture and stirred them continuously for 30 minutes at 35 – 40 C, the reaction was monitored by TLC. Pour it in to ice. Excess iodine was removed by adding fresh saturated sodium bisulphite solution. Formed material 1–(2-hydroxy–4–benzyloxy-3-iodo phenyl) ethanone was passed through filter out and washes them two to three times with distilled water. Synthesized material was recrystallized in ethanol.

Synthesis of 1-(4-benzyloxy-2-hydroxy-3-iodo phenyl)-3-(substituted phenyl) prop-2-en-1-one from 1–(4–benzyloxy-2-hydroxy–3-iodo phenyl) ethanone :(B_1-B_{10})

General procedure:1-(4-benzyloxy-2-hydroxy-3-iodophenyl) ethanone (0.01 mol) and substituted aromatic aldehydes (0.01 mol) were dissolved in ethanol (25 ml) was added 10% sodium hydroxide solution, (25 ml) was added slowly and the mixture stirred for 4 hrs, the reaction monitored by TLC. Then it was poured into 400 ml of water with constant starring and neutralized with 10% hydrochloric acid solution and left overnight in refrigerator. The precipitate obtained was filtrated, washed and recrystallized from ethanol.

Synthesis of 2-Amino-1-(4-benzyloxy-3-iodo-2-hydroxy- phenyl)-6-(substituted aryl)-6H-1, 3-Oxazine (C_1-C_{10}) from 1-(4-benzyloxy-2-hydroxy-3-iodo phenyl)-3-(substituted phenyl) prop-2-en-1-one from 1-(4-benzyloxy-2-hydroxy-3-iodo phenyl) ethanone.

General procedure:A mixture of 1-(4-benzyloxy-2-hydroxy-3-iodo phenyl)-3-(substituted phenyl)prop-2-en-1-one (0.01 mol), Urea (0.01mol) were dissolved in ethanolic sodium hydroxide solution (10 ml) was stirred for 3 hrs, the reaction was monitored by TLC. Then it was poured into 400 ml of cold water with continuous stirring for 1 hr then left overnight. The precipitate formed was filtered, washed and recrystallized from ethanol.

Results And Discussion

All the synthesis compounds were characterized by elemental analysis, IR, 1NMR, and 13CNMR as following.

1-(4-benzyloxy-2-hydroxy-3-iodophenyl) ethanone (A)
Mass;368.17 ; IR(KBr cm-1): 2870(C-H str. vib.) 3032(-Aromatic C-H),1573,1489, (C=C str.Vib.),879(-C – H o.o.p multi sub. benzene),1280, 1080(C-O-C str.vib), 3634(O-H str.vib), 1620(-C=O str.vib),501(C-I str.vib),;1H NMR 6.44 – 7.77 (s,7H,of the Ar-H) ,13.5 (s,1H, Ar-OH), 5.16 (2H,s, -CH2-O-), 2.5 (3H,s, O=CCH3),;Yield 64.30%;

1-(4-Benzyloxy-2-hydroxy- 3-iodophenyl)-3- phenylprop-2-en-1-one [B1]:
Mass;456.27 IR(KBr cm-1): 3063(-Aromatic C-H),1573, 1489,(C=C str. Vib.),817(-C – H o.o.pmulti sub. benzene),1273, 1072(C-O-C str.vib), 3634(O-H str.vib), 1627(-C=O str.vib),501(C-I str.vib),972(CH=CH bending),;1H NMR:8.0 -7.7 (m,12H, of the Ar-H) ,6.44-6.52 (m, 2H, -CH=CH-), 5.17 (d,2H, -CH2-O-), Yield 57.23%;

1-(4-benzyloxy -2-hydroxy-3-iodophenyl)-3-(4-chlorophenyl) prop-2-en-1-one [B2]:
Mass; 490.72 IR(KBr cm-1): 3032(Aromatic C-H),1573, 1489,(C=C str. Vib.),817(-C – H o.o.p multisub. benzene),1280, 1041(C-O-C str.vib), 3201(O-H str.vib), 1627(-C=O str.vib), 786(C-Cl str.vib) 578(C-Istr.vib),972(CH=CH bending), 1H NMR:8.0 -7.2 (m,12H, of the Ar-H) ,6.44-6.49 (m, 2H, -CH=CH-), 5.16(d,2H, -CH2-O-),; Yield 59.91%;

1-(4-benzyloxy- 2-hydroxy-3-iodophenyl)-3-(3-hydroxyphenyl) prop-2-en-1-one [B3]:
Mass;472.27 IR(KBr cm-1): 3050(Aromatic C-H),1489, 1404,(C=C str. Vib.),817(-C – H o.o.p multisub. benzene),1273, 1080(C-O-C str.vib), 3201(O-H str.vib), 1627(-C=O str.vib), 501(C-I str.vib),964(CH=CHbending), 1H NMR:8.0 -7.7 (m,12H, of the Ar-H) ,6.44-6.52 (m, 2H, -CH=CH-), 5.17 (d,2H, -CH2-O-),; Yield 64.32%;

1-(4-benzyloxy- 2-hydroxy- 3-iodophenyl)-3-(4-methoxyphenyl) prop-2-en-1-one [B4]:
Mass:486.3; IR(KBr cm-1): 3063(Aromatic C-H),1558, 1404,(C=C str. Vib.),825(-C – H o.o.p multisub. benzene),1280, 1072(C-O-C str.vib), 3194(O-H str.vib), 1627(-C=O str.vib), 540(C-I) str.vib),972(CH=CH)bending), 1H NMR:8.0 -7.2 (m,12H, of the Ar-H) ,6.44-6.52 (m, 2H, -CH=CH-), 5.17 (d,2H, -CH2-O-),; Yield 62.81%;

1-(4-benzyloxy -2-hydroxy-3-iodophenyl)-3-(2-chlorophenyl) prop-2-en-1-one [B5]:
Mass; 490.72 IR(KBr cm-1): 3063(Aromatic C-H),1573, 1450,(C=C str. Vib.),864(-C – H o.o.p multisub. benzene),1226, 1049(C-O-C str.vib), 3649(O-H str.vib), 1627(-C=O str.vib), 732(C-Cl str.vib) 509(C-Istr.vib),972(CH=CH bending), 1H NMR:8.0 -7.2 (m,12H, of the Ar-H) ,6.44-6.49 (m, 2H, -CH=CH-), 5.16(d,2H, -CH2-O-),; Yield 58.50%;

1-(4-benzyloxy- 2-hydroxy-3-iodophenyl)-3-(3-hydroxyphenyl) prop-2-en-1-one [B6]:
Mass;472.27 IR(KBr cm-1): 3032(Aromatic C-H),1489, 1404,(C=C str. Vib.),817(-C – H o.o.p multisub. benzene),1219, 1080(C-O-C str.vib), 3518(O-H str.vib), 1620(-C=O str.vib), 501(C-I str.vib),941(CH=CHbending), 1H NMR:8.0 -7.7 (m,12H, of the Ar-H) ,6.44-6.52 (m, 2H, -CH=CH-), 5.17 (d,2H, -CH2-O-),; Yield 59.37%;

1-(4-benzyloxy- 2-hydroxy- 3-iodophenyl)-3-(4-methoxyphenyl) prop-2-en-1-one [B7]:
Mass:486.3; IR(KBr cm-1): 3063(Aromatic C-H),1573, 1489,(C=C str. Vib.),856(-C – H o.o.p multisub. benzene),1280, 1072(C-O-C str.vib), 3194(O-H str.vib), 1627(-

C=O str.vib), 509(C-I) str.vib),972(CH=CH)bending), 1H NMR:8.0 -7.2 (m,12H, of the Ar-H) ,6.44-6.52 (m, 2H, -CH=CH-), 5.17 (d,2H, -CH2-O-),; Yield 53.17%;

1-(4-benzyloxy -2-hydroxy-3-iodophenyl)-3-(4-chlorophenyl) prop-2-en-1-one [B8]:
Mass; 490.72 IR(KBr cm-1): 3032(Aromatic C-H),15723, 1492,(C=C str. Vib.),817(-C – H o.o.p multisub. benzene),1273, 1041(C-O-C str.vib), 3439(O-H str.vib), 1620(-C=O str.vib), 786(C-Cl str.vib) 501(C-Istr.vib),972(CH=CH bending), 1H NMR:8.0 -7.2 (m,12H, of the Ar-H) ,6.44-6.49 (m, 2H, -CH=CH-), 5.16(d,2H, -CH2-O-),; Yield 58.23%;

1-(4-benzyloxy- 2-hydroxy-3-iodophenyl)-3-(3-hydroxyphenyl) prop-2-en-1-one [B9]:
Mass;472.27 IR(KBr cm-1): 3032(Aromatic C-H),1489, 1404,(C=C str. Vib.),879(-C – H o.o.p multisub. benzene),1280, 1080(C-O-C str.vib), 3510(O-H str.vib), 1620(-C=O str.vib), 501(C-I str.vib),966(CH=CHbending), 1H NMR:8.0 -7.7 (m,12H, of the Ar-H) ,6.44-6.52 (m, 2H, -CH=CH-), 5.17 (d,2H, -CH2-O-),; Yield 59.71%;

1-(4-benzyloxy- 2-hydroxy- 3-iodophenyl)-3-(4-methoxyphenyl) prop-2-en-1-one [B10]:
Mass:486.3; IR(KBr cm-1): 3032(Aromatic C-H),1489, 1404,(C=C str. Vib.),815(-C – H o.o.p multisub. benzene),1280, 1041(C-O-C str.vib), 3510(O-H str.vib), 1627(-C=O str.vib), 501(C-I) ,648 (C-I) str.vib),966(CH=CH)bending), 1H NMR:8.0 -7.2 (m,12H, of the Ar-H) ,6.44-6.52 (m, 2H, -CH=CH-), 5.17 (d,2H, -CH2-O-),; Yield 54.33%;

2-Amino-1-(4-Benzyloxy-2-hydroxy -3-iodophenyl)-6-(phenyl)-6H-1, 3-Oxazine [C1]:
Mass;500.23 IR(KBr cm-1): 3032(-Aromatic C-H),1573, 1489,(C=C str. Vib.),825(-C – H o.o.pmulti sub. benzene),1234, 1041(C-O-C str.vib), 3580(O-H str.vib),3580(NH_2(amine) (N-H straching),1627(-N-H (Bending)),1280(-C-N str.vib), 563(C-Istr.vib), ,;1H NMR:7.9 -7.2 (m,12H, of the Ar-H) ,6.3-6.6 (s, CH), 5.2 (d,2H, -CH2-O-),2.5(s, Ar-NH2) Yield 51.67%;

2-Amino-1-(4-Benzyloxy-2-hydroxy -3-iodophenyl)-6-(4-chlorophenyl)-6H-1, 3-Oxazine [C2]:
Mass;534.77 IR(KBr cm-1): 3032(-Aromatic C-H),1573, 1489,(C=C str. Vib.),825(-C – H o.o.pmulti sub. benzene),1172, 1041(C-O-C str.vib), 3510(O-H str.vib),3510(NH_2(amine) (N-H straching),1627(-N-H (Bending)) 1280(-C-N str.vib), 563(C-I str.vib) 786(C-Cl str.vib),1H NMR:7.8 -7.2 (m,12H, of the Ar-H) ,5.2 (s, CH), 5.3 (d,2H, -CH2-O-),2.5(s, Ar-NH2) Yield 56.33%;

2-Amino-1-(4-Benzyloxy-2-hydroxy -3-iodophenyl)-6-(3-hydroxyphenyl)-6H-1, 3-Oxazine [C3]:
Mass;516.33 IR(KBr cm-1): 3032(-Aromatic C-H),1589, 1489,(C=C str. Vib.),828(-C – H o.o.pmulti sub. benzene),1228, 1072(C-O-C str.vib), 3510(O-H str.vib), 3510(NH_2(amine) (N-H straching),1627(-N-H (Bending)),1273(-C-N str.vib), 563(C-I str.vib),1H NMR:8.1 -7.2 (m,12H, of the Ar-H) ,6.3-6.6 (s, -CH), 5.1 (d,2H, -CH2-O-)2.5(s, Ar-NH2), Yield 81.33%;

2-Amino-1-(4-Benzyloxy-2-hydroxy -3-iodophenyl)-6-(4-methoxyphenyl)-6H-1, 3-Oxazine [C4]:
Mass;530.35 IR(KBr cm-1): 3032(-Aromatic C-H),1550, 1489,(C=C str. Vib.),828(-C – H o.o.pmulti sub. benzene),1257, 1033(C-O-C str.vib), 3618(O-H

str.vib),3618(NH_2(amine) (N-H straching),1627(-N-H (Bending)) 1257(-C-Nstr.vib), 563(C-Istr.vib)1H NMR:8.0 -7.2(m,12H, of the Ar-H) ,6.3-6.6 (m, 2H, -CH=CH-), 3.8 (d,2H, -CH2-O-), 2.5(s, Ar-NH2) Yield 65.33%;

2-Amino-1-(4-Benzyloxy-2-hydroxy -3-iodophenyl)-6-(2-chloroyphenyl)-6H-1, 3-Oxazine [C5]:
Mass;534.77 IR(KBr cm-1): 3032(-Aromatic C-H),1550, 1489,(C=C str. Vib.),825(-C – H o.o.pmulti sub. benzene),1188, 1033(C-O-C str.vib), 3510(O-H str.vib),3580(NH_2(amine) (N-H straching),1627(-N-H (Bending)), 1273(-C-N str.vib), 563(C-I str.vib), 740(C-Clstr.vib),1H NMR:7.8 -7.2 (m,12H, of the Ar-H) ,5.2 (s, CH), 5.3 (d,2H, -CH2-O-),2.5(s, Ar-NH2) Yield 56.33%;

2-Amino-1-(4-Benzyloxy-2-hydroxy -3-iodophenyl)-6-(2-hydroxyphenyl)-6H-1, 3-Oxazine [C6]:
Mass;516.33 IR(KBr cm-1): 3047(-Aromatic C-H),1550, 1496,(C=C str. Vib.),810(-C – H o.o.pmulti sub. benzene),1228, 1063(C-O-C str.vib), 3510(O-H str.vib),3510(NH_2(amine) (N-H straching),1627(-N-H (Bending)), 1265(-C-N str.vib), 563(C-Istr.vib),1H NMR:8.1 -7.2 (m,12H, of the Ar-H) ,6.3-6.6 (s, CH), 5.1 (d,2H, -CH2-O-),2.5(s, Ar-NH2) Yield 59.43%;

2-Amino-1-(4-Benzyloxy-2-hydroxy -3-iodophenyl)-6-(2-methoxyphenyl)-6H-1, 3-Oxazine [C7]:
Mass;530.35 IR(KBr cm-1): 3047(-Aromatic C-H),1550, 1496,(C=C str. Vib.),810(-C – H o.o.pmulti sub. benzene),1180, 1041(C-O-C str.vib), 3510(O-H str.vib),3510(NH_2(amine) (N-H straching),1627(-N-H (Bending)), 1265(-C-N str.vib), 563(C-Istr.vib), 1H NMR:8.0 -7.2 (m,12H, of the Ar-H) ,6.3-6.6 (s, CH), 5.2(d,2H, -CH2-O-),2.5(s, Ar-NH2) Yield 55.80%;

2-Amino-1-(4-Benzyloxy-2-hydroxy -3-iodophenyl)-6-(3-chlorophenyl)-6H-1, 3-Oxazine [C8]:
Mass;534.77 IR(KBr cm-1): 3047(-Aromatic C-H),1550, 1489,(C=C str. Vib.),810(-C – H o.o.pmulti sub. benzene),1228, 1049(C-O-C str.vib), 3201(O-H str.vib),3580(NH_2(amine) (N-H straching),1627(-N-H (Bending)), 1273(-C-N str.vib), 740(C-Cl str.vib),563(C-I str.vib),1H NMR:7.8 -7.2 (m,12H, of the Ar-H) ,5.2 (m, 2H, -CH=CH-), 5.3 (d,2H, -CH2-O-), 2.5(s, Ar-NH2) Yield 57.53%;

2-Amino-1-(4-Benzyloxy-2-hydroxy -3-iodophenyl)-6-(4-hydroxyphenyl)-6H-1, 3-Oxazine [C9]:
Mass;516.33IR(KBr cm-1): 3047(-Aromatic C-H),1550, 1489,(C=C str. Vib.),810(-C – H o.o.pmulti sub. benzene),1188, 1041(C-O-C str.vib), 3510(O-H str.vib), 3510(NH_2(amine) (N-H straching),1627(-N-H (Bending)),1273(-C-Nstr.vib), 563(C-I str.vib)1H NMR:8.1 -7.2 (m,12H, of the Ar-H) ,6.3-6.6 (s, CH), 5.1 (d,2H, -CH2-O-), 2.5(s, Ar-NH2) Yield 58.30%;

2-Amino-1-(4-Benzyloxy-2-hydroxy -3-iodophenyl)-6-(3-methoxyphenyl)-6H-1, 3-Oxazine [C10]:
Mass;530.35 IR(KBr cm-1): 3047(-Aromatic C-H),1550, 1496,(C=C str. Vib.),810(-C – H o.o.pmulti sub. benzene),1180, 1041(C-O-C str.vib), 3510(O-H str.vib),3510(NH_2(amine) (N-H straching),1627(-N-H (Bending)), 1265(-C-N str.vib), 563(C-Istr.vib),1H NMR:8.0 -7.2 (m,12H, of the Ar-H) ,6.3-6.6 (s, CH), 5.2 (d,2H, -CH2-O-),2.5(s, Ar-NH2) Yield 56.33%;

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References:

1. P. Anusha and P. Mani Chandrika.; *world. J. Pharm. Pharm. Sci*., 4(11), 885-895(2015).
2. G. R. Kamala and L. Patnaik.; *J. Med. Chem. Drug. Discovery*., 8, 2332(2016).
3. H. Jamal and W. H. Ansari.; *Biol. Med*., 1, 107-115(2009).
4. Y. Zuo.; *Euro. J. Med. Chem*., 50, 393-404(2012).
5. V. Kotra.; *Indian. J. Chem*., 49, 1109-1116(2010).
6. N. S. Parmar.; *Indian. J. Phy. Pharma. Col*., 42, 343-351(1998).
7. H. P. Singh.; *Sch. Res. Lib*., 2, 460-472(2010).
8. F. V. Lauro, D. C. Franciso and L. R. Marcela.; *J. Chem*., 1-9(2014).
9. K. Elumalai, M. A. Ali and M. Elumalai.; *J. Acute. Disease*., 316-321(2013).
10. N. A. Khalaf, K. Ashok, Shakya and A. Al-Othman.; *Turk. J. Biol*., 32, 51-55(2008).
11. Duffine, W. M. Rollo, I. M.; *J. Pharm. Col*., 12, 171(1957).
12. M. E. Kuehne and E. A. Konopke.; *J. Med. Chem*., 5, 257(1962).
13. J. B. Chylinska and Urbanski.; *J. Med. Chem*., 6, 484(1963).
14. L. Y. Hsu and C. H. Lin.; *Heterocycles*., 43, 2687(1996).
15. O. S. Pedersen and E. B. Pedersen.; *Synthesis*., 2000(4), 479-495(2000).
16. A. J. Cocuzza, D. R. Chidester and B. C. Cordova.; *Bioorg. Med.Chem.Lett*., 11, 1177(2001).
17. T. N. Don and D. T. Tran.; *Pharmacol. Pharm*., 2, 282-288(2011).
18. C. M. Bhalgat, M. I. Ali and B. Ramesh.; *Ara. J. Chem*., 1-8(2011).
19. O. V. Singh and H. Han.; *Tetrahydron Lett*., 48, 2345(2007).
20. N. Zonatta and Squizani.; *J. Braz. Chem. Soc*., 16, 1255-1261(2005).
21. Shingare, A. H. Kategaonkar and S. S. Sonar.; *Bull. Korean. Chem.Soc*., 31(6), 1657(2010).

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38 Synthesis and Characterization of Some Novel Chalcone Derivatives

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Abstract

Chalcones possess a diverse pharmacological profile, rendering them excellent structural components for drug discovery and the production of bioactive chemicals.The new substituted chalcone is prepared by the condensation of aromatic aldehydes and aromatic ketone usingClaisen-Schmidt reaction.The characterization of newly synthesized chalcones were established on the basis of spectral data likeFourier transform infrared(FT-IR) and Mass spectrometry.

Key words : Chalcone,pharmacological, condensation

Introduction

The term "Chalcone" was coined by Kostanecki and Tambor (1899). Chalcones,1,3-diphenylprop-2-en-1-ones belong to the flavonoid family and consist of two aromatic rings connected by an aliphatic three-carbon chain α,β-unsaturated carbonyl system. The conjugate double bonds and π-electron system on both benzene rings are present in chalcone.The other names for chalkones are phenyl styryl ketone and benzalacetophenone.The presence of the reactive keto-ethylenic group –CO–CH=CH–, which contributes to their color and biological activity[1,2].

The substituted chalcones are prepared by condensation of aromatic aldehydes and aromatic ketones by Claisen-Schmidt condensation reaction in the presence of aqueous alkaline solutions.A chalcone is a straightforward chemical framework made up of numerous substances that are found in nature.Chalcones are naturally abundant in edible plants, including vegetables, fruits, spices, tea, and other food stuffs[3,4].

In 21st century, natural and synthetic chalcones are significant part of medicinal chemistry because of their various pharmacological activities and characteristics such as antibacterial, anti-inflammatory, analgesic, anticholinergic, antiplatelet, antiulcer, antioxidant,antimalarial, anticancer, antiviral, antileishmanial, antidiabetic, immunomodulatory, etc[5-8]. Chalcones can be used as precursors to obtain various heterocyclic rings through ring closure reactions[9,10]. Examples of heterocyclic compounds synthesized using chalcones include cyanopyridines, pyrazolines, isoxazoles, and pyrimidines[11,12].Chalcone-based heterocycles are numerous, many of which have been shown to be life saving medicinal agents[13-15].

In this study we synthesis verious chalcone from 1-(2-(benzyloxy)-5-bromo-4-methoxyphenyl)ethanone using Claisen-Schmidt condensation reaction. This synthesised compounds were characterized by FT-IR and Mass spectrometry.Furthermore all synthesised compounds will be given for investigating various biological

activities.Also the compounds will be optimise DFT analysis. On an addition to this, different heterocyclic compounds can be synthesized from these chalcones which may give excellent biological activities.

MATERIAL AND METHOD

Analytical-grade chemicals were used in the experiment.The melting points were determined using the open capillary method and were uncorrected. The purity was assessed using thin layer chromatography (TLC). The structures of the obtained compounds were confirmed by Fourier transform infrared (FTIR) and mass spectroscopic methods.

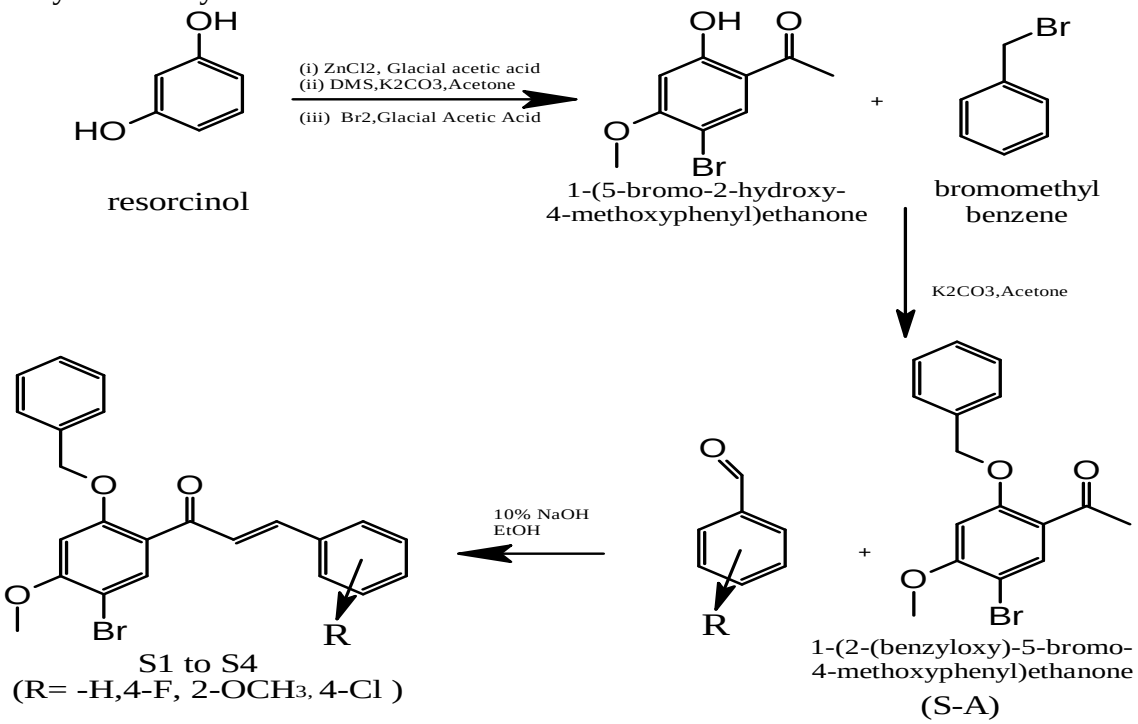
Synthesis

Synthesis of 1-(2-(benzyloxy)-5-bromo-4-methoxyphenyl)ethanone (S-A)

A mixture of 0.1 mole of 1-(5-bromo-2-hydroxy-4-methoxyphenyl)ethanone (which was synthesized from the resorcinol, step -01, scheme-01), 0.1 moles of benzyl bromide, and 0.1 moles of potassium carbonate in 100 ml acetone was stirred at 50-60°C temperature for 7 hours. Then the mixture was allowed to cool at room temperature and quenched with 200ml water. The product was filtered and washed with water. It was recrystallized by ethanol.

Synthesis of 1-(2-(benzyloxy)-5-bromo-4-methoxyphenyl)-3-phenylprop-2-en-1-one (S1 to S4)

0.01 moles of 1-(2-(benzyloxy)-5-bromo-4-methoxyphenyl)ethanone and0.01 moles of substituted aromatic aldehydes were dissolved in 25 ml of ethanol. In this mixture 10% sodium hydroxide solution added dropwise with continuous stirring. The mixture was stirried for 4 hour at room temperature using magnetic stirrer.The reaction was monitored by TLC. After the 4 hour the reaction mixture was poured into 400 ml of cold water and neutralised with 10% HCl. The product was filtered ,washed and recrystallized by ethanol.



Scheme-01

1-(2-(benzyloxy)-5-bromo-4-methoxyphenyl)ethanone (S-A)

M.p.: 124°C ; Yield:72.76%; Mass: m/z 335.3
IR:3059,3029(Aromatic C-H),2939(C-H str.vib.),1588(C=C str.vib), 1273,1054(C-O-C str.vib), 1645(-C=O str.vib),632(C-Br str.vib.)

1-(2-(benzyloxy)-5-bromo-4-methoxyphenyl)-3-phenylprop-2-en-1-one(S-1)
M.p. 98°C ; Yield: 65.89% ; Mass: m/z 423.4
IR: 3062,3025,(Aromatic C-H),2987,2922,2853(C-H str.vib.),1582(C=C str.vib), 1259,1055(C-O-C str.vib.),1648(-C=O str.vib), 971(CH=CH bending),688(C-Br str.vib)

1-(2-(benzyloxy)-5-bromo-4-methoxyphenyl)-3-(4-fluorophenyl)prop-2-en-1-one(S-2)
M.p. 110°C : Yield: 58.95 % ; Mass: m/z 441.0
IR: 3032(Aromatic C-H),2915,2847(C-H str. vib.),1571(C=C str.vib.), 1259,1049(C-O-C str.vib), 1643 (-C=O str.vib), 979(CH=CH bending), 642 (C-Br str.vib)

1-(2-(benzyloxy)-5-bromo-4-methoxyphenyl)-3-(2-methoxyphenyl)prop-2-en-1-one(S-3)
M.p. 115°C; Yield:58.98% ; Mass: m/z 453.3
IR: 3037 (Aromatic C-H),2846 (C-H str. vib.),1588(C=C strVib), 1242,1200(C-O-C str.vib), 1639 (-C=O str.vib), 993 (CH=CH bending), 681(C-Br str.vib)

1-(2-(benzyloxy)-5-bromo-4-methoxyphenyl)-3-(4-chlorophenyl)prop-2-en-1-one(S-4)
M.p. 92°C ; Yield: 68.86% ; Mass: m/z 457.3
IR: 3053 (Aromatic C-H),2913,2846 (C-H str. vib.),1582 (C=C str.vib), 1242,1201(C-O-C str.vib), 1644(-C=O str.vib), 995(CH=CH bending), 673(C-Br str.vib)

Conclusion:

The synthesized compounds were characterized by FT-IRspectroscopy and mass spectroscopy. The results of this study confirmed the formation of the product.Considering these properties, additional compounds can be synthesized and subjected to pharmacological evaluation. These chalcone derivatives may have various biological activities, such as anti-inflammatory,antiviral, antioxidant and antibacterial activities, and may provide a way to synthesize and characterize some new chalcone derivatives.

References:

1. Prasad, Y.R.; Rao, A.L.; Rambabu, R. Synthesis and antimicrobial activity of some chalcone derivatives. J. Chem. 2008, 5, 461-466
2. Mahapatra, D. K., Bharti, S. K., & Asati, V. (2015). Chalcone scaffolds as anti-infective agents: Structural and molecular target perspectives. European journal of medicinal chemistry, 101, 496-524.
3. Alidmat, M. M., Khairuddean, M., Norman, N. M., Asri, A. N. M., Suhaimi, M. H. M., & Sharma, G. (2021). Synthesis, characterization, docking study and biological evaluation of new chalcone, pyrazoline, and pyrimidine

derivatives as potent antimalarial compounds. Arabian Journal of Chemistry, 14(9), 103304.
4. Asiri, A. M., & Khan, S. A. (2012). Synthesis, characterization, and in vitro antibacterial activities of macromolecules derived from bis-chalcone. Journal of Heterocyclic Chemistry, 49(6), 1434-1438.
5. Mellado, M., Espinoza, L., Madrid, A., Mella, J., Chávez-Weisser, E., Diaz, K., & Cuellar, M. (2020). Design, synthesis, antifungal activity, and structure–activity relationship studies of chalcones and hybrid dihydrochromane–chalcones. Molecular diversity, 24, 603-615.
6. Higgs, J., Wasowski, C., Marcos, A., Jukič, M., Paván, C. H., Gobec, S., ... & Marder, M. (2019). Chalcone derivatives: synthesis, in vitro and in vivo evaluation of their anti-anxiety, anti-depression and analgesic effects. Heliyon, 5(3).
7. Asiri, A. M., & Khan, S. A. (2011). Synthesis, characterization and optical properties of mono-and bis-chalcone. Materials Letters, 65(12), 1749-1752.
8. Rozmer, Z., & Perjési, P. (2016). Naturally occurring chalcones and their biological activities. Phytochemistry reviews, 15, 87-120.
9. Karaman, İ., Gezegen, H., Gürdere, M. B., Dingil, A., & Ceylan, M. (2010). Screening of biological activities of a series of chalcone derivatives against human pathogenic microorganisms. Chemistry & biodiversity, 7(2), 400-408.
10. Andrade, J. T., Santos, F. R., Lima, W. G., Sousa, C. D., Oliveira, L. S., Ribeiro, R. I., ... & Ferreira, J. M. (2018). Design, synthesis, biological activity and structure-activity relationship studies of chalcone derivatives as potential anti-Candida agents. The Journal of antibiotics, 71(8), 702-712.
11. Siddiqui, Z. N., Asad, M., & Praveen, S. (2008). Synthesis and biological activity of heterocycles from chalcone. Medicinal Chemistry Research, 17, 318-325.
12. Sharma, P., Kumar, S., Ali, F., Anthal, S., Gupta, V. K., Khan, I. A., ... & Satti, N. K. (2013). Synthesis and biologic activities of some novel heterocyclic chalcone derivatives. Medicinal Chemistry Research, 22, 3969-3983.
13. Dandawate, P., Ahmed, K., Padhye, S., Ahmad, A., & Biersack, B. (2021). Anticancer Active heterocyclic chalcones: recent developments. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 21(5), 558-566.
14. Zhuang, C., Zhang, W., Sheng, C., Zhang, W., Xing, C., & Miao, Z. (2017). Chalcone: a privileged structure in medicinal chemistry. Chemical reviews, 117(12), 7762-7810.
15. Yazdan, S. K., Sagar, G. V., & Shaik, A. B. (2015). Biological and synthetic potentiality of chalcones. J Chem Pharm Res, 7(11), 829-42.

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39 Synthesis, Evaluation of Antimicrobial Activities, and Characterization of Novel Oxazine and Thiazine Compounds Derived

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Abstract

In this ongoing research, our objective is to synthesize Oxazines and Thiazines by employing Chalcones, providing an uncomplicated approach for generating diverse heterocyclic derivatives. These substances show a wide spectrum of biological impact, i.e. analgesic, anti-phlogistic, and anti-convulsant properties. To characterize these newly synthesized compounds, we conducted comprehensive analyses employing techniques such as infrared spectroscopy (IR), proton nuclear magnetic resonance (1H NMR), carbon-13 nuclear magnetic resonance (13C NMR), and mass spectrometry. Additionally, we evaluated the antimicrobial potential of these compounds.

Key Words: Chalcone, Antimicrobial, Oxazine, Thiaz

INTRODUCTION

Oxazines and Thiazines, two classes of organic compounds, exhibit a diverse array of biological activities, making them subjects of significant interest in the field of chemical and pharmaceutical research. In this comprehensive report, the researcher provides an intricate account of the synthesis of a series of chalcones denoted as 1C1 to 1C4. These chalcones were meticulously crafted through Claisen-Schmidt condensation reactions, wherein a variety of substituted benzaldehydes were skillfully combined with acetophenone in the presence of an alkali catalyst, a well-established methodology as outlined in previous scientific investigations [(1-2)].Following the synthesis process, the chalcones underwent a rigorous purification regimen and a thorough characterization that encompassed both physical and spectral analyses. This multifaceted characterization was crucial in confirming the structural integrity and purity of the chalcones.The next pivotal step in this research involved the transformation of the synthesized chalcones into novel Oxazines[(3-5)] and Thiazines[(6-8)], denoted as 1C1OX to 1C4OX and 1C1TH to 1C4TH, respectively. These transformations were achieved through distinct reactions with urea and thiourea, resulting in the formation of compounds with significant pharmacological potential. To elucidate the structures of these newly synthesized compounds, the research team relied on a robust array of analytical techniques, including IR spectroscopy, ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy, and mass spectrometry. These methods provided invaluable insights into the molecular compositions and arrangements of the Oxazines and Thiazines. Furthermore, the study encompassed antimicrobial screening assays [(9-10)] to evaluate the potential bioactivity of these compounds. The screening

assays were conducted with the use of DMSO as the solvent, and the results were meticulously recorded. This phase of the research aimed to assess the compounds' efficacy against various microbial strains, shedding light on their potential as antimicrobial agents. Subsequently, the resulting product underwent a process of recrystalization in ethanol, a crucial step in enhancing the purity and crystalline structure of the synthesized compounds, thereby ensuring their suitability for further studies and potential applications. This research represents a significant contribution to the field, offering a comprehensive exploration of the synthesis, characterization, and potential bioactivity of these newly developed Oxazines and Thiazines.

MATERIAL AND METHODS

In our experimental procedures, we employed analytical-grade chemicals exclusively. The determination of uncorrected melting points was conducted using the open capillary technique, and the assessment of purity was executed via thin-layer chromatography (TLC). For spectroscopic analysis, Fourier-transform infrared (FTIR) spectra and proton nuclear magnetic resonance (1H NMR) spectra were acquired utilizing a Varian 400MHz spectrometer, with CDCl3 serving as the solvent and tetramethylsilane (TMS) as the reference standard. Elemental analysis was carried out using a Thermofinigan Flash EA instrument (Italy), and the sulfur and halogen content was calculated using the carious method. To evaluate antibacterial and antifungal activity, our research team conducted Broth Dilution method tests against a diverse spectrum of both Gram-positive and Gram-negative bacteria, as well as fungi, as detailed in Table 1.

Synthesis of 1-(3-fluoro-4-hydroxyphenyl)ethanone (A)

In carbon di sulphide, a mixture of 2 fluro phenol (0.1mole) and Acetyl chloride (0.1mole) reflux for 30 miniutes in presence of aluminium chloride and it was allowed to stay for around 30 minutes until the hydrochloride gas solution stopped. The carbon di sulphide was then distilled out. Thereafter, the whole Product was steam distilled. The nonvolatile product was 3 fluro 4 hydroxyAcetophenone.

Synthesis of 1-[4-(benzyloxy)-3-fluorophenyl] ethanone(BFE)(1)

In a 100ml flask, we combined 1-(3-fluoro-4-hydroxyphenyl)ethanone (0.1 mol), (bromo methyl) benzene (0.1 mol), and Di pottasium carbonate (0.1 mol) with acetone as the solvent. The reaction mixture was vigorously agitated for 7 hours under reflux conditions at a degree varying from 50^0C to 60°C. After the reaction mixture had cooled to room temperature, 100 ml of cold water was added to quench it. The resulting 1-[4-(benzyloxy)-3-fluorophenyl]ethanone was then filtered and thoroughly rinsed.

Synthesis of 1-(4-(benzyloxy)-3-florophenyl)-4-phenyl)-3-(sub.phenyl) prop-2-en-1-one(1C1to 1C4)

General Procedure:

A solution was prepared by dissolving 1-[4-(benzyloxy)-3-fluorophenyl]ethanone (0.01 mol) and replaced aromatic aldehydes (0.01 mol) in 30 mililiters of C_2H_5OH. In this solution, a 10% caustic soda solution was steadily added. TLC was used to track the reaction's development after the mixture was agitated for 4 hours. Subsequently, the mixture was added into 400 mililitersof icy water were mixed consistently and then counterbalanced using a 1:9 hydrochloric acid and water solution. The reaction

mixture was left to stay as it is for a night in a freezer. The resulting expedite was filtered, cleaned, and subjected to recrystallization via ethanol.

Synthesis of Oxazines and Thiazines ViaChalcones

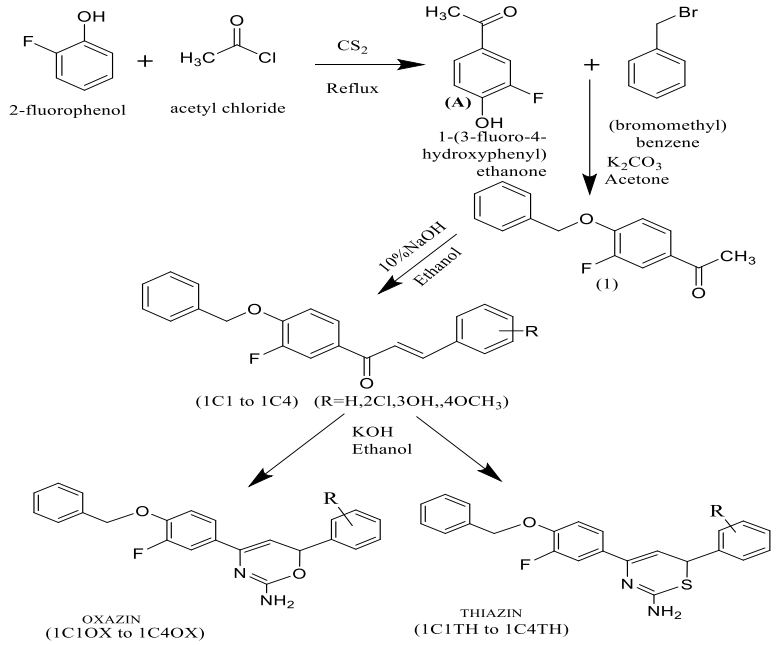
A mixture containing 0.01 mol of 1-(4-(benzyloxy)-3-fluorophenyl)-4-phenyl)-3-(sub. phenyl) prop-2-en-1-one, 1.0 gm of potassium hydroxide (KOH), and 0.01 mol of either Urea or Thiourea was prepared and dissolved in 30 ml of ethanol. This mixture was refluxed in a water bath at a temperature range of 70-80°C for 3 hours, with continuous monitoring using thin-layer chromatography (TLC). Afterward, the solid product that formed was left undisturbed overnight and then purified through recrystallization using ethanol. Notably, The interaction between chalcone and urea led to the production of Oxazine, while the reaction with thiourea produced Thiazine.

RESULTS AND DISCUSSION

Table 1- Result of Antimicrobial activity of oxazine and thiazine derevitives.

CONCENTRATION ON MINIMAL INHIBITION µg/ml]				
	Coding	Escherichia coli	Staphylococcus aureus	Candida albicans
NO	No.	MTCC 443	MTCC 96	MTCC 227
1	1C1OX	125	100	500
2	1C2OX	250	125	1000
3	1C3OX	250	100	>1000
4	1C4OX	125	250	500
5	1C1TH	100	50	500
6	1C2TH	62.5	100	1000
7	1C3TH	100	62.5	>1000
8	1C4TH	50	62.5	>1000
Standard drug	CHLORAMPHENICOL	50	50	
	CIPROFLOXACIN	25	50	
	GRESEOFULVIN			500

Fig-1 Reaction Scheme



Characterization of BFE and chalconederevatives

1-[4-(benzyloxy)-3-fluorophenyl]ethanone(BFE) (1)

Product-66.20%,M.P-103^0-105^0C, LC-MS: m/z244.27,

FT-IR(KBr $Cm^{-1)}$: 2930 (C-H Str.Vib) 3047 (Aromatic C-H) 1511,1455 (C=C strVib), 1081 C-O-C str.vib, 1620 (-C=O str.vib),611 (C-Fstr.vib)

^{1}HNMR(400MHz,$CDCl_3$):5.314(s,2H,O-CH_2-),2.539(s,3H,$COCH_3$), 7.0-7.7(m,8H,Aromatic)

^{13}CNMR(400MHz,$CDCl_3$):26.50(1C,-CO<u>C</u>H_3),71.30(1C,O-CH_2),110-175(12C Aeromatic),196.07(1C,-CO-)

Theoretical for $C_{15}H_{13}FO_2$:C-73.36,H-5.36

Obtained:C-73.33,H-5.34

1-[4-(benzyloxy)-3-fluorophenyl]-4-phenylbut-2-en-1-one (1C1)

Product-61.33% ,M.P-111^0-113^0C, LC-MS: m/z332.37

FT-IR(KBr $Cm^{-1)}$:3060(Aromatic C-H), 1593,1494(C=C strVib), 1043(C-O-C str.vib), 1654(-C=O str.vib), 982(CH=CH bending,761(C-F str.vib)

^{1}HNMR:(400MHz,$CDCl_3$):5.217(s,2H,O-CH_2-), 7.55-8.0(m,2H,CH=CH) 7.0-7.8(m,13H,Aromatic).

^{13}CNMR:71.11(1C,O-CH_2),110-175(18C,Aeromatic),187.758(1C,-CO-),100-150(2C,CH=CH)

Theoretical for $C_{22}H_{17}FO_2$: C-79.50,H-5.16

Obtained:C-79.48,H-5.15

(1-(4-(benzyloxy)-3-fluorophenyl)-3-(2-chlorophenyl)prop-2-en-1-one (1C2)

Product-60.37% ,M.P- 141^0-143^0C, LC-MS: m/z366.82

FT-IR(KBr $Cm^{-1)}$:3028(Aromatic C-H), 1567,1517(C=C strVib), 1655(-C=Ostr.vib),972(CH=CHbending,624(C-F multi sub.Benzene),1039(C-O-Cstr.vib),787(C-Clstr.vib).

^{1}HNMR (400MHz,$CDCl_3$):5.214(s,2H,O-CH_2-), 7.55-8.0(m,2H,CH=CH),7.0-7.8(m,12H,Aromatic).

^{13}CNMR:71.09(1C,O-CH_2),110-175(18C,Aeromatic),163.52(1C,-CO-),100-150(2C,CH=CH).

Theoretical for $C_{22}H_{16}ClFO_2$: C-72.04, H-4.40

Obtained C-72.02, H-4.42

1-[4-(benzyloxy)-3-fluorophenyl]-4-(3-hydroxyphenyl)but-2-en-1-one (1C3)

Product-58.22% ,M.P-160^0-162^0C ,LC-MS: m/z348.37

FT-IR(KBr $Cm^{-1)}$:3056(Aromatic C-H), 1509,1455(C=C strVib), 1061(C-O-C str.vib), 1667(-C=O str.vib), 985(CH=CH bending,636(C-F str.vib),3554(C-OH str.vib).

^{1}HNMR(400MHz,$CDCl_3$):5.219(s,2H,O-CH_2-),7.55-8.0(m,2H,CH=CH),7.0-7.8(m,12H,Aromatic),4.0-7.0(s,1H,Ar-OH)

^{13}CNMR:71.10(1C,O-CH_2),110-175(18C,Aeromatic),187.758(1C,-CO-),100-150(2C,CH=CH).

Theoretical for $C_{22}H_{17}FO_3$: C-75.85, H-4.92

Obtained C-75.83, H-4.93

1-[4-(benzyloxy)-3-fluorophenyl]-4-(4-methoxyphenyl)but-2-en-1-one (1C4)

Product-66.89% ,M.P- 118^0-120^0C, LC-MS: m/z362.40

FT-IR(KBr $Cm^{-1)}$:3063(Aromatic C-H), 1573,1431(C=C strVib), 1021(C-O-C str.vib), 3576(O-H str.vib), 1652(-C=O str.vib), 978(CH=CH bending,728(C-F str.vib)

^{1}HNMR (400MHz,$CDCl_3$:5.242(s,2H,O-CH_2-), 7.55-8.0(m,2H,CH=CH)

7.0-7.8(m,12H,Aromatic),3.859(s,3H,Ar-OCH_3).
^{13}CNMR:71.06(1C,O-CH_2),110-175(18C,Aeromatic),187.720(1C,-CO-),100-150(2C,CH=CH),55.35(1C,O-CH_3)
Theoretical for $C_{22}H_{19}FO_3$: C-76.23, H-5.28. Obtained- C-76.22, H-5.30
Characterisation of Synthesized Oxazines
Synthesis of 4-(4-(benzyloxy)-3-fluorophenyl)-6-phenyl-6H-1,3-oxazin-2-amine (1-C-1 OX)
Product- 55.33% ,M.P- 256^0-258^0C, LC-MS: m/z374FT-IR(KBrCm$^{-1)}$:C-O-C(symmetric)1230,C-O-C(asymmetric)1036,NH_2 (amine) (N-H starching)3435,N-H (bending) 1596, C-N stretching1337
^{1}HNMR(400MHz,$CDCl_3$):2.544(s,2H,-NH_2),5.219(s,2H,-O-CH_2,),7.0-7.8(m,15H,Aromatic).
^{13}CNMR:71.104(1C,–OCH_2),157.601(1C,C-NH_2),100-160(18C,Aeromatic),115-125(1C,-C=N),115-140(2C,C=C).
Theoretical for $C_{23}H_{19}FNO_2$:C-76.65,H-5.31,N-3.89
Obtained;C-76.62,H-5.28,N-3.88
4-(4-(benzyloxy)-3-fluorophenyl)-6-(2-chlorophenyl)-6H-1,3-oxazin-2-amine (1-C-2 OX)
Product-61.11% ,M.P-233^0-2355^0C, LC-MS: m/z 409.58 FT-IR(KBrCm$^{-1)}$:C-O-C(symmetric)1227,C-O-C (asymmetric)1041, ,NH_2(amine)(N-Hstarching)3435,N-H(bending)1528,C-N stretching1337.
^{1}HNMR(400MHz,$CDCl_3$):2.543(s,2H,NH_2),5.127-5.220(s,2H,-OCH_2,),7.07.7(m,14H,Aromatic).
^{13}CNMR(400MHz,$CDCl_3$):71.073(1C,–OCH_2),100-160(18C,Aeromatic),115-125(1C,C=N)157.456(1C,C-NH_2),115-140(2C,C=C).
Theoretical for $C_{23}H_{18}ClFNO_2$:C-69.96,H-4.59,N-3.55.
Obtained:C-69.93,H-4.56,N-3.58
Synthesis of 3-(2-amino-4-(4-(benzyloxy)-3-fluorophenyl)-6H-1,3-oxazin-6-yl)phenol (1-C-3 OX)
Product-56.71%,M.P- 211^0-213^0C, LC-MS: m/z389.03
FT-IR(KBr Cm$^{-1)}$:C-O-C (symmetric)1230, C-O-C (asymmetric)1052,NH_2 (amine) (N-H starching)3435,N-H(bending)1528,C-Nstretching1337.
^{1}HNMR(400MHz,$CDCl_3$):2.543(s,2H,-NH_2),5.235(s,2H,-O-CH_2,),7.0-8.0(m,15HAromatic),4.0-7.0(s,1H,Ar-OH)
^{13}CNMR(400MHz,$CDCl_3$):70.737(1C,–OCH_2),100-160(18C,Aeromatic),115-125(1C,-C=N),157.787(1C,C-NH_2),115-140(2C,C=C).
Theoretical for $C_{23}H_{19}FN_2O_3$:C-64.87,H-4.50,N-6.58
Obtained:C-64.88,H-4.52,N-6.55
4-(4-(benzyloxy)-3-fluorophenyl)-6-(4-methoxyphenyl)-6H-1,3-oxazin-2-amine (1-C-4 OX)
Product- 66.33% ,M.P-266^0-268^0C,LC-MS: m/z405.1
FT-IR(KBr Cm$^{-1)}$:C-O-C (symmetric)1230, C-O-C (asymmetric)1072,NH_2 (amine) (N-H starching)3436,N-H (bending) 1596, C-N stretching1336
^{1}HNMR(400MHz,$CDCl_3$):2.544(s,2H,-NH_2),5.220(s,2H,-O-CH_2,),7.0-7.8(m,14H Aromatic),3.864(s,3H,O-CH_3)

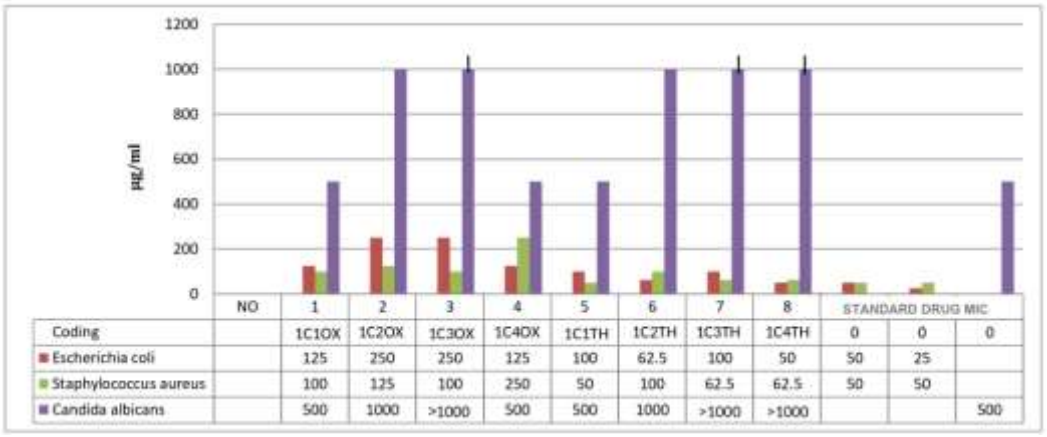
^{13}CNMR(400MHz,$CDCl_3$):70.102(1C,–OCH_2),100-160(18C,Aeromatic),115-125(1C,-C=N),157.913(1C,C-NH_2),55.334(1C,O-CH_3),115-140(2C,C=C)
Theoretical for $C_{24}H_{21}FN_2O_3$:C-71.27,H-5.23,N-6.93.
Obtained: C-71.24,H-5.21,N-6.91
Characterisation of Synthesized Thiazines
4-(4-(benzyloxy)-3-fluorophenyl)-6-phenyl-6H-1,3-thiazin-2-amine(1-C-1 TH)
Product- 63% ,M.P- 211^0-213^0C, LC-MS: m/z390.2
FT-IR(KBr$Cm^{-1)}$:C-O-C(symmetric)1275,C-O-C (asymmetric)1018,NH_2 (amine) (N-H starching)3435,N-H (bending) 1561, C-N stretching1327.
^{1}HNMR(400MHz,$CDCl_3$):2.547(s,2H,-NH_2),5.220(s,2H,-O-CH_2,),7.0-7.9(m,15HAromatic)
^{13}CNMR(400MHz,$CDCl_3$):70.834(1C,–OCH_2),100-160(18C,Aeromatic),115-125(1C,-C=N)157.838(1C,C-NH_2)115-140(2C,C=C).
Theoretical for :$C_{23}H_{19}FN_2OS$: C-70.75,H-4.86,N-7.17,S-8.21
Obtained:C-70.73,H-4.84,N-7.15,S-8.21
4-(4-(benzyloxy)-3-fluorophenyl)-6-(2-chlorophenyl)-6H-1,3-thiazin-2-amine (1-C-2 TH)
Product-61.90% ,M.P- 244^0-246^0C, LC-MS: m/z425.2
FT-IR(KBr $Cm^{-1)}$:C-O-C (symmetric)1248,C-O-C (asymmetric)1025,NH_2 (amine) (N-H starching)3434,N-H (bending) 1607, C-N stretching1330
^{1}HNMR(400MHz,$CDCl_3$):2.543(s,2H,-NH_2),5.220(s,2H,-O-CH_2,),7.0-7.7(m,14HAromatic).
^{13}CNMR(400MHz,$CDCl_3$):71.073(1C,–OCH_2),100-160(18C,Aeromatic),115-125(1C,-C=N),115-140(2C,C=C),157.190 (1C,C-NH_2).
Theoretical for :$C_{23}H_{18}FN_2OS$: C-65.01,H-4.27,N-6.59,S-7.55
Obtained- C-65.00,H-4.25,N-6.56,S-7.53
3-(2-amino-4-(4-(benzyloxy)-3-fluorophenyl)-6H-1,3-thiazin-6-yl)phenol (1-C-3 TH)
Product-55.33 ,M.P- 267^0-269^0C, LC-MS: m/z406.3
FT-IR(KBr$Cm^{-1)}$:C-O-C(symmetric)1276,C-O-C (asymmetric)1079,NH_2 (amine) (N-H starching)3435,N-H (bending) 1667, C-N stretching1276
^{1}HNMR(400MHz,$CDCl_3$):2.544(s,2H,-NH_2),5.220(s,2H,-O-CH_2,),7.0-7.7(m,14H Aromatic),4.0-7.0(s,1H,Ar-OH)
^{13}CNMR(400MHz,$CDCl_3$):71.401(1C,–OCH_2),100-160(18C,Aeromatic),115-125(1C,-C=N),115-140(2C,C=C),157.168 (1C,C-NH_2)
Theoretical for :$C_{23}H_{19}FN_2OS$: C-67.96,H-4.71,N-6.89,S-7.89
Obtained:C-67.98,H-4.73,N-6.90,S-7.91
4-(4-(benzyloxy)-3-fluorophenyl)-6-(4-methoxyphenyl)-6H-1,3-thiazin-2-amine (1-C-4 TH)

Product-68.66% ,M.P- 281^0-283^0C , LC-MS: m/z421.4
FT-IR(KBr $Cm^{-1)}$:C-O-C (symmetric)1247,C-O-C (asymmetric)1037,NH_2 (amine) (N-H starching)3435,N-H (bending) 1583, C-N stretching1269
^{1}HNMR(400MHz,$CDCl_3$):2.544(s,2H,-NH_2),5.221(s,2H,-O-CH_2,),7.0-7.8(m,14HAromatic),3.865(s,3H,O-CH_3)
^{13}CNMR(400MHz,$CDCl_3$):71.064(1C,–OCH_2),100-160(18C,Aeromatic),115-125(1C,-C=N),157.890(1C,C-NH_2) 55.304(1C,O-CH_3),115-140(2C,C=C)
Theoretical for $C_{24}H_{21}FN_2O_2S$: C-68.55,H-5.03,N-4.52,S-7.63.
Obtained:C-68.56,H-5.04,N-4.54,S-7.64

ANTIMICROBIALACTIVITY

We conducted in vitro antimicrobial assessments of the synthesized compounds, namely 1C1OX, 1C2OX, 1C3OX, 1C4OX, 1C1TH, 1C2TH, 1C3TH, and 1C4TH, against bacterial strains S. aureus and E. coli, in addition to the fungal strain C. albicans. Using the Broth Dilution Method, these compounds' antibacterial activity was assessed. Chloramphenicol and Ciprofloxacin served as standard drugs for bacterial strains, while Greseofulvin was used as a reference for fungi.

Fig- 2 Histogram of antimicrobial activity of derivatives



	NO	1	2	3	4	5	6	7	8	STANDARD DRUG MIC		
Coding		1C1OX	1C2OX	1C3OX	1C4OX	1C1TH	1C2TH	1C3TH	1C4TH	0	0	0
Escherichia coli		125	250	250	125	100	62.5	100	50	50	25	
Staphylococcus aureus		100	125	100	250	50	100	62.5	62.5	50	50	
Candida albicans		500	1000	>1000	500	500	1000	>1000	>1000			500

RESULTSANDDISCUSSION

In this article, we have outlined our preliminary endeavors aimed at uncovering novel and potentially active oxazine and thiazine compounds derived from chalcones. Notably, Compound 1C1TH exhibited the most significant inhibitory effect on the growth of gram-positive S. aureus, with a Minimum Inhibitory Concentration (MIC) value as low as 50 µg/mL.were compound 1C3TH and 1C4TH shows moderate MIC value 62.5 µg/mL.The gram negative E.Coli bacterial growth is inhibited by compound 1C1TH at A minimum inhibitory concentration (MIC) of 50 g/mL.were compound 1C3TH,1C4TH shows moderate MIC value 62.5 µg/mL.Oxazines and Thiazines exhibited potent antifungal activity.Among all the synthesized oxazine and thiazine compounds, specifically 1C1OX, 1C1TH, and 1C4OX, these three compounds demonstrated notable antifungal efficacy. They exhibited strong activity against C. albicans fungi at a concentration of 500 µg/mL, comparable to Griseofulvin[11].Newly discovered Oxazines and Thiazines are chemically active components that readily undergo different types of substitutions on their heterocyclic rings, enabling them to display various biological effects. These novel chemical

compounds offer medicinal chemists an opportunity to create and advance Oxazine and Thiazine derivatives as potential lead compounds in drug invention.

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REFERENCES

1. Nowakowska Z., *A review of anti-infective and antiinflammatorychalcones. Eur J. Med Chem*., 2007,*42(2)*, 125-137.
2. Go M L.; Wu X.; Liu X L. *Chalcones: An Update on Cytotoxic and Chemoprotective Properties. Current Medicinal Chemistry*.,2005, *12(4)*, 483-499.
3. Sawant RL.; Bhatia MS.,*Applications of Topliss modified approach in the design and synthesis of GABA-nergic anticonvulsants. Res J Pharm Tech*.,2008,*1*, 273-275.
4. Mathew BP.; Kumar A.; Sharma S.; Shukla.;NathM.,*An eco-friendly synthesis and antimicrobial activities of dihydro-2H-benzo- and naphtho-1,3-oxazine derivatives. Eur J Med Chem*.,2010*45*, 1502-1507.
5. Ouberai M.; Asche C.; Carrez D.; Croisy A.; Dumy P.; DemeunynckM.,*3,4-Dihydro-1H-[1,3]oxazino[4,5-c]acridines as a new family of cytotoxic drugs. Bioorg Med Chem, Lett*.,2006*16*, 4641-4643.
6. Koketsu M.; Tanaka K, Takenaka Y.; Kwong C D.; Ishihara H., *Synthesis of 1,3-Thiazin derivatives and their Evaluationas Potential Antimycobacteruial Agents. Eur J Pharm Sci*., 2002,*15*, 307-310.
7. Bozsing D.; Sohar P.; Gigler G.; Kovacs G.,*Synthesis and Pharmacological Study of New 3,4-Dihydro 2H,6Hpyrimido (2,1-b) (1,3) thiazines. Eur J Med Chem*.,1996,*31*, 663-668.
8. Koketsua M.;Tanakaa K.; Takenakaa Y.; Kwongb C D.;IshiharaaH.,*Synthesis of 1,3-Thiazine derivatives and their Evaluationas Potential Antimycobacterial Agents. Eur J Pharma Sci.*, 2002, *15*, 307–310,
9. Jyothi MV.;VenkateshP.,*Preparation and Biological Evaluation of Novel Pyrimidines from Novel Chalcones.Oriental Journal of Chemistry.*,2012,*28 (3)*,1437-1442.
10. H W Seely.;Van Demark P J.;*Microbes in action. Alaboratory manual of Microbiology, D.B Taraporewala sons, Bombay*.;1975, *55*.
11. S. M. Prajapati et al., *"Synthesis and In Vitro Antibacterial and Antifungal Evaluation of Quinoline Analogue Azetidin and Thiazolidin Derivatives", International Letters of Chemistry, Physics and Astronomy*.;2014, *39*. 195-210.

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17 Limnological study of Malav Talav of Ahmedabad with Reference to Physico-Chemical Properties and Plankton

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Abstract

Water is one of the most important factors for all living beings. Only 3% of total available water on earth is freshwater. Of this less than 1% is in the form which can be utilized by living forms. Urbanization, Industrialization, increase in population causes pollution which affects water bodies and their quality. Therefore, its conservation is very much important. Water quality can be determined by conducting study of physico-chemical and some biological properties. Plankton are one of the pioneer sp. in aquatic food chain. They are very sensitive for their environment hence is considered as an ecological indicator to predict water quality and status of water body. They are indicative of eutrophication process of water body also. Therefore, study of plankton diversity along with its physico-chemical factors was carried out.

Keywords: Malav talav, Plankton, Physico-chemical factors.

INTRODUCTION

Freshwater is one of the basic necessities for the existence and sustenance of life. Urbanization, Industrialization, Population growth is the key factors for over exploitation which causes the water pollution.

Malav talav is located in south western side of Ahmedabad. Initially it was a natural pond. But govt. authorities constructed the pond and made walkway surrounding it inweding a garden on one side of the talav. There is a temple of goddess amba near the pond; the waste water of the temple is discharge into the talav. Some portion of the talav becomes dry during the summer season. The talav covers an area of 16,196 m^2. And its latitude and longitude are $23^0$00'24.52" N and $72^0$32'22.49" E respectively.

The present study involves effects of physico-chemical properties with reference to plankton diversity and population.

MATERIALS AND METHODS

The present study was carried out to assess water quality by using physico-chemical and biological parameters. The water samples were collected separately

by applying standard methods suggested by APHA and other workers. The physico-chemical parameters were estimated in the laboratory by applying standard methods while plankton population was considered as biological parameter.

Plankton:

The total count per ml and per liter was recorded for the study. Plankton are observed carefully under 4x, 10X or 45 X objective lens wherever required. Then with the help of digital camera attached to the microscope, images of plankton were captured. Identification of plankton was done by using various online sources and print sources like book Fresh Water Algae-Prescott, Indian freshwater microalgae –Dr.N. Anand (1998), "Freshwater Biology" by Ward & Whipple, Freshwater zooplankton of India by Battish S. K. (1992).

RESULTS AND DISCUSSION

Physico-chemical parameters play an important role in the productivity of plankton. Physico-chemical parameters are highly important with regard to the occurrence and abundance of plankton species. (Koorosh Jalil Zadeh et al. (2009) The consideration of physico-chemical parameters in the study of limnology is basic in understanding the trophic dynamics of the water body. Each factor does play its individual role but at the same time the final effect is really the result of interaction of all the factors (Hulyal & Kalwal,2008).

In study area the water level was quite low and almost shallow during the winter and summer hence such huge difference in physico-chemical factors was recorded. The pH recorded indicates somewhat alkaline nature of water. There is a high degree of temperature fluctuation is observed. The temperature is slightly towards higher limit especially in winter and summer as water body turns into shallow water body. Usually high value of EC is recorded during monsoon months. Because of the decreasing level of water in summer months, life in water body is badly affected. Most of them may die. Hence salts and ions in the water body are not utilized that will lead to increase in their concentration in water which ultimately responsible for high EC values. The result of the present study also shows that EC recorded higher in monsoon. The decomposition of aquatic plants and animal's ions are released back in water after summer, Vora et al., (1998), Ahluwalia, (1999) and Solanki and Pandit, (2006). High turbidity of water body during monsoon season is due to inflow of clay, silt, and various other pollutants along with rain water from

the surroundings into lake. Similar results were noted by Ansari and Prakash (2000); Solanki (2001); Dagaonkar and Saksena (1992); and Garg et al., (2006).

DO is vital parameter for aquatic organisms. Temperature plays an important role in determining DO in aquatic body (Vasumathi Reddy et al. (2009). It is recorded less in summer and monsoon, compared to winter. As new water enters into water body it also increases the turbidity which reduces the DO in monsoon while, in summer low water level were the main causes for decreasing DO level. BOD: A requirement of oxygen needed for biochemical degradation of organic material. By assessing BOD pollution level of water body can be determined.

Among biological parameters phytoplankton and zooplankton were counted by using Sedwitch Rafter chamber. The total count per ml and per liter was recorded for all the four sites. There are total 33plankton species were recorded. Out of this 27 were phytoplankton while rest zooplankton species. Among phytoplankton Chlorophyceae was more abundant during monsoon season while Bascillariophyceae were recorded maximum during the summer and winter season.

The physico-chemical factors and plankton species recorded during the study were shown in Table 1, 2 and 3 respectively.

Table:1 Seasonal variations in physico-chemical parameters at Malav talav

Sr.No.	Parameters	Season		
		Monsoon Mean ±SE	**Winter Mean ±SE**	**Summer Mean ±SE**
1	Temperature ^{0}C	29.5 ± 0.29	18.5 ± 4.22	23.5 ± 3.93
2	pH	7.60 ± 0.3	7.8 ± 0.11	8.23 ±0.07
3	Turbidity in NTU	4.1 ± 1.89	0.67 ± 0.13	0.38 ± 0.07
4	EC mhos/cm	1.28 ± 0.34	0.99 ± 0.09	0.50 ± 0.11
5	TDS	365 ± 24.67	545 ± 119.55	480 ± 21.22
6	TA	160 ± 17.80	220 v 13.54	217.5 ± 2.50
7	TH	155 ± 22.55	207.5 ± 20.57	202.5 ± 10.31
8	Mg $^{2+}$ hardness	14.03 ± 3.50	15.76 ± 3.74	19.38 ± 4.08
9	Ca^{2+} hardness	39 ± 4.13	42.75 ±4.27	50 ± 5.04
10	Nitrate	0.84 ± 0.07	0.75 ± 0.09	0.77 ± 0.05
11	Phosphate	0.69 ± 0.04	0.7 ± 0.05	0.71 ± 0.06
12	DO	4 ± 0.93	6.04 ± 0.39	5.82 ± 0.90
13	BOD	6.5 ± 1.85	6.25 ± 1.03	6.25 ± 1.11
14	Chloride	68.75 ± 18.97	100 ± 46.86	95 ± 15.55

Table: 2. Seasonal population of Phytoplankton at Malav talav

Plankton		Seasonal population		
Class	**Genera**	**Monsoon**	**Winter**	**Summer**
Cyanophyceae	*Oscillatoria sp.*	10	12	08
	Cylindrospermum sp.	05	03	04
	Gleocapsa sp.	05	12	28
	Nostoc sp.	10	04	05
	Anabaena sp.	08	04	03
	Merismopedia sp.	03	08	02
	Total	**41**	**43**	**50**
Bascillariophyceae	*Gomphonema sp.*	04	08	03
	Cymbella sp.	08	04	03
	Navicula Sp.	23	12	15
	Nitzschia sp.	03	06	04
	Synedra sp.	10	04	06
	Cyclostella sp.	08	03	05
	Total	**56**	**37**	**36**
Chlorophyceae	*Spirogyra sp.*	15	10	02
	Cosmarium sp.	04	06	10
	Pediastrum sp.	06	05	08
	Scenedesmus sp.	10	12	07
	Closterium sp.	08	07	02
	Ankistrodesmus sp.	08	03	00
	Zygnema sp.	10	04	08
	Total	**61**	**47**	**37**
Total plankton count/ml		**158**	**127**	**123**
Total plankton count/L		**94800**	**76200**	**73800**

Table: 3. Seasonal population of Zooplankton at Malav talav

Plankton		**Seasonal population**		
Group	**Genera**	**Monsoon**	**Winter**	**Summer**
Flagellata	*Euglena sp.*	06	08	09
	Total	**06**	**08**	**09**
Copepoda	*Cyclops sp.*	08	05	07
	Diaptomus sp.	12	07	08
	Total	**20**	**12**	**15**
Rotifera	*Brachionus sp.*	14	05	07
	Rotifera sp.	02	06	08
	Total	**16**	**11**	**15**
Total plankton count/ ml		**42**	**31**	**39**
Total plankton count/L		**25200**	**18600**	**23400**

CONCLUSION

During study total 19 phytoplankton species were recorded which represented three different groups. Of this, *chlorophyceae* and *Bascillariophyceae* were most abundant during winter, while *cyanophyceae* is more abundant during summer. Among 05 zooplankton species, copepods and rotifers were more recorded than flagellates. It is indicated it may lead to organic evolution which may cause eutrophication of water body.

REFERENCES

APHA 20th edition *(1998)* Dr. N. Anand *(1998),* 'Indian Fresh water microalgae'.
Ward and Whipple, "Fresh water Biology".
Koorosh Jalilzadeh, Sadanand, M., Yamakanamardi and K. Altaff, (2009). Abundance of copepods from three contrasting lakes of Mysore city, Karnataka state. *J. Aqua.Biol., Vol.24(2):1-9*

Hulyal, S.B. and B.B. Kaliwal *(2008)*. Water quality assessment of Almatti reservoir of Bijapur, Karnataka State, India with special reference to zooplankton

Philipose, M. T., (1967). Chlorococcales*, pp.365, ICAR, New Delhi, India. Poll.Res.,22(1):89-100*

Yeole, S. M. and Patil G. P. (2005) Physico-chemical status of Yedshi lake in relation to water pollution. *J. AquaticBiol.,20(1):41-44.*

Vasumathi Reddy K. Laxmi Prasad, K. Swamy, M. and Ravinder Reddy, T. (2009). Physico-chemical parameters of Pakhal lake of Warangal district, Andhra Pradesh, India. *J.Aqua.Biol.Vol.24(1),2009:77-80.*

Garg, R. K., Rao, R. J., Saksena, D. N., (2006). Assesment of physicochemical water quality of Harsi Reservoir, District Gwalior, Madhya Pradesh. *J. Ecophysiol. Occupat. Health* **6:** 33-40.

Dagaonkar and Saksena, D.N. (1992) Physico-chemical and biological characterization of a tample tank, Kaila Sagar, Gwalior, M.P., *J. Hydrobiol;8(1):*11-19

Solanki and Pandit, B.R. (2006) Tropic status of lentic waters of ponds water of Vadodara, Gujarat state, India. *Int. J. of Biosciences Reporter 4(1):191*-198

Vora, A.B., Ahluwalia, A.A., Gupte, R.Y**.** (1998) Study on water and soil, vegetation, zooplankton and zoo-benthos. In: Environmental Impact Assessment of Sardar Sarovar Project on Nalsarovar Bird Sanctuary, Gujarat Ecological Education and Research (GEER) foundation, Gandhinagar

MALAV TALAV

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19

Communal Roosting Habitat of Some Cormorants Near Hijadiya Pond, Gujarat, India

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ABSTRACT:

The communal roosting of wetland birds is poorly known and even less is known for many species. Roosting of family Phalacrocoracidae birds includes cormorants and shags in whichLittle cormorants (*Phalacrocorax niger*), Great cormorants (*Phalacrocorax carbo)* and Indian cormorants (*Phalacrocorax fuscicollis)* was studied during last two years. We have studied selection of trees for roost, height of trees, roosting population, flock size and arrival and departure time of birds. The investigation found that little cormorants come in larger number in flocks at roosting sites which is up to 15-17 in number. During departure flock size is larger than the arrival time. It was also observed that departure time was comparatively lesser than arrival time in almost all of three birds.

KEY-WORDS: Roosting, Cormorants, waterbirds, Phalacrocoracidae, India

INTRODUCTION

Communal roosting is defined as gathering of more than two species or individuals during day or night time to take rest or sleep (Koli et al., 2019). Bird who are individuals have the choices to use same roosting place repeatedly or live in community, or roost individually while not mandatory to returning the same place every time(Beauchamp, 1999). Roosting birds uses caves, climber plants or trees as their roosting sites. Some use seasonally while others utilize them throughout the year(Jayson, 2008).

A roosting site is a place where not only roosting took place but also contribute in reduces predator attack, increased heat regulation and efficiency of foraging(Beauchamp, 1999). The birds who roost in large number have higher surveillance and reduce predatory pressure. Mixed species roost have higher benefits in reduction from predation and higher feeding efficiency (Eiserer, 1984). Geometry of roosting flocks provides a shielded place for individual in the inner side of the flock while members on the periphery of roosting site act as buffer zone from predation. Some scientists suggested that birds share body heat to control thermoregulations. Apart from that they also used to share information related to feeding places which makes the better feeding ability of individuals (Beauchamp, 1999).

The ecology of wetland birds is prejudice towards breeding and foraging studies while roosting behavior is poorly known for many bird species. Roost sites can be dissimilar from breeding sites (Ogden, 1990), suggesting that lack of information about these create a gap in interpretation of ecology and conservation of wetland birds. Communal roosting is not put to use in many bird groups but it is common in waterbirds (Harrison & Whitehouse, 2011). Communal roosting of waterbirds are documented in several parts of the world including USA (Beauchamp, 1999) Canada (Harrison & Whitehouse, 2011) Sri Lanka (Wickramasinghe & Diwakara, 2017) and India (Jayson, 2008). Several studies were performed on the roosting behavior of waterbirds in cities (Toloa et al., 2017) but due to habitat destruction and loss, waterbirds use to be prone to urbanized area. Most of them are egrets, herons and cormorants prefer to roost in mixed flocks. Roosting behavior has been documented in several waterbirds species (Ali et al., 1987), but is very poorly understood for cormorants.

Our objective was to document cormorants (Little, Great and Indian) roosting ecology focusing on site and selection of trees, height of trees, accompany roosting species, flock sizes and arrival and departure time in an urban area of Hijadiya pond (District- Sabarkantha)

MATERIAL AND METHODS

Study Area

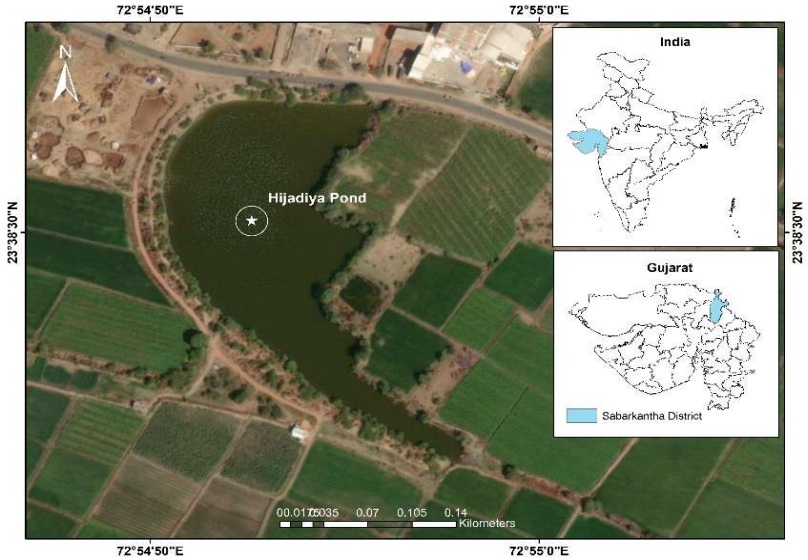

Figure 1. Location and map of the study area of Hijadiya pond, Gujarat, India

The study was conducted near Hijadiya pond (District- Sabarkantha) located near Ilol village on Ilol-Himmatnagar road (Fig.1). The region gets exposure of strong seasonality with distinct winter (Nov-Feb), Summer (March-June) and monsoon (July-Oct). the highest temperature (~42 ^{0}C) was recorded in summer and lowest temperature (~9 ^{0}C) in the winter season. The pond is covered from so many trees in periphery. Roosting of these birds took place in these trees.

Data collection and analysis

The study took place between January 2022 to December 2023. During field visits, roosting birds were identified using Birds of the Indian Subcontinent (Grimmett et al., 2016) using direct observation. Through point count their abundance was recorded using a tally counter. The counting of birds was performed after settled of birds on the roosting trees to avoid counting error. Change in the abundance of birds were noted. Census was conducted at evening time followed by next morning for 2-6 consecutive days.

Tree species used for roosting were identified and height of tree and roosting were measured using laser rangefinder. Number of trees for roosting were also recorded. Sunset and sunrise time were recorded, to determine relationship with peak arrival as well as departure timing of birds. The flock size and flight path weredetermined by recording departing and arriving birds. All the observation made only on clear weather day to avoid a bias. Observation was made from the distance of about 40 m with the help of 10x50 binocular.

Results

Roosting population

Figure 2. Community roosting of cormorantsHijadiya pond, Gujarat, India

In the study area, month wise census conducted by direct count method. The population size of the cormorants varied seasonally as well as annually between 5 to 125 individuals during the study area (Fig.2).

Selection of Roosting tree

Selection of roosting site was mainly dependent on tree species and safety. For the purpose of study, it was observed that cormorants selected tall and bushy trees. All these selected birds showed communal roosting pattern in which roost with other species on same tree but at different canopy and height. Total five tree species were recorded for roosting. Out of these *Acacia nilotica* (Desi baval) was most favorable for cormorants. Cormorants have roosted in close proximity of

aquatic foraging habitat and roosting of trees height ranging from 10.12 m to 19.14 m.

Table 1. Preference of roosting tree species and average height.

Name of tree	Height of tree (m)
Ficus benghalensis (Banyan tree)	19.14
Acacia nilotica (Desi baval)	10.12
Ficus religiosa (Pipal tree)	13.18
Azadirecta indica (Limdo)	18.17
Peltrophorum pterocarpum (Yellow gulmohar)	13.08

Flock size

The flock size is one of the important parameters of roosting ecology. Flock size varies from one to seventeen individuals per flock (table 2). Maximum flock sizeup to seventeen was observed of little cormorant, while great cormorants flock sizes were one to three. It was observed that departure flock sizes were comparatively fewer that arrival flocks.

Table 2. Flock size in roosting species.

Species name	No of individual/flock
Little cormorant	15-17
Great cormorant	1-3
Indian cormorant	2-6

Arrival and Departure time

Roosting of any living organism depend upon sunset and sunrise time. Throughout the year, this times various because it depends on photoperiodism. So, arrival and departure timing for roosting also do not remain constant. The peak arrival time is determined by an average time. Cormorants arrived at roosting place before sunset throughout the year. On an average, little cormorants took 58 min, great cormorants took 55 min while Indian cormorants took 52 min to enter in the roost. Departure time is comparatively lesser in all of three species compare to arrival. Little cormorants took 36 min, great cormorants took 33 min and Indian cormorants took 29 min to depart the roosting place. Month wise data

of cormorants' arrival and departure is described in table 3 and 4. The number of birds recorded during evening were not exactly same as the number of birds recorded in the morning. Average flock size of arrival and departure birds are not corelated. Juveniles arrived early at roosting sites compared to adults. It was also observed that juveniles follow the adults at the time of departure.

Table. 3 Monthly comparison of sunset with starting of arrival of cormorants.

Month	Sunset	Arrival rhythm of birds		
		Little cormorant	Great cormorant	Indian cormorant
Jan	18:13	17:33	17:36	17:39
Feb	18:33	18:06	18:10	18:13
Mar	18:47	18:39	18:42	18:45
Apr	19:03	18:53	18:56	18:59
May	19:13	18:45	18:47	18:50
Jun	19:26	18:24	18:26	18:29
Jul	19:27	17:52	17:55	17:58
Aug	19:11	17:10	17:13	17:16
Sep	18:41	16:49	16:52	16:56
Oct	18:12	16:48	16:51	16:53
Nov	17:53	16:43	16:46	16:48
Dec	17:54	17:15	17:17	17:21

Table. 4 Monthly comparison of sunrise with starting of departure of cormorants.

Month	Sunrise	Departure rhythm of birds		
		Little cormorant	Great cormorant	Indian cormorant
Jan	7:21	6:00	6:04	6:09
Feb	7:11	5:52	5:54	5:58
Mar	6:49	5:46	5:48	5:51
Apr	6:17	5:26	5:28	5:32
May	5:57	5:37	5:40	5:43
Jun	5:52	5:30	5:34	5:37
Jul	6:01	5:46	5:48	5:54
Aug	6:14	5:53	5:56	5:59
Sep	6:25	6:05	6:09	6:13
Oct	6:36	6:28	6:32	6:36
Nov	6:53	6:43	6:45	6:48
Dec	7:13	6:30	6:33	6:36

It was also recorded that roosting birds did not change its place during overnight, as an individual seen during the evening at any particular spot was found on the same spot next morning. Juveniles' birds were seen in the lower canopy of the

roost site while adults observed on different height of canopy. It was also observed that first birds arrived took inside canopy and gradually birds took outer canopy. All the birds taken two or three round of roosting place before entering into roosting site.

Discussion

Cormorants have exclusively their dependency on aquatic foraging habitat all over the world (Cramp et al., 1983). According to Senma (Senma & Acharya, 2010) black headed ibises prefer to roost on ground instead of tree, but in present study cormorants prefer to roost on trees. The cormorants preferred live and unbroken canopy of tall trees. Light duration most certainly influence the roosting time (Vasundriya Ranjana & Shehla, 2019). Arrival and departure time vary depending upon photoperiod (fig 2&3). During evening arrival of the cormorants at intervals happened to take a longer period to fill the roost.

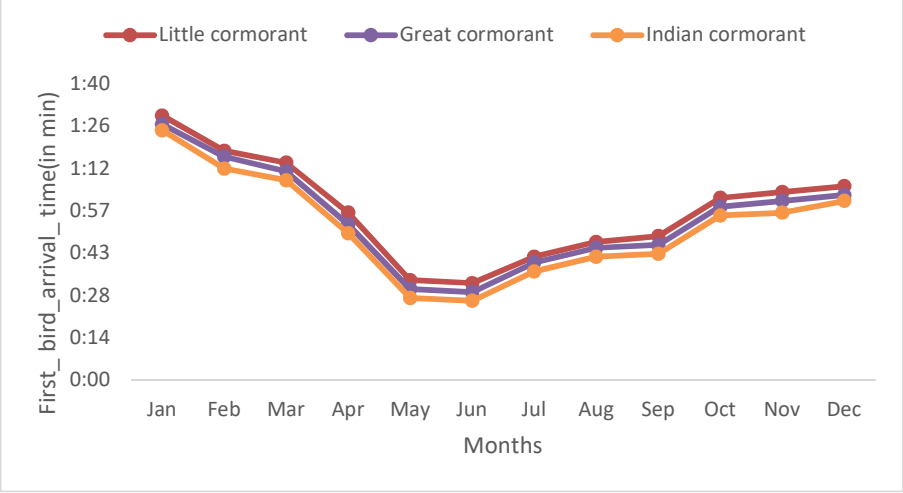

Fig 2. Month wise time taken by cormorants before sunset to arrive at roosting place.

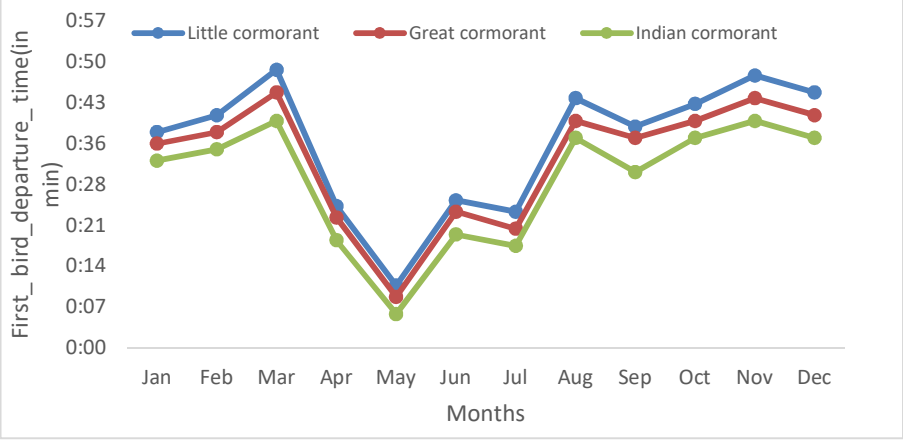

Fig 3. Month wise time taken by cormorants before sunriseto depart roosting place.

During the month of May and June arrival of birds was very late before sunset. The conclusion may be because they spend more time on foraging ground to intake energy to nourish young ones. Pre-roosting gathering has been seen particularly among the species that feed together and roost communally, which serves as a roosting advertisement and acts as an information centre related to the potential food sources.

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References

Ali, S., Ripley, S. D., & Dick, J. H. (1987). *Compact handbook of the birds of India and Pakistan*. Oxford University Press, New Delhi.

Beauchamp, G. (1999). The evolution of communal roosting in birds: Origin and secondary losses. *Behavioral Ecology*, *10*(6), 675–687. https://doi.org/10.1093/beheco/10.6.675

Cramp, S., Simmons editors, K. E. L., Brooks, D. C., Collar, N. J., Dunn, E., Gillmor, R., Hollom, P. A. D., Hudson, R., Nicholson, E. M., & Ogilvie, M. A. (1983). *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the Western Palearctic: 3. Waders to gulls*. Oxford University Press.

Eiserer, L. A. (1984). Communal roosting in birds. *Bird Behavior*, *5*(2–3), 61–80.

Grimmett, R., Inskipp, C., & Inskipp, T. (2016). *Birds of the Indian Subcontinent: India, Pakistan, Sri Lanka, Nepal, Bhutan, Bangladesh and the Maldives*. Bloomsbury Publishing.

Harrison, N. M., & Whitehouse, M. J. (2011). Mixed-species flocks: an example of niche construction? *Animal Behaviour*, *81*(4), 675–682.

Jayson, E. A. (2008). *Factors affecting roosting ecology of birds in Kerala*. India. Technical report. DOI: 10.13140/RG. 2.2. 16637.87521.

Koli, V. K., Chaudhary, S., & Sundar, K. S. G. (2019). Roosting Ecology of Black-Headed Ibis (Threskiornis melanocephalus) in Urban and Rural Areas of Southern Rajasthan, India. *Waterbirds*, *42*(1), 51–60. https://doi.org/10.1675/063.042.0106

Ogden, J. C. (1990). Habitat management guidelines for the wood stork in the southeast region. *Prepared for the US Fish and Wildlife Service*.

Senma, R. C., & Acharya, C. A. (2010). First record: Selection of an electric pole as a roosting site by Black Ibis in North Gujarat region. *Journal of the Bombay Natural History Society*, *107*(1), 59.

Toloa, E., Kibuule, M., Blasberg, D., & Pomeroy, D. (2017). The increasingly urban status of the cattle egret Bubulcus ibis in Uganda, with some observations on its variable breeding seasons and associated species. *Scopus: Journal of East*

African Ornithology, *37*(1), 14–23.

Vasundriya Ranjana, R., & Shehla, I. (2019). "Photoperiod Influence Roosting Time And Other Activity In Common Myna (Acridotheres tristis). *Condor*, *62*, 126–129.

Wickramasinghe, S., & Diwakara, D. (2017). Diversity, Abundance and Conservation of Communal Roosting Waterbirds in Kotuattawala Nature Reserve in Sri Lanka. *An International Journal of Environment and Biodiversity*, *8*(2), 98-103pp.

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25 STUDIES SOME PHYSICO – CHEMICAL PARAMETERS OF 'NAVDI OVARA' SITE OF TAPI RIVER, SURAT, GUJARAT

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Abstract:

The present study was conducted to evaluate the water quality of the Navdi ovara site of Tapi river of Surat city, India. To evaluate the quality of water its physico-chemical analysis is necessary so during the period of April – May-2009 to February – March – 2010 to evaluate of water quality bimonthly water samples were taken. The present study was undertaken to know the water pollution level at Navdi ovara site of Tapi River, therefore some parameters like color, odour, temperature, pH, electrical conductivity, turbidity, alkalinity, total hardness, TDS, dissolved oxygen, nitrate, phosphate, chloride, sodium and potassium were analyzed at regular bimonthly during study period. The result shows various seasonal effects as well as influence of human at site.

Key words: Navdi ovara site, Physico-chemical parameters, Water pollution

Introduction:

The Present study was conducted to find out the variation in the physico - chemical parameters of 'Navdi ovara' site of Tapi River in Surat city of Gujarat, India. The objective of study was to determine physico – chemical properties of 'Navdi ovara' site of Tapi river and to study the level of water pollution as well as seasonal effects on water quality. Due to climate change and anthropogenic pressure the quality of water is day by day much affected, so there is a need to find out what change is occurred in the water quality so the present study was designed to monitor the variations of water quality parameters.

As we know Water is the most precious gift to mankind. Life on earth is not possible without water; it is the soul of nature. Water is a vital resource used for various activities such as drinking, irrigation, fish production, industrial cooling, power generation and many others (Sanjay. S. Sathe *et al*. 2001). Almost 70 % of the earth surface area is covered by water. The available fresh water to man is hardly 0.3 % to 0.5 % of total water available on the earth and therefore its judicious use is imperative. Without the knowledge of waters quality, it is difficult to understand the biological phenomenon fully, because the increasing anthropogenic influence in

recent years in and around aquatic system and their catchment area have attributed to a large extent to deterioration of water quality and dwindling of water bodies leading to their accelerated eutrophication (Bhatt et al. 1999). In today's scenario, unplanned urbanization, rapid industrialization and indiscriminate use of artificial chemicals cause of heavy and varied pollution in aquatic environments leading to deterioration of water quality and depletion of aquatic fauna including fish. Without the knowledge of water chemistry, it is difficult to understand the biological phenomenon fully, because the chemistry of water reveals much about metabolism of the ecosystem and explains the general hydro biological interrelationship (Deshmukh and Ambore, 2006). The quality of water can be assessed by studying by its physical and chemical characteristics. Limnological studies provide a basic understanding of nature and generally help to monitor the environment (forsberg, 1982)

Material and method:

The water samples were collected in glass bottles from each site. Water samples were collected bimonthly during April – May - 2009 to February – Mar - 2010. Water samples were collected during morning hours between 7:30 am to 8:30 am, during study period. For dissolve oxygen water samples were collected in separate BOD bottles and fixed on the spot, the other parameters like pH and temperature was measured by pH meter and thermometer. Rest of the physico–chemical parameters were analyzed according to the 'Standard methods for the examination of water and waste water (APHA-1998) and Environmental analysis of water, soil and air (M. M. Saxena 1998).

Results and discussion:

The result of physico – chemical parameters of 'Navdi ovara site of Tapi river' during April-May-2009 to February – March -2010 are shown in table 01.

Pure water has no color. The presence of humic acids, fulvic acids, metallic ions, suspended matter, phytoplankton, weeds and industrial effluents may give color to natural water. At site the color unit was recorded which is shown in below table 01. During the study period at all sites different odour was noted in different seasons there is no any remarkable odour at site but mostly it was odorless and unobjectionable and some time it was agreeable which is shown in below table 01.

The present study shows the highest water temperature was 31^0c during April – May and Jun - July it means in summer and lowest was 30^0c in December – January and in Feb. - March. The fluctuation in river water temperature usually depends on the season, geographic location, sampling time and temperature of effluents entering the stream (Ahipathy, 2006). The variation in water temperature found in the present investigation may be due to the normal climatic fluctuation and effect of seasons and different times of collection or may be due to the effects of atmospheric temperature as reported by Jayaraman et al., (2003); Tiwari et al. (2004); and Zingade (1981) respectively.

pH is a measure of the acidity and alkalinity of water, expressed in terms of its concentration of hydrogen ions. The pH scale ranges from 0 to 14. A pH, 7 is considered to be neutral. Substances with pH less than 7 are acidic; substances with pH greater than 7 are basic. The pH of water determines the solubility (amount that can be dissolved in the water) and biological availability (amount that can be utilized by aquatic life) of chemical constituents such as nutrients (phosphorus, nitrogen, and carbon) and heavy metals (lead, copper, cadmium, etc.). In present study pH range was 6.7 (December- January) to 7.7 (Oct. – Nov.)

pH was high during winter months and summer months this is because of high photosynthetic activity similar result was noted by Vasumathi Reddy *et al* (2009) and maximum pH in summer was reported in different water bodies by Kushwah (1989), Ingole, S.B. *et al.* (2009). High pH value during summer could be due to the uptake of CO_2 by phytoplankton, Sanathanan, 1976. Abhaykumar *et al*. (1995) also stated that pH profile altered with seasons. The pH of river water was usually showing alkaline character throughout the study period.

Electrical conductivity usually used for indicating the total concentration of ionized constituents of water (Huq and Alam, 2005). Electrical conductivity is the measure of total concentration of dissolved salts in water. When salts dissolve in water, they give off electrically charged ions that conduct electricity. Hard water contains more salts, and therefore more ions, has a high electrical conductivity. Electrical conductivity (EC) estimates the amount of total dissolved salts (TDS), or the total amount of dissolved ions in the water.

Electrical conductivity was recorded between range 464 µmho/cm. (Aug. – Sep.) to 42400 µmho/cm. (Jun– July). The higher conductivity was observed during summer and monsoon season this may be due to the evaporation of water in summer season similar result was noted by Vasumathi Reddy et al. 2009. While several factors influence the conductivity including temperature, ionic mobility and ionic valencies. The higher conductivity values may be due to more concentration of organic matter and also due to human intervention (Claymo, 1983; Koshy and Nayar, 2000; Dakshini and Soni, 1979; Madhavan and Krishnaswami, 1983; Datta *et al,* 1988; Mathew Vergis, 1995; Mathew Koshy, 2005). The site is also near to estuarine region so during high Electrical conductivity was very high.

Turbidity in water is caused by suspended matter like clay, silt, organic matter, phytoplankton and other microscopic organisms. It is an expression of optical property (Tyndall effect) in which light is scattered by the suspended particle present in water. During the present study turbidity show range between 3.2 NTU (Apr. - MaY) to 670 NTU (Dec- Jan). Turbidity is normally increased during and after rainy season and in summer season similar result was reported by Jawale, C. A., *et al*. (2009) Vol.24. Normally turbidity increases after heavy rain because the rain runs along the ground picking up small particles of dirt before emptying into water sources hence increasing turbidity level. Similar work reported by Patil, *et al.* (2009), Govind Balde, Vasumathi Reddy *et al*. (2009). In accordance with general

observation turbidity was recorded high in rainy and after rainy season. The turbidity also depends on the rainfall in monsoon period.

Usually, water shows alkalinity due to presence of salts of weak acids and strong bases. Alkalinity in water is caused due to presence of Carbonates (CO_3^-), Bicarbonates (HCO_3^-) and Hydroxides (OH^-). Alkalinity is the buffering capacity of a water body. It measures the ability of water bodies to neutralize acids and bases thereby maintaining a stable pH.

In present study the minimum alkalinity was recorded 110 mg/l in Aug. – Sep. and highest was 544mg/l in February – March. As per the Bureau of Indian standards the desirable level of total alkalinity for drinking water is below 200 mg/l and permissible level in the absence of alternate source is 600 mg/l (BIS, 1992). At site total alkalinity was high values mostly during summer season while minimum during monsoon season similar observation was recorded by Jawale C.A. (2009), Patil (2009). However most of the water is rich in carbonate, bicarbonate with little concentration of other alkalinity imparting ions (Trivedi and Goel 1984). In this study low alkalinity was recorded in the rainy season this is because the inflow of more rain water in to the river. Sometime high alkalinity was in winter and summer months' similar results were also reported by Mishra *et al.* (1989), Jain *et al*. (1996), Vasumathi (2009); Furhan Iqbal *et al*. (2006).

Hardness can also be defined as water that doesn't produce lather with soap solutions, but produces white precipitate. High levels of total hardness are not considered a health concern. On the contrary, calcium is an important component of cell walls of aquatic plants, and of the bones or shells of aquatic organisms. Magnesium is an essential nutrient for plants, and is a component of chlorophyll. In present study the highest total hardness was 3800 mg/l (Jun – July) and lowest 90 mg/l (August – September). In general, at site total hardness values were high observed in summer and monsoon months and in winter values were low. The monsoon value of hardness was in low level during August indicating influence of dilution of water caused by monsoon water flow (Deka *et al*. 2001) similar observation was made by Koorosh jalizadeh *et al*. 2009.

TDS refer to any minerals, salts, metals, cations or anions dissolved in water. Salts like carbonate, bicarbonates, chlorides, sulphates, phosphates and nitrates of calcium, magnesium, sodium, potassium, iron etc. are dissolved in natural water. The high content of dissolved solids increases the density of water and influences osmoregulation of fresh water organisms. They reduce solubility of gases (like oxygen) and utility of water for drinking, irrigation and industrial purposes.

In the present study minimum TDS was 331 mg/l (August – September)) and maximum 31400 mg/l (Jun – July). TDS gradually increased after monsoon season similar result was noted by Jawale A.K. *et al.* (2009), N. Vijay kumar (2009). The observed variation could be attributed to dilution effect associated with rainy season, while to high evaporation of water during summer (Sitaramassamy -1995). The human interference also contributed to the enrichment of dissolved solids (Devi 1997).

Dissolved oxygen in water is an index of physical and biological processes going on, non- polluted surface water generally saturated with dissolved oxygen. Dissolved oxygen is probably the most crucial and important water quality variable in freshwater body. Dissolved oxygen analysis measures the amount of gaseous oxygen (O_2) dissolved in an aqueous medium. During the study period minimum dissolved oxygen was noted 1.2 mg/l (Apr. - May) and maximum 4.0 mg/l (Feb. - March). Study shows that minimum dissolved oxygen was noted in summer months and usually maximum was noted during winter months, similar results were observed by Patil, Anil R. (2009), Jindal, R et al. (2009). Dissolved oxygen also had an inverse relationship with photoperiod. When the photoperiod was long, the dissolve oxygen value was low and when photoperiod was short, dissolved oxygen value was high. Ali *et al*. (2000) and Chaudhary *et al*. (1990) also arrived at the same conclusion.

Nitrate is the highest oxidized form of nitrogen and in water its most important source is biological oxidation of nitrogenous organic matter of both autochthonous and allochthonous origin. Domestic sewage and agricultural runoff are the chief source of allochthonous nitrogenous organic matter. Metabolic waste of aquatic community and dead organisms add to the autochthonous nitrogenous organic matter. The high concentration of nitrate in water is indicative of pollution.

The present study shows the range of nitrate 0.19 mg/l (February – March) to 1.3 mg/l (Dec. – Jan.). At site the minimum nitrate was recorded in starting of summer months (March) Similar data was recorded by Jawale A.K. *et al.* (2009)., however the data revealed no significant seasonal changes during study period.

Chlorides are salts resulting from the combination of the gas chlorine with a metal. Some common chlorides include sodium chloride (NaCl) and magnesium chloride ($MgCl_2$). Chlorides are troublesome in irrigation water and also harmful to aquatic life (Rajkumar, 2004). Chlorine alone as Cl_2 is highly toxic and it is often used as a disinfectant. In combination with a metal such as sodium it becomes essential for life. Small amounts of chlorides are required for normal cell functions in plant and animal life. During the study period range of chloride was between 140mg/l (Aug. – Sep.) to 10997 mg/l (Jun – July). Minimum chloride was noted during rainy days of August and highest during the monsoon and mostly during summer, during summer chloride values were high and during monsoon it were low recorded it may be due to inflow of rain water and similar results were reported by Koorosh Jalilzadeh *et al*. 2009, Jindal, R. *et al*., N. Vijaykumar *et al*. (2009). Higher chloride content during summer could be due to continuous evaporation of water especially during summer season (Nair *et al*., 1983, Harikantra and Paruleker 1990; Sampathkumar and Kannan 1998; Borase and Bhave, 2001

Sodium compounds naturally end up in water. As was mentioned earlier, sodium stems from rocks and soils. Not only seas, but also rivers and ponds contain significant amounts of sodium. Concentrations however are much lower, depending on geological conditions and wastewater contamination Sodium is a dietary mineral for animals. Plants however hardly contain any sodium.

During the present study minimum sodium was 16 mg/l (Aug. – Sep.) and maximum 5100 mg/l (Jun – July). Above results show minimum value of sodium recorded in monsoon season and maximum during summer and early of monsoon months it may be due to evaporation.

Potassium plays a central role in plant growth, and it often limits it. Potassium from dead plant and animal material is often bound to clay minerals in soils, before it dissolves in water. Potassium is weakly hazardous in water, but it does spread pretty rapidly, because of its relatively high mobility and low transformation potential. In present study lowest potassium was recorded 1.2 mg/l in Oct. – Nov. and in Dec. – Jan. where highest was recorded 95 mg/l in in Feb. – Mar.

Physico – Chemical Parameters of Tapi River - Navdi Ovara Site Apr.- May 2009 to Feb.-Mar. 2010										
Sr. No.	Parameters	April-May	Jun.-July	Aug.-Sep.	Oct.-Nov.	Dec.-Jan.	Feb.-Mar.	Min.	Max.	Avg.
1	Colour	<5	0.7	<0.5	< 0.5	<1.0	<1	-	-	-
2	Odour	Agreeable	Unobjectional	Odorless	Odorless	Odorless	Unobjectional	-	-	-
3	Temperature(ºC)	31	31	30	31	30	30	30	31	30.5
4	pH	6.72	7.43	7.38	7.7	6.7	7.16	6.7	7.7	7.18167
5	EC(µmho/cm)	21150	42400	464	920	6800	13722	464	42400	14242.7
6	Turbidity (NTU)	3.2	6.78	4.32	15.03	670	(not detected)	3.2	670	139.866
7	Alkalinity (mg/l)	186	216	110	206	280	544	110	544	257
8	Total hardness(mg/l)	2740	3800	90	236	1102	1500	90	3800	1578
9	TDS(mg/l)	14700	31400	331	656	4700	9608	331	31400	10232.5
10	Dissolved Oxygen(mg/l)	1.2	3.2	3.2	3.3	3.4	4	1.2	4	3.05
11	Nitrate(mg/l)	1.2	1.19	0.283	0.944	1.3	0.193	0.193	1.3	0.85167
12	Phosphate(mg/l)	1.1	3.41	2.73	4.84	1	6.68	1	6.68	3.29333
13	Chloride(mg/l)	7397	10997	140	149	2900	3961	140	10997	4257.33
14	Sodium(mg/l)	4086	5100	16	90	28	2600	16	5100	1986.67
15	Potassium(mg/l)	20	90	3.4	12	1.2	95	1.2	95	36.9333

(Table-01 Physico – Chemical Parameters of Tapi River – 'Navdi Ovara' Site Apr. - May 2009 to Feb.-Mar. 2010)

Conclusion:

During the study period physico - chemical parameters shows vast variation in salinity, DO, EC, Alklinity, Total hardness, TDS, Turbidity, Chloride, etc. Because many reasons are responsible like, anthropogenic activities at site and it is near to estuarine region so it is highly fluctuated site by every tide then once again it become shallow and site with very complex environment during summer and monsoon season.

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References:

A.P.H.A (1998): "Standard Methods for the Examination of Water and Waste water" 20th ed. American Public Health Association, New York.

Abhaykumar, Zofair, S.M. and Duke, H.C. (1995): "Physico-chemical features of two intertidal sediments of Bhavnagar port". *Poll. Res.* 14(1): **1-5**

Ahipathi M.V., and Puttaiah, E.T. (2006). "Ecological Characteristics of Vrishabhavathi River in Bangalore (India)". Environmental Geology, 49: **1217-1222**.

Ali, M., A. Salam, A. Azeem (2000): "Studies on the effects of seasonal variations on physical and chemical characteristics of mixed water from Rivers Ravi and Chenab at union site in Pakistan", J. Res. B. Z. Univ. Multan, 2: **1-17**

Bhatt L.R., Lacoul. P, Lekhak. H.D. and Jha P.K. (1999): Physico-chemical characteristics and phytoplankton of Taudha Lake, Kathmandu. *Poll Res. 18(4),* **353-358**.

BIS (1992): Indian Standards for drinking water specification, Bureau of Indian Standarda, New Delhi. BIS- 10500: **2-4**

Borase, P.V. and S. K. Bhave (2001): "Seasonal variation in Temperature, Dissolved oxygen, pH and Salinity and their influence on planktons Inaner River Water, Jalgaon, Maharashtra". *Poll. Res*., 20 (1): **79-82**

Chaudhary, A. A. and T. Inayat (1990): "Ecological studies at Kharal lake Dist. Okara", P. J. Zool. Abstract, Series No. **10**

Clymo, R.S. (1983): Peat. In. Micro-swamps, Bog. Fen and moor, Ecosystem of the world. (Ed.) *A.J.P. Gore, Elsevier, Amsterdam*

Dakshini, K.M.M. and J.K. Soni (1979): "Water quality of sewage drains entering Yamuna in Delhi. Indian Journal of Environ. Hlth. 21(4): **354-361**

Datta, N.C., B.K. Bandyopadhya, A. Mujumdar and D. Ahuja (1988): "Hydrological profile of Hooghly sector (Bally to Bandel) of river Ganga" (in: R.K. Trivedi Ed.) Ecology and pollution of Indian River: **129-133**

Deka, R.M., Baruah, B.K. and Kalita, J. (2007): "Physico-chemical characteristics of water of Kapla beell, a freshwater wetland in Barpeta district, Assam", *Poll. Res*.,26 (4):**699-701**

Deshmukh, J.U. and Ambore, N.E. (2006): "Seasonal variation in physical aspects of pollution in Godavari river at Nanded, MS, India". *j.Aqua.Biol., Vol. 21(2)*, **93-96.**

Devi, B.S. (1997): "Present status, Potentialities management and Economics of fisheries of two minor reservoir of Hyderabad". Ph.D. Thesis, Osmania University, Hyderabad

Forsberg, C (1982): Limnological research can improve and reduce the cost of monitoring and control of water quality. *Hydrobiologia*, 86: **143-146.**

Furhan Iqbal, M. Ali (2006): "Seasonal variations of physic-chemical characteristics of River Soan Water at Dhoak Pathan Bridge (Chakwal), Pakistan", *International J. of Agriculture and Biology*, 1560-8530/2004-06-1: **89-92**

Govind Balde (2009): "Studies on physical parameters of reservoir Purna district, Parbhani", M.S.", *The Asian J. of Animal Science*, 4(1): **105:108**

Harikantra, S.N. and A.H. Parulekar (1990): "Population distribution of Meiofauna in relation to some environmental features in a sandy intertidal region of Goa, West cost of India. *Indian J. Mar. Sci*., 18: **259-264**

Huq S. M.I and Alam, M. D. (2005). A Handbook on Analysis of Soil, Plant and Water. BACER-DU, Univerversity of Dhaka, Bangladesh. pp. xxii-246. IS:10500. (1991) Indian standards of Drinking Water Specification, Bureau to Indian Standards (BIS), New Delhi, Indi

Ingole, S.B. (2009): "Water quality studies of Majalgaon dam, Beed District, Maharashtra", *J. Aqua. Biol*., 24 (1), 2009: **71-76**

Ingole, S.B., R.G. Pawle and P.N. Wavde (2009): "water quality studies at majalgaon dam, beed district, Maharashtra", *J. Aqua. Biol.,* 24 (1), 2009: **1-6**

Jain, S.M. (1996): "Seasonal variations in physic-chemical parameters of Halali reservoir of Vidisha district, India". *J. Ecobiol*., 8(3): **181-188**

Jawale, C.A. (2009): "Seasonal variation in different pysico-chemical parameters of second terna makani, reservoir in relation to pisciculture, Osmanabad District, Maharashtra", *J. Aqua. Biol*., 24 (1), 2009: **1-4**

Jayaraman, P. R., ganga Devi, T. and Vasudevan Nayar, T. (2003): "Water quality studies on Karmana River, Thiruvananthpuram district, South Kerala", India. *Poll. Res*., 22(1):**89-100**

Jindal, R. and R. Thakur (2009): "Biodiversity and trophic status in relation to hydrobiological factors of rewalsar wetland" (district Mandi, Himachal Pradesh) *Indian J. Aqua. Biol*., 24 (2), 2009: **50-56**

Koorosh Jalilzadeh, Sadanand, M. Yamkanamardi and K. Altaf (2009): "Physico – chemical parameters of three contrasting lakes of Mysore, Karnataka, India", *J. Aqua. Biol*., 24 (2), 2009: **1-9**

Koshy, M. and T.V. Nayar (2000): "Water quality of river pamba at Kozhencherry". *Poll. Res*., 19(4): **665-668**

Kushwah M.K.S. (1989): "Limnological studies of Harsi dam dist. Dabara (M.P.) with special reference to primary production". Ph.D. Thesis, Jiwaji University, Gwalior

M.M. Saxena (1998): "Environmental Analysis Water, Soil and Air" Agro Botanica, Bikaner.

Mahadavan, Koshy (2005): "A comparative study of the lotic systems of Mavelikara Taluk in Kerala". *Poll. Res*., 24 (4): **809-814**

Mishra P.C. (1989): "Effect of municipal waste on water quality and ecology of Burla Lake near Hirakund dam reservoir. *Poll. Res*., 8(3): **145-152**

N. Vijaykumar (2009): "Studies on Physico- chemical parameters in Thengaithittu estuary, Puducherry, South India", *J. Aqua. Biol.*, 24 (1), **104-106**

Nair, V.R., S.N. Gajabhiye and F.Y. Sayed (1983): "Organic carbon content of zooplankton from the near shore water of Bombay", *Asian J. of Mar. Sci*.12: **183-185**

Patil, Anil R. and Lohar Prakash S. (2009): "Seasonal variations in physic-chemical parameters of river Patalganga, Raigad district, Maharashtra", J. *Aqua. Biol*., 24(2):**1-4**

Rajkumar, S., Velmurugan, P., Shanthi, K., Ayyasamy, P.M., and Lakshmanaperumalasamy, P. (2004). Water Quality of Kodaikanal lake, Tamilnadu inRelation to Physico-Chemical and Bacteriological Characteristics, Capital Publishing Company, Lake, pp. 339-346

Sampathkumar, P. and L. Kannan (1998): "Seasonal variations in Physic-Chemical characteristics in the Tranquebar – Nagapattinam Region, Southeast coast of India" *Poll.Res*., 17(4):**397-402**

Sanjay S Sathe, Suresh A. Khabade and Millind S. hajare (2001): "Hydrobiological studies on two manmade reservoirs from Tasgaon Tahsil (Maharashtra), India". *J. Aqua. Biol*.

Santhanam, R. (1976): "Hydrobiological in Porto Novo waters. "Ecology of phytoplankton indifferent biotopes". Ph.D. Thesis, Annamalai University, India, pp**101**

Tiwari, S., Dixit, S. and Gupta, S. K. (2004): "An evaluation of various Physico-chemical parameters in surface waters of Shahpura lake, Bhopal". *Poll.Res*., 23(4): **829-832**

Trivedi, R.K. and Goel P.K. (1984): "Chemical and Biological Methods for water pollution studies. Envi. Media. Pub. Karad. India PP **215**

Vasumathi Reddy (2009): "Physico-chemical parameters of Pakhal lake of Warangal District, Andhra Pradesh, India", *J. Aqua. Biol.,* 24 (1) **1-4**

Zingade, M.D. (1981): "Base line water quality of river Narmada (Gujarat), India". *J. Mar. Sci*., 10:**161**

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27

A Comparative Study on Microplastic Contamination in Four Brachyuran Crabs along the Bhavnagar Coast

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Abstract

Microplastics (MPs) pollution has been increased drastically worldwide, causing harmful effects on biodiversity. In this study, occurrence of MP contamination in the digestive tracts of four species of marine brachyuran crabs, *Mutata victor* (50 individuals), *Scylla serrata* (100 individuals), *Dotilla blanfordi* (50 individuals) and *Charybdis hellerii* (50 individuals) collected from Sartanpar, Gopnath and Zanzmer Gulf of Khambhat, Gujarat was carried out. The collected samples were dissected, and guts were removed to check for MP contamination. Organic tissue digestion was conducted using a 10% potassium hydroxide solution. Subsequently, a density gradient was established using a supersaturated sodium chloride solution to facilitate the flotation of MP. Filtration of the supernatant was performed utilizing Ashless Whatman filter paper. Each filter paper was scrutinized using a stereomicroscope to quantify the physical characteristics of MPs, including their count, shape, size, and color. The average prevalence of MP contamination was observed to be highest in *Mutata victor* crabs (8.42 ± 3.78 MPs/individual), followed by *Scylla serrata* (5.86 ± 3.23 MPs/individual), *Dotilla blanfordi* (3.46 ± 1.27 MPs/individual), and *Charybdis hellerii* (2.08 ± 0.87 MPs/individual). The classification based on shape indicated a prevalence of MP with a fiber-like structure across four species of crabs. In terms of colour-wise MPs quantification, black coloured MPs were found dominantly than other colours in four crab species. The size of MPs in the range of 1-2mm were

predominantly observed in all four species followed by 2-3mm, 3-4mm and 4-5mm. The polymer composition analysis revealed that the MPs isolated consisted primarily of PET (polyethylene terephthalate) and PU (polyurethane). The results underscored the extent of MP contamination in invertebrates inhabiting the Gulf of Khambhat, raising concerns about the environmental impact. Furthermore, the results will serve as foundational data for designing additional investigations into the eco-toxicological effects of MPs on marine taxa.

Keywords: brachyuran crabs, microplastic pollution, Gulf of Khambhat, KOH, density gradient, Morphometric analysis

Introduction

Marine debris pollution has grown to be a severe issue that affects all members of the global community, including other living things (Patria et al., 2020). The term "plastic" has its roots in the Greek word "plastikos," which signifies its ability to be shaped into various forms. (Rajmohan et al. 2019; Chia et al., 2021). In aquatic ecosystems, plastic contamination was first noted in the 1970s (Dusaucy et al., 2021). Plastic garbage makes up the majority of the waste found in the water (Dias and Lovejoy 2012). Due to heightened plastic production, disposal practices, and human-induced activities, the issue of plastic pollution has evolved into a significant and escalating concern across global ecosystems. (Horton and Barnes, 2020). According to Crawford and Quinn (2017), 90% of marine trash is made of plastic, and 80% of that plastic comes from sources on land. The correlation between land-based sources and both quickly expanding populations and a lack of waste disposal infrastructure is the cause of the link. (Jambeck et al., 2015; Sanchez et al., 2022). Plastic is regarded as having an adverse effect on food security, socioeconomic well-being, and the environment globally (Botterell et al., 2019; Walkinshaw et al., 2020; Sanchez et al., 2022).

Accumulated plastic in the marine environment undergoes degradation and fragmentation processes, leading to its conversion into a variety of sizes. The range of particles between 1μm to 5 mm are classified as microplastics (MPs). MPs can be generated at the microscale and are categorized as either primary or secondary, depending on their origin and breakdown processes in the

environment (Crawford and Quinn, 2017; Watts et al., 2014). MPs manifest in various forms such as pellets, films, foam, fragments, and microfibers. (Lozano et al., 2021; Rabari et al., 2023a). According to Rochman (2015), The physical and chemical assessment of MPs, including parameters such as size, shape, color, and polymer composition, is vital for promptly identifying the primary sources of contamination, understanding wildlife exposure, and determining the eventual fate of MPs in the environment. (Rodríguez-Seijo and Pereira, 2017; Sanchez et al., 2022).

MP contamination has been observed in various marine organisms, spanning amphipods (Iannilli et al., 2019), coral (Patterson et al., 2020), sea cucumbers (Mohsen et al., 2019), mussels (Hariharan et al., 2021), prawns (Daniel et al., 2021), crabs (Waddell et al., 2020), and fish (Munno et al., 2022). MP contamination has been reported in various crab species, including *Lithodes santolla* (Andrade and Ovando, 2017), *Emerita analoga* (Horn et al., 2019), *Pleuroncodes planipes* (Choy et al., 2019), and *Callinectes sapidus* (Waddell et al., 2020). MPs are known to negatively impact an organism's ability to grow and develop in a number of ways. According to Wright and Kelly (2017), MPs could induce oxidative stress and cellular damage in living organisms. Moreover, several reports have underscored the function of MPs as carriers for diverse harmful substances, such as infections, heavy metals, dyes, and pigments naturally associated with the surface of the MPs.

Gujarat has a 1,600-kilometer coastline that hugs rich marine biodiversity including crabs. The coastal regions of Gujarat have documented a total of 163 crab species, encompassing 93 genera and 29 families. (Trivedi et al., 2015, 2018; Gosavi et al., 2021). Nevertheless, there have been only two studies conducted to assess the prevalence of MP contamination in commercially significant crabs, namely *P. segnis* and *P. sanguinolentus*, sourced the Saurashtra coast and Gulf of Kachchh (Rabari et al., 2023a; 2023c). Hence, there is a critical need to evaluate the degree of MP contamination in marine crab species situated in the Gulf of Khambhat. The main goal of this study was to evaluate the extent of

MP contamination in four brachyuran crab species (*Mutata victor, Scylla serrata, Dotilla blanfordi, and Charybdis hellerii)* along the Bhavnagar Coast.

2. Material and Methods

2.1 Study area and sample collection

The study was conducted at three main fishing harbors (Sartanpar, Gopnath, and Zanzmer) along the Bhavnagar Coast, Gujarat, India, during from March 2023. **(Figure 1)**. Sartanpar is located at (21°17' 56.0904'' N,72° 6' 22.3344'' E) Bhavnagar district of Gujarat State. Gopnath is situated at (21°14′52.4436" N, 72°04′ 20.8272″E) Bhavnagar district of Gujrat State. Zanzmer is situated at (21°10'59.3"N, 72°03'57.9"E) Bhavnagar district of Gujrat State. This led to the establishment of multiple seafood companies and processing facilities, which export a wide range of high-quality meals to Southeast Asia, Japan, Europe, and the USA. A collective total of 250 crab specimens was obtained from each site, including 25 males and 25 females of *Mutata Victor* (Fabricius, 1781), 50 males and 50 females of *Scylla serrata* (Forsskal, 1775), 25 males and 25 females of *Charybdis hellerii* (Milne-Edwards, 1867), and 25 males and 25 females of *Dotilla blanfordi* (Alcock, 1900). The gathered samples were immediately placed in an icebox to prevent any degradation of the tissues. (Daniel et al., 2021) **(Figure 2)**. The gender of a crab, whether male or female, can be distinguished by examining the shape of its abdomen. Male abdomens are usually pointed and narrow, like a "V" shape. In comparison to the male crab, the female crab's abdomen is rounder and broader. It's commonly called the "U" shaped abdomen. Following collection, the samples were promptly transported to a laboratory for further examination.

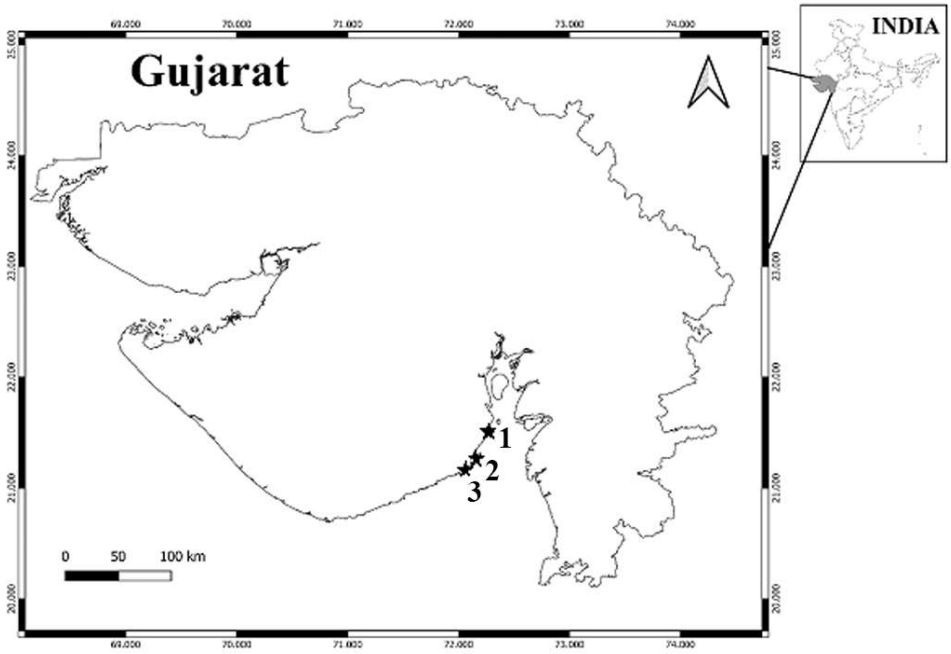

Figure:1 Locations of Sampling stations (1. Sartanpar, 2. Gopnath, 3. Zanzmer).

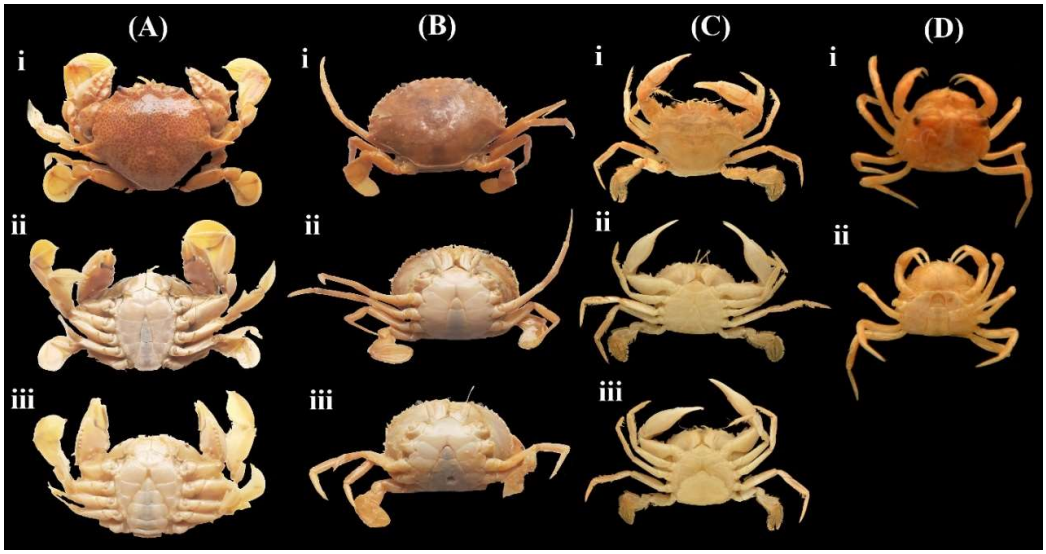

Figure:2 Collected crab species (A: *Mutata victor* (Fabricius,1781), B: *Scylla serrata* (Forsskal, 1775), C: *Charybdis hellerii* (Milne-Edwards,1867), and D: *Dotilla blanfordi* (Alcock,1900): i- dorsal view, ii- male ventral view, and iii- female ventral view).

2.2. Extraction, identification, and quantification of MP

The collected samples were prewashed in the laboratory using double-distilled water to eliminate any potential contaminants from the crab surfaces. Later, measurements of the crabs' morphological characteristics, such as their overall weight, carapace width, and length, were made. Following that, the digestive tracts of each crab were dissected, and they were individually placed into distinct beakers. Each beaker was filled with a 10% KOH solution and exposed to heating at 60 °C in a hot air oven until complete breakdown of all organic tissues was accomplished. (Hara et al., 2020). As per Rabari et al. (2023a), MPs were isolated by buoying them in a hyper-saline NaCl solution (1.2 kg NaCl L-1). Filtration of the supernatant solution was carried out using ashless Whatman filter paper (Grade No. 41, pore size: 20µm). Following the filtration, Petri dishes were placed on top of the filter papers, and they were allowed to dry at room temperature. The physical properties of MPs, such as size, colour, and shape, were noted as each filter paper was examined under a zoom stereomicroscope.

Polymer identification of the extracted MPs was conducted at the CIMF Laboratory, Department of Chemistry, HNGU, Patan, utilizing ATR-FTIR (Bruker-Alpha). Polymer identification was conducted on 10% of the selected samples, representing distinct classes of extracted MPs, following the protocols outlined by Daniel et al. (2020) and Rabari et al. (2023b). The obtained spectra were compared with known primary and secondary MP libraries (FLOPP and FLOPP-e; n = 762 spectra) using the FTIR Essential software, which includes the library developed by De Frond et al. (2021). According to Xu et al. (2020), Rabari et al. (2022) have noticed that spectral matches with primary plastic polymers may be influenced by fragmentation and biofouling. As a result, spectral matches more than 70% were classified as MPs.

2.3. Contamination control

Throughout the process of sample collection and laboratory analysis, precautionary measures were implemented to prevent any potential contamination. Prior to dissection, the collected samples were cleaned and shielded with aluminum foil. Milli Q water was used to clean a metal tray and stainless-steel utensils. For every set of sample analysis, three filter papers were maintained as controls. The whole laboratory work was carried out in a secluded location with little foot traffic in or der to further reduce environmental contamination (Rabari et al., 2023a). To confirm that the retrieved MPs were present, the hot needle method was used. Additionally, MPs were identified through ATR-FTIR analysis. Concurrently, three salt-free blank samples were analyzed to address potential MP contamination arising from sample preparation. No MPs recorded in blanks.

3. Result and discussion

3.1. MPs abundance

In this research, the contamination of MPs was evaluated in the gut of four brachyuran crab species, namely *M. victor, S. serrata*, *D. blanfordi*, and *C. hellerii*, collected from three sample sites along the Bhavnagar Coast in Gujarat. A total of 421, 586, 173 ,104 MPs were found in the guts of *Mutata victor*, followed by *Scylla serrata*, *Dotilla blanfordi* and *Charybdis hellerii*. Crabs *M. victor* (8.42 ± 3.78 MPs/individual) had the highest average abundance of MP contamination, followed by *S. serrata* (5.86 ± 3.23 MPs/individual), *D. blanfordi* (3.46 ± 1.27 MPs/individual), and *C. hellerii* (2.08 ± 0.87 MPs/individual). **(Figure 3)**. MPs showed significant differences in average abundance between species ($\chi 2$ = 47.49, $p < 0.001$, df = 169). Similarly, Watts et al. (2014) reported that in the crab *Carcinus maenas,* the GIT was identified as the initial route of MP entrance. Conversely, it was discovered that females were more likely to consume MPs (Kleawkla, 2019). Males may be able to consume more MP than females because they engage in more energy-intensive and demanding tasks, like reproduction. Sartanpar had the highest average abundance of MP contamination (7.14 ± 1.81 MPs/individual), followed by Gopnath (3.46 ± 1.27 MPs/individual) and Zanzmer (2.08 ± 0.87 MPs/individual). **(Figure 4)**. MPs varied significantly in

average abundance between study locations ($\chi 2 = 31.68$, $p < 0.001$, $df = 169$). According to Horn et al. (2019), Differences in anthropogenic activity levels at the study sites could be a contributing factor to the variations in MP contamination observed in crabs. MP pollution was found in higher concentrations in urbanized areas that were marked by increased human activities and poor management of plastic trash. (Vaid et al., 2021; Rabari et al., 2022).

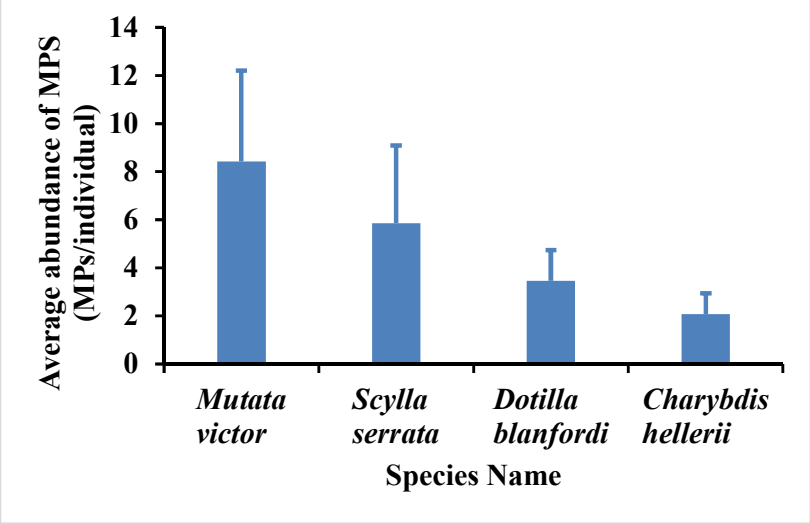

Figure 3. Abundance (Mean ± SD) of MPs (MPs/individual) between species.

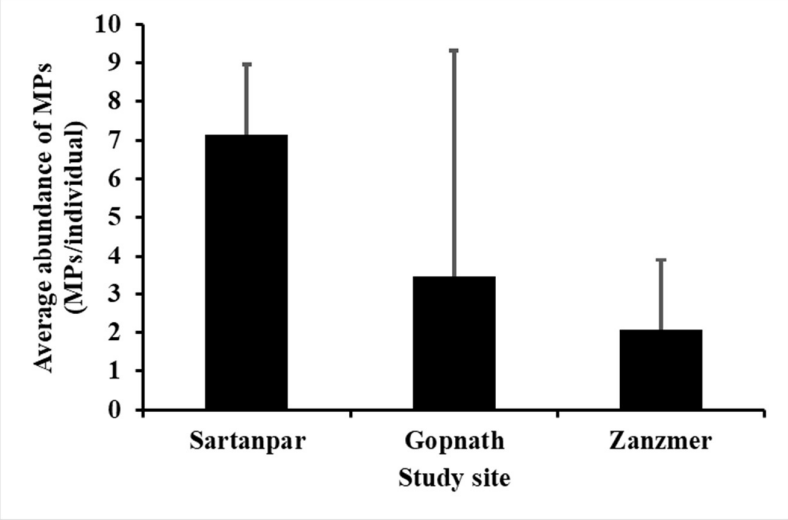

Figure 4. Abundance of MPs (MPs/individual) between study sites.

3.2. Physical characterization of microplastics

3.2.1. Shape of microplastics

MPs that were extracted were characterized by their physical attributes, such as size, colour, and shape. Microfiber emerged as the predominant shape of MPs in *Mutata victor, Scylla serrata, Dotilla blanfordi*, and *Charybdis hellerii*, as indicated by the percentage composition analysis. A stereomicroscope was used to acquire pictures of the retrieved MPs **(Figure 5)**. Studies on MPs in *Carcinus aestuarii* (Piarulli et al., 2019), *Pachygrapsus transversus* (de Barros and dos Santos Calado, 2020), and *Leptuca festae* (Villegas et al., 2021) all showed consistent results, emphasizing fibers as the most common shape. The introduction of fibers into the marine environment may occur through sources such as fishing nets and wastewater discharge. (Feng et al., 2019). Additionally, investigations into both terrestrial and marine activities along beaches have indicated potential influences on the forms of MPs (Browne et al., 2008; Rabari et al., 2023b).

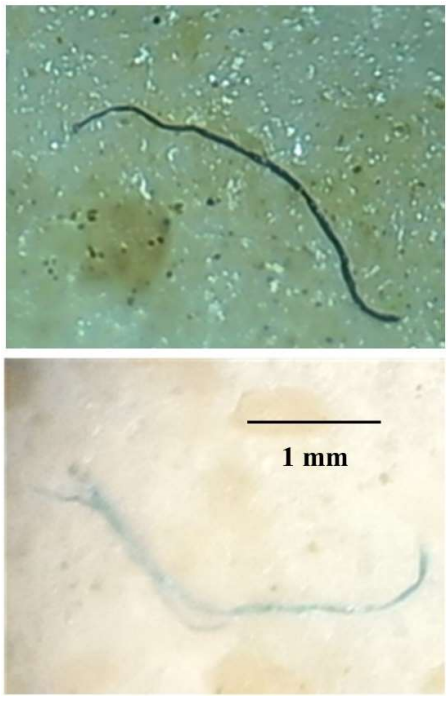

Figure 5: Representative photographs for microfiber.

3.2.2. Colour of MPs

MPs were categorised according to colour, it was found that black-colored particles predominated in every study site, followed by green, red, and blue. **(Figure 6)**. The maritime environment's prevalence of coloured MPs may be caused by blue or black fishing nets. (Rabari et al., 2022). Wright et al. (2013) discovered that MPs with blue and black colors can fool marine creatures into mistakenly consuming them because they resemble their prey.

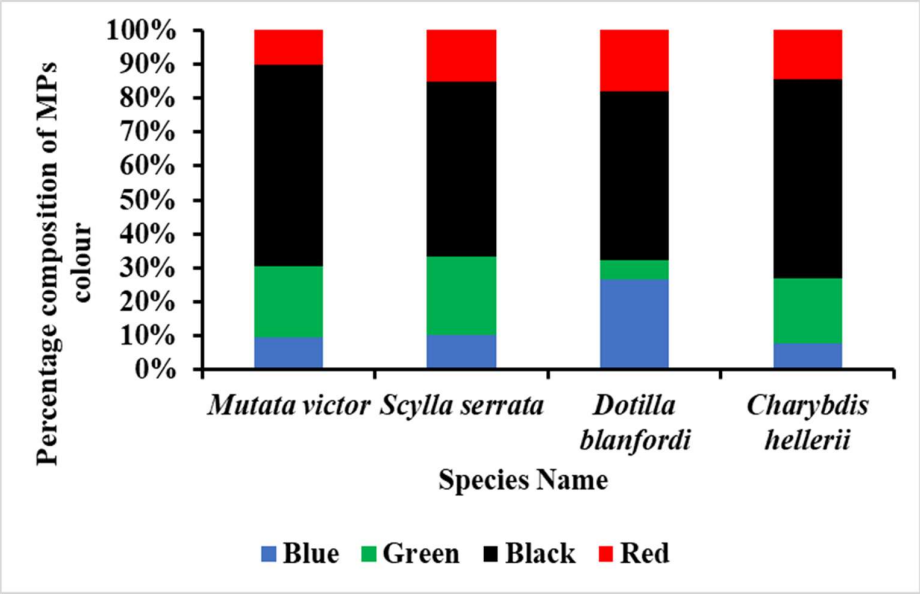

Figure 6: Percentage Composition of MP colours found in four crab species.

3.2.3. Size of MPs

The size-based classification of MPs, the 1–2 mm size was found predominant throughout the all study sites, followed by 2–3 mm, 3–4 mm, and 4–5 mm. **(Figure 7)**. D'Costa (2022) asserts that depending on a species' feeding habits, level of MPs contamination can be varied. The identification of different-sized MPs in crabs' gastrointestinal tracts (GITs) may be a sign that bigger plastic items have fragmented in the marine environment. (Rabari et al., 2023a). Smaller-sized MPs have the potential to cause detrimental effects on the biota because of their greater surface area, which makes them more likely to acquire sticky pollutants. (Robin et al., 2020).

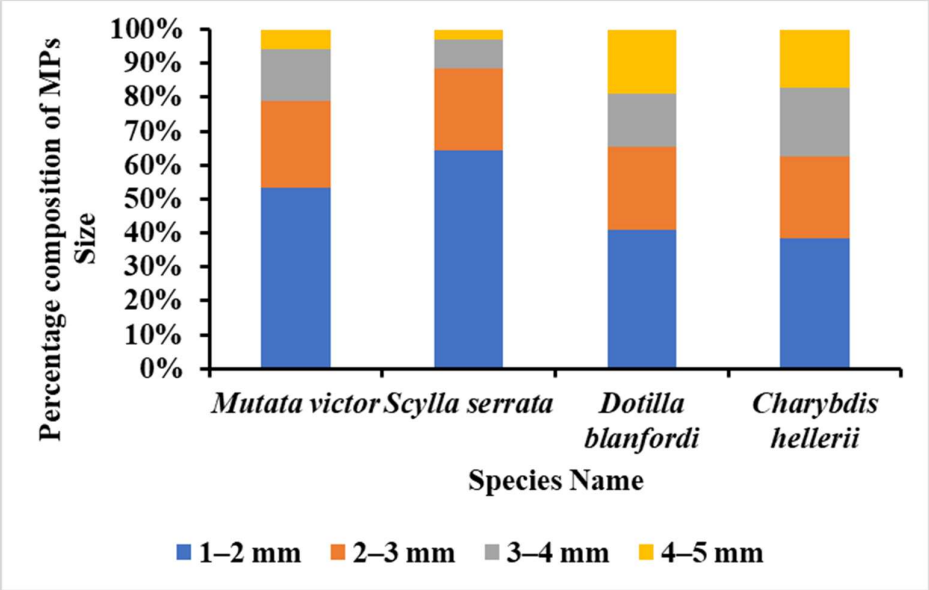

Figure 7: Percentage Composition of MP size-classes found in four crab species.

3.2.4. Polymer composition of MPs

ATR-FTIR was used to determine the isolated MPs' polymer composition. Using libraries of known plastic materials, obtained spectra were compared to estimate the polymer compositions of MPs, which turned out to be polyethylene tetrathionate (PET) and polyurethane (PU). **(Figure 8)**. The sources of extracted MPs in marine ecosystems can be predicted with the help of chemical identification. (Rabari et al., 2023b). PET can be used to packaging materials, plastics containers and fishing products (Rabari et al., 2023a). According to Zia et al. (2007), PU is utilized in adhesives and marine equipment. The current study emphasized the presence of MP contamination in four brachyuran crab species along the Bhavnagar Coast in the state of Gujarat, India. According to Rabari et al. (2022) and Song et al. (2023) MP buildup in the marine biota may have detrimental impacts on species, including starvation, growth suppression, aberrant reproduction, gastrointestinal tract blockage, and mortality. Additionally, humans and higher taxa have the ability to biomagnified accumulated MPs, indicating a concern to food safety.

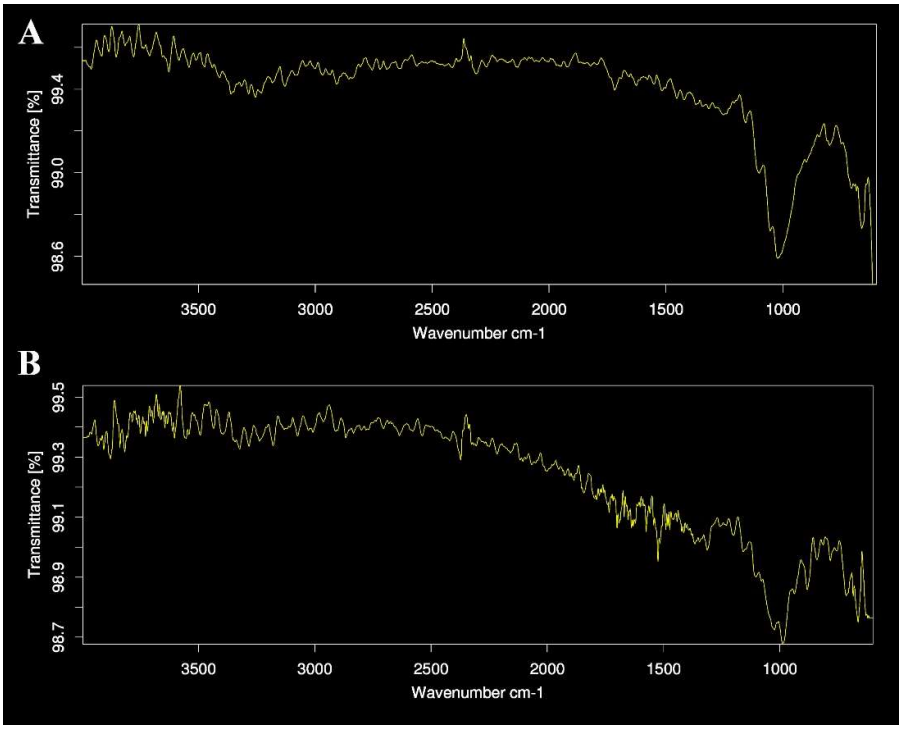

Figure 8: ATR-FTIR spectra of MPs: A) PET, and B) PU.

Conclusion

The current study examined the contamination of MPs in four brachyuran crab species along the Bhavnagar Coast. The mean abundance of MP contamination was higher in *Mutata victor* crab, followed *by Scylla serrata*, *Dotilla blanfordi*, and *Charybdis hellerii.* Microfibers were the predominant form observed at each research site. Shape, size, and colour polymorphism were discovered through physical characterization of MPs. The polymer compositions of PET and PU were identified by the chemical analysis of the isolated MPs. The current investigation has offered initial insights into the concentration of MPs, providing foundational knowledge that can be instrumental in designing studies to assess the ecotoxicological effects of MPs on marine biota. Moreover, the study recommended the need for effective plastic pollution mitigation action to achieve the clean coast concept in marine eco-systems of India.

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References

Aguirre-Sanchez, A., Purca, S., & Indacochea, A. G. (2022). Microplastic presence in the mangrove crab Ucides occidentalis (Brachyura: Ocypodidae) (Ortmann, 1897) derived from local markets in Tumbes, Peru, *Air Soil and Water Research*, *15*, 1–12.

Andrade, C., & Ovando, F. (2017). First record of microplastics in stomach content of the southern king crab Lithodes santolla (Anomura: Lithodidae), Nassau bay, Cape Horn, Chile. *Anales Del Instituto de La Patagonia*, *45*(3), 59–65. https://doi.org/10.4067/S0718-686X2017000300059

Botterell, Z. L. R., Beaumont, N., Dorrington, T., Steinke, M., Thompson, R. C., & Lindeque, P. K. (2019). Bioavailability and effects of microplastics on marine zooplankton: A review. *Environmental Pollution*, *245*(245), 98–110. https://doi.org/10.1016/j.envpol.2018.10.065

Browne, M. A., Dissanayake, A., Galloway, T. S., Lowe, D. M., & Thompson, R. C. (2008). Ingested microscopic plastic translocate to the circulatory system of the mussel, Mytilus edulis(L.). *Environmental Science & Technology*, *42*(13), 5026–5031. https://doi.org/10.1021/es800249a

Chia, R. W., Lee, J.-Y., Kim, H., & Jang, J. (2021). Microplastic pollution in soil and groundwater: a review. *Environmental Chemistry Letters*, *19*(6), 4211–4224. https://doi.org/10.1007/s10311-021-01297-6

Choy, C. A., Robison, B. H., Gagne, T. O., Erwin, B., Firl, E., Halden, R. U., Hamilton, J. A., Katija, K., Lisin, S. E., Rolsky, C., & S. Van Houtan, K. (2019). The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. *Scientific Reports*, *9*(1), 7843. https://doi.org/10.1038/s41598-019-44117-2

Crawford, C. B., & Quinn, B. (2017). *Microplastic Pollutants* (1st ed.). Elsevier. https://doi.org/10.1016/c2015-0-04315-5

D costa, A. H. (2022). Microplastics in decapod crustaceans: accumulation, toxicity and impacts, a review. *Sci. Total Environ.* , 154963. https://doi.org/10.1016/j

Daniel, D. B., Ashraf, P. M., Thomas, S. N., & Thomas, K. T. (2020). Abundance, characteristics and seasonal variation of microplastics in Indian white shrimps (Fenneropenaeus indicus) from coastal waters off Cochin, Kerala, India. *Sci. Total Environ*, *737*, 139839. https://doi.org/10.1016/j

Daniel, D. B., Ashraf, P. M., Thomas, S. N., & Thomson, K. T. (2021). Microplastics in the edible tissues of shellfishes sold for human consumption. *Chemosphere*, *264*(pt2), 128554. https://doi.org/10.1016/j.

De Barros, M. S. F., & Dos Santos Calado, T. C. (2020). Plastic ingestion lead to reduced body condition and modified diet patterns in the rocky shore crab Pachygrapsus transversus (Gibbes, 1850) (Brachyura: Grapsidae). *Mar. Pollut. Bull., 156*, 111249. https://doi.org/10.1016/j

De Frond, H., Rubinovitz, R., & Rochman, C. M. (2021). µATR-FTIR Spectral Libraries of Plastic Particles (FLOPP and FLOPP-e) for the Analysis of Microplastics. *Analytical Chemistry*, *93*(48), 15878–15885. https://doi.org/10.1021/acs.analchem.1c02549

Dias, B. F. D. S., & Lovejoy, T. E. (2012). Impacts of marine debris on biodiversity: Current status and potential solutions. *CBD Technical Series, 67*, 11–26.

Dusaucy, J., Gateuille, D., Perrette, Y., & Naffrechoux, E. (2021). Microplastic pollution of worldwide lakes. *Environmental Pollution*, *284*, 117075. https://doi.org/10.1016/j.envpol.2021.117075

Feng, Z., Zhang, T., Li, Y., He, X., Wang, R., Xu, J., & Gao, G. (2019). The accumulation of microplastics in fish from an important fish farm and mariculture area, Haizhou Bay, China. *Science of the Total Environment*, *696*, 133948. https://doi.org/10.1016/j.scitotenv.2019.133948

Gosavi, S., Purohit, B., Mitra, S., Patel, K., Vachhrajani, K., & Trivedi, J. (2021). Annotated checklist of marine decapods (Crustacea: Decapoda) of Gujarat state with three new records. *Proceedings of the "Marine Biology Research Symposium – MBRS,* 45–66.

Hara, J., Frias, J., & Nash, R. (2020). Quantification of microplastic ingestion by the decapod crustacean Nephrops norvegicus from Irish waters. *Marine Pollution Bulletin*, *152*, 110905. https://doi.org/10.1016/j.marpolbul.2020.110905

Hariharan, G., Purvaja, R., Anandavelu, I., Robin, R. S., & Ramesh, R. (2021). Accumulation and ecotoxicological risk of weathered polyethylene (wPE) microplastics on green mussel (Perna viridis). *Ecotoxicology and Environmental Safety*, *208*, 111765. https://doi.org/10.1016/j.ecoenv.2020.111765

Horn, D., Miller, M., Anderson, S., & Steele, C. (2019). Microplastics are ubiquitous on California beaches and enter the coastal food web through consumption by Pacific mole crabs. *Marine Pollution Bulletin*, *139*, 231–237. https://doi.org/10.1016/j.marpolbul.2018.12.039

Horton, A. A., & Barnes, D. K. A. (2020). Microplastic pollution in a rapidly changing world: Implications for remote and vulnerable marine ecosystems. *Science of the Total Environment*, *738*. https://doi.org/10.1016/j.scitotenv.2020.140349

Iannilli, V., Pasquali, V., Setini, A., & Corami, F. (2019). First evidence of microplastics ingestion in benthic amphipods from Svalbard. *Environmental Research*, *179 Pt A*, 108811. https://doi.org/10.1016/j.envres.2019.108811

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R., & Law, K. L. (2015). Plastic waste inputs from land into the ocean. *Science*, *347*(6223), 768–771. https://doi.org/10.1126/science.126035

Kleawkla, N. (2019). Microplastic fragments in stomach content of blue swimming crab, Portunus pelagicus from Wonnapha coastal wetland, Chonburi Province, Thailand. *Ramkhamhaeng International Journal of Science &Technology*, *2*(3), 7–16.

Lozano, Y. M., Lehnert, T., Linck, L. T., Lehmann, A., & Rillig, M. C. (2021). Microplastic shape, polymer type, and concentration affect soil properties and plant biomass. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.616645

Mohsen, M., Wang, Q., Zhang, L., Sun, L., Lin, C., & Yang, H. (2019). Microplastic ingestion by the farmed sea cucumber Apostichopus japonicus in China. *Environmental Pollution*, *245*, 1071–1078.

Munno, K., Helm, P. A., Rochman, C., George, T., & Jackson, D. A. (2022). Microplastic contamination in Great Lakes fish. *Conservation Biology*, *36*, 13794.

Patria, M. P., Santoso, C. A., & Tsabita, N. (2020). Microplastic ingestion by periwinkle Snail Littoraria scabra and mangrove crab Metopograpsus quadridentata in Pramuka Island, Jakarta Bay, Indonesia. *Sains Malaysiana*, *49*(09), 2151–2158. https://doi.org/10.17576/jsm-2020-4909-13

Patterson, J., Jeyasanta, K. I., Sathish, N., Edward, J. K. P., & Booth, A. M. (2020). Microplastic and heavy metal distributions in an Indian coral reef ecosystem. *Science of the Total Environment*, *744*, 140706. https://doi.org/10.1016/j.scitotenv.2020.140706

Piarulli, S., Scapinello, S., Comandini, P., Magnusson, K., Granberg, M., Wong, J. X., & Airoldi, L. (2019). Microplastic in wild populations of the omnivorous crab Carcinus aestuarii: a review and a regional-scale test of extraction methods, including microfibres. *Environmental Pollution*, *251*, 117–127. https://doi.org/10.1016/j.envpol.2019.04.092

Rabari, V., Patel, K., Patel, H., & Trivedi, J. (2022). Quantitative assessment of microplastic in sandy beaches of Gujarat state, India. *Marine Pollution Bulletin*, *181*, 113925. https://doi.org/10.1016/j.marpolbul.2022.113925

Rajmohan, K. V. S., Ramya, C., Raja Viswanathan, M., & Varjani, S. (2019). Plastic pollutants: effective waste management for pollution control and abatement. *Current Opinion in Environmental Science & Health*, *12*, 72–84. https://doi.org/10.1016/j.coesh.2019.08.006

Robin, R. S., Karthik, R., Purvaja, R., Ganguly, D., Anandavelu, I., Mugilarasan, M., & Ramesh, R. (2020). Holistic assessment of microplastics in various coastal environmental matrices, southwest coast of India. *Science of the Total Environment*, *703*, 134947. https://doi.org/10.1016/j.scitotenv.2019.134947

Rochman, C. M. (2015). The complex mixture, fate and toxicity of chemicals associated with plastic debris in the marine environment. *Marine Anthropogenic Litter*, 117–140.

Rodríguez-Seijo, A., & Pereira, R. (2017). Morphological and physical characterization of microplastics. *Comprehensive Analytical Chemistry*, *75*, 49–66. https://doi.org/10.1016/bs.coac.2016.10.007

Song, X., Du, L., Si, M., Zou, D., & Qiu, X. (2023). Effects of micro (nano) plastics on the reproductive system: a review. *Chemosphere*, *139138*.

Trivedi, D. J., Trivedi, J. N., Soni, G. M., Purohit, B. D., & Vachhrajani, K. D. (2015). Crustacean fauna of Gujarat state of India: a review. *Electron. J. Environ. Sci., 8*, 23–31.

Trivedi, J. N., Trivedi, D. J., Vachhrajani, K. D., & Ng, P. K. L. (2018). An annotated checklist of the marine brachyuran crabs (Crustacea: Decapoda: Brachyura) of India. *Zootaxa*, *4502*(1), 1–83. https://doi.org/10.11646/zootaxa.4502.1.1

Vaid, M., Mehra, K., & Gupta, A. (2021). Microplastics as contaminants in Indian environment: a review. *Environmental Science and Pollution Research*, 1–28. https://doi.org/10.1007/s11356-021-16827-6

Vasantkumar Rabari, Patel, H., Ali, D., Yadav, V. K., Patel, A., Dipak Kumar Sahoo, & Trivedi, J. N. (2023a). Ingestion and polymeric risk assessment of microplastic contamination in commercially important brachyuran crab Portunus sanguinolentus. *Frontiers in Marine Science*, *10*, 1286782. https://doi.org/10.3389/fmars.2023.1286782

Vasantkumar Rabari, Patel, H., Patel, K., Patel, A., Snehal Bagtharia, & Trivedi, J. N. (2023b). Quantitative assessment of microplastic contamination in muddy shores of Gulf of Khambhat, India. *Marine Pollution Bulletin*, *192*, 115131. https://doi.org/10.1016/j.marpolbul.2023.115131

Vasantkumar Rabari, Rakib, M. R. J., Trivedi, J. N., Idris, A. M., & Guilherme Malafaia. (2023c). Microplastic occurrence in commercial crab (Portunus segnis) from the western coast of India and pollution indices: First investigation and evidence. *Science of the Total Environment*, *905*, 167500. https://doi.org/10.1016/j.scitotenv.2023.167500

Villegas, L., Cabrera, M., & Capparelli, M. V. (2021). Assessment of microplastic and organophosphate pesticides contamination in fiddler Crabs from a Ramsar site in the estuary of Guayas River, Ecuador. *Bulletin of Environmental Contamination and Toxicology*, *107*(1), 20–28. https://doi.org/10.1007/s00128-021-03238-z

Waddell, E. N., Lascelles, N., & Conkle, J. L. (2020). Microplastic contamination in Corpus Christi Bay blue crabs, *Callinectes sapidus*. *Limnology and Oceanography Letters*, *5*(1), 92–102. https://doi.org/10.1002/lol2.10142

Walkinshaw, C., Lindeque, P. K., Thompson, R., Tolhurst, T., & Cole, M. (2020). Microplastics and seafood: lower trophic organisms at highest risk of contamination. *Ecotoxicology and Environmental Safety*, *190*, 110066. https://doi.org/10.1016/j.ecoenv.2019.110066

Watts, A. J. R., Lewis, C., Goodhead, R. M., Beckett, S. J., Moger, J., Tyler, C. R., & Galloway, T. S. (2014). Uptake and retention of microplastics by the Shore Crab Carcinus maenas. *Environmental Science & Technology*, *48*(15), 8823–8830. https://doi.org/10.1021/es501090e

Wright, S. L., & Kelly, F. J. (2017). Plastic and human health: A micro issue? *Environmental Science & Technology,* *51*(12), 6634–6647. https://doi.org/10.1021/acs.est.7b00423

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution*, *178*, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031

Xu, Q., Xing, R., Sun, M., Gao, Y., & An, L. (2020). Microplastics in sediments from an interconnected river-estuary region. *Science of the Total Environment*, *729*, 139025. https://doi.org/10.1016/j.scitotenv.2020.139025

Zia, K. M., Bhatti, H. N., & Ahmad Bhatti, I. (2007). Methods for polyurethane and polyurethane composites, recycling and recovery: A review. *Reactive and Functional Polymers*, *67*(8), 675–692. https://doi.org/10.1016/j.reactfunctpolym.2007.05.004

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4 Micropropagation: Advance technology for Commercial propagation of Medicinal plants

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Abstract

Micropropagation or clonal propagation is a specific aspect of plant tissue culture dealing with the aseptic vegetative multiplication of plants *in vitro*. The application is to produce large number of true to type aseptic plants in limited period of time and space. Micropropagation of plants achieved by forced proliferation of shoots from axillary or apical buds, production of adventitious buds and somatic embryogenesis. Establishment of micropropagation protocol allows fast and continuous commercial propagation of valuable medicinal plant and *in vitro* propagation ultimately protects this type of rare and endangered plant by does not destroy the mother works. Established micropropagation protocol serves as an indispensable tool to preserve medicinal plants and improve secondary metabolite production.

Introduction

Medicinal plants play a vital role in the maintenance of human health throughout the world. In fact, they are of critical importance in poor communities. Medicinal plants also play an important cultural role as well as important economical role. Knowledge of their use is wide spread and their efficacy is trusted, based on a long history of use (Chopra et al, 1956). Medicines derived from plants include aspirin, taxol, morphine, reserpine, colchicine, digitalis and vincristine. There are hundreds of herbal supplements such as *Ginkgo, Echinacea*, *Eucalyptus*, *Aloe, Withania* etc (Kokate et al, 1997).

The World Health Organization (WHO) estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary healthcare needs. Also, modern pharmacopoeia contains at least 25% drugs derived from plants. Demand for medicinal plants are increasing in both developing countries due to growing recognition of natural products, being non-toxic, having no side-effects, easily available and that too at affordable prices.

The cultivation of medicinal plants is necessary because of the following reasons (Kokate et al, 1997)-

i. In nature, there remains a wide variation among the plant with regards to their active principle. As only the best among them are used for cultivation, it enables us to obtain raw material of homogenous quality and high potency.

ii. It is easy to grow and fulfill the commitment of large-scale supply through cultivated sources rather than from natural resources, which mainly depends on nature for their regeneration and availability.

iii. In many cases, plant collectors are engaged in destructive collection/extractive methods, which have resulted in many plants becoming extinct or being listed as threatened.

iv. The growing pressure of population/urbanization and the development of roads to remote area have resulted in deforestation and loss of natural plant resources.

v. Despite the fact that our forests are major resource base for medicinal plants, as many of them appear in the wild, the importance of this has been government agencies.

Tissue culture techniques are becoming more popular as an alternative means of plant vegetative propagation. The idea of cell and tissue culture was coined by a German plant physiologist Gattlieb Haberlandt (1854-1945), who is regarded as the father of plant tissue culture (Chawla, 2002). Tissue culture is used in its broadest sense to include the aseptic culture of plant parts of widely different organizational complexities including controlled conditions (Gamborg and Phillips, 1996). The last three decades have seen a very rapid rise in the number of plant scientists using the techniques of organ, tissue and cell culture for plant improvement.

Tissue culture techniques have been exploited to increase the number of desirable stock of germplasm available to the plant breeder, to create genetic variability for plant improvement programmes and to improve the state of health of the planting materials (Kannan, 1998).

Among the various applications of plant tissue culture, micropropagation of plant species has attained the status of large plant based industry. The developments in the study of various aspects of plant growth and differentiation were rapid during 1960s and 1970s (Chand and Ramawat, 2000).

Micropropagation:

Clonal propagation *in vitro* is called micropropagation. Webber first used the word 'clone' for cultivated plants that were propagated vegetatively. Clone means twig, spray or a slip, like those broken off as propagules for multiplication. It signifies that plants grown from such vegetative parts are not individuals in the modernity sense, but are simply transplanted parts of the same individual and such plants are identical. Thus, clonal propagation is the multiplication of genetically identical individuals by asexual reproduction. Murashige (1974) out lined three major stages involved in micropropagation (Fig-1)-

Stage I: selection of suitable explants, their sterilization and

transfer to nutrient media for establishment. That means initiation of a sterile culture of the selected explant.

Stage II: proliferation or multiplication of shoots from the explant on medium.

Stage III: transfer of shoots to a rooting medium followed later by planting into soil.

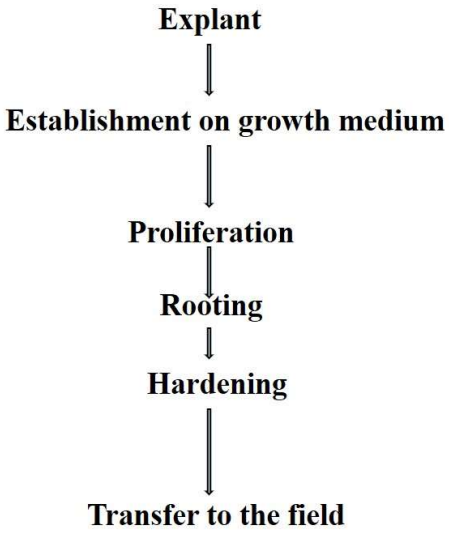
Micropropagation and plant regeneration can be grouped into the following categories-

i. **Enhanced release of axillary bud proliferation**: i.e. by multiplication through growth and proliferation of existing meristems through shoot tip culture, single node/axillary bud culture.

ii. **Organogenesis:** It is the formation of individual organ such as shoots and roots from the explant directly or indirectly through callus.

iii. **Somatic embryogenesis**: It is the formation of a bipolar structure containing both shoot and root meristem either directly from the explant or *de novo* origin from the explant.

Fig-1: Micropropagation steps as proposed by Murashige (1974)



Various advantages of micropropagation:

i. Shoot multiplication can be achieved in small space because miniature plantlets are produced.

ii. Propagation is carried out under sterile conditions. No damage is caused due to insects and disease, and plantlets produced are free from microbes.

iii. Through virus elimination by meristem culture, a large number of virus-free plants can be obtained.

iv. Cultures are carried out under defined conditions of environmental, nutritional and tissue system; therefore, it is a highly reproducible system under the defined set of conditions.

v. This production is unaffected by seasonal variations as uniform conditions are maintained.

vi. No care is requiring between two subcultures as compared to conventional vegetative propagation system like watering, weeding.

vii. Small greenhouse facilities are sufficient because of miniature size of plantlets.

viii. Mother plant or genotype of stock plant can be stored and maintained *in vitro* without damage through environmental factors and stock plants.

ix. The plants that are difficult to propagate vegetatively (recalcitrants) by conventional method can also be propagated by this method.

Various plant species (woody and herbaceous) have been successfully propagated through micropropagation for example *Bamboo* (Raste and Bhojwani, 1998), *Emblica officinalis* (Jasrai et al, 1998), *Morus laevigata* (Ahlawat et al, 1999), *Azadericta indica* (Sharma et al, 1999), *Leucaena leucocephalla* (Bhat et al, 2000), *Courupita guianensis* (Kathiravan et al, 2000), *Acacia species* (Beak and Dunlap, 2001), *Lagerstomia parviflora* (Tiwari et al, 2001), *Phyllanthus amarphus* (Bhattacharya and Bhattacharya, 2001), *Jatropha curcus* (Rajor et al, 2002), *Centella asiatica* (Nath and Buragohain, 2003), *Rose* (Roy, 2004).

References:

Ahlawat ST, Singh UV and Deori ML (1999), Micropropagation through shoot tip culture of mature tree of *Morus laevigata* (Wall), In: Abst vol of Natl Symp on Role of Plant Tissue Culture in Biodiversity Conservation and Economic Development, Plant Institute of Himalayan Environment and Development, Almora, pp: 5.

Beak SL and Dunlap RW (2001), Micropropagation of The *Acacia* sp, A review, *In vitro* Cell Dev Bio-Plant, 37: 531-538.

Bhat B, Venkatesh VB, Gupta MG and Kumar S (2000), Micropropagation of elite hybrid of *Leucaena,* In: Abst vol of Natl Symp on prospects and potentials of plant bio-technology in India on the 21st century and 23rd Annual meeting of PTCA, JNV University, Jodhpur, pp:50.

Bhattacharya R and Bhattachrya S (2001), High frequency *in vitro* propagation of *Phyllanthus amarus* Schum and Thom by shoot tip culture, Ind J Exp Biol, 39: 1184-1187.

Chand S and Ramawat KG (2000), Plant Biotechnology, S chand and company Ltd, New Delhi, India.

Chawla HS (2002), A Handbook of Introduction to Plant Biotechnology, Oxford and IBH Publ, New Delhi, India.

Chopra RN, Nayar SL and Chopra IC (1956), Glossary of Indian Medicinal Plants, CSIR, New Delhi, India.

Gamborg OL and Phillips GC (1995), Plant Cell Tissue Culture and Organ Cultures, Narosa Publishing House, New Delhi, Springer-verlag, Berlin, Heidelberg, In: Raval K (2005), Tissue culture studies on *Musa paradisiaca* L, M. Phil thesis, Botany Department, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India.

Jasrai YT, Ramakanthan A and Kannan VR (1998), *In vitro* propagation of *Gmelina, Vitex and Emblica*, In: Abst vol of Natl Symp on commercial aspects of plant tissue culture, Molecular biology and madicinal plant biotechnology, Jamia Hamdard, New Delhi, pp: 16.

Kannan RV (1998), Tissue Culture Studies with Trees, Clonal multiplication, Ph.D. Thesis, The Maharaja Siyajirao University of Baroda, Gujarat, India.

Kathiravan K, Prakash S, Sehadri S and Ignacimuthu S (2000), *In vitro* Multiplication of *Cannan- ball tree (Courupita guianensis),* In: Abst vol of Natl Symp on prospects and potentials of plant Bio-technology in India on the 21st century and 23rd annual meeting of PTCA, JNV University, Jodhpur, pp: 56.

Kokate CK, Purohit AP and Gokhale SB (1997), Pharmacognosy-General Introduction, Nirali prakashan, Pune, India.

Murashige T (1974), Plant propagation through tissue culture, Avn Rev Plant Physiol, 25: 135-166.

Murashige T and Skoog F (1962), A revised medium for rapid growth and bioassays with tobacco tissue cultures, Physiol Plant, 15: 473-497.

Nath S and Buragohain AK (2003), *In vitro* method for propagation of *Centella asitica* L Urban by shoot tip culture, J Plant Biochem and Biotech, 12: 167-169.

Rajor S, Shardana J and Batra A (2002), *In vitro* cloning of *Jatropa curcus* L, J of Plant Biol, 29: 195-198.

Raste EP and Bhojwani SS (1998), *In vitro* clonalpropagation of *bamboo,* In: Srivastav PS, Plant Tissue Culture and Molecular Biology- Applications and Prospects, Narosa Publ House, New Delhi, pp: 538-553.

Roy P, Mamun ANK and Ahmed G (2004), *In vitro* plantlets regeneration of *Rose,* Plant Tissue Culture, 14: 149-154.

Sharma AK, Sharma M and Chaturvedi HC (1999), Conservation of phytodiversity of *Azadiracta indica* A Juss through *in vitro* strategies, In: Abst vol of Natl Symp on role of plant tissue culture in biodiversity conservation and economic development, G B Plant Institute of Himalayan Environment and Development, Almora, pp: 60.

Tiwari SK, Kashyap MK, Ujjaini MM and Aggarwal AP (2001), *In vitro* micropropagation of *Lagerstromia parviflora* Roxb from abuit tree, Ind J Exp Biol, 40(2): 212-215.

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1 A Study on Micro Tuber Production Optimization for Sustainable Potato Cultivation in Gujarat, India

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Abstract

In Gujarat, potatoes are a major cash crop. Potato production has increased drastically all over the states. The food processing field, home consumption, and potential for export stimulate potato demand. While potato production in India has increased, the business is infected by various difficulties. Post-harvested losses due to unsuitable handling and storage. The availability of quality potato seeds is a major problem. This study shows the impact of different culture systems and nutritional supplements on developing and optimising an appropriate system for in vitro shoot development, microtubers formation, and preservation conditions. Three distinct, primarily suited potato cultivars were grown *in vitro,* and the shoot formation methodology was standardized. The microtubers technique was adjusted using four-week-old shoots, and a mean of four microtubers per shoot was seen on the Murashige and Skoog medium supplemented with sucrose (8%). Harvested microtubers were used to test storage conditions, and shoot development from microtubers was studied in vitro and ex-vitro. After 18 days of storage at 4 °C in vitro and ex-vivo, all micro tubers grew healthy shoots, and the resulting plantlets demonstrated >90% survival in the greenhouse. A suitable microtubarization process may assist in the distribution of high-quality potato seeds, which are in great demand in Gujarat. This study confirms the viability of long-term germplasm preservation and micro tubers-based farming strategies in Gujarat.

Key-Words: Micropropagation, liquid culture; sucrose; microtuber storage; germplasm

Introduction

Potatoes serve as a versatile staple in the Indian diet, offering nutritional diversity and playing a crucial role in ensuring a stable food supply for the country's growing population. India is a significant contributor to global potato production, ranking second worldwide. The cultivation of potatoes spans an extensive area of 2.13 million hectares in the country, yielding a substantial production of 43.77 million metric tonnes, as reported by Viswanath et al. in 2018.Gujarat, holding the fourth position in the cultivation of potatoes, considers the potato as a crucial cash crop. The state leads the nation in terms of productivity, cultivating the crop across 125,000 hectares of land. The remarkable surge in potato productivity and production, referred to as the "Brown Revolution," positioned India as the second-largest potato producer globally.

With a projected population increase of 19% by 2050, India faces a tremendous challenge to increase its production of all food crops, including potatoes, to meet future demands (Bamberg *et al.,* 2005).

Potatoes are predominantly cultivated through vegetative propagation methods (Srivastava *et al.,*2012). Farmers primarily obtain seed tubers from the Central Potato Research Institute (CPRI) located in Himachal Pradesh, India. Despite the availability of cultivars with enhanced resistances and tolerances to abiotic and biotic stresses, the challenges faced by potato farmers persist, indicating that these improved varieties alone are insufficient to address the underlying issues. Nevertheless, potato farmers continue to face challenges, as the issue persists despite the availability of cultivars with enhanced resistance and tolerance to both abiotic and biotic stresses. Complete fields being destroyed due to late blight epidemics are reported relatively frequently. The extent of yield reduction caused by late blight in India fluctuates annually, ranging from 20% to 75%. The pathogen induces water-soaked lesions surrounded by chlorotic borders, initially small but rapidly expanding under humid conditions. This leads to the swift blighting of the entire plant within a few days, followed by the decay of developing tubers, ultimately resulting in substantial yield losses under favourable conditions (Sundaresha et al., 2015) [10]. The cost of seed tubers constitutes approximately 34% of the overall production expenses, along with additional costs related to transportation and storage. Over the past 50 years, the production of potatoes in the state of Gujarat has greatly increased. In terms of productivity in 2003–04, Gujarat was the second-most productive state.

Plant tissue cultureor micropropagation, offers numerous benefits, including the production of true-to-type plants that are clean, disease-free, can be cultivated year-round, and are easily transportable. Microtubers, also known as "in vitro" formed tubers, are tiny seed potatoes found in between "in vitro" plantlets and minitubers (Nistor *et al.,* 2010). The initial generation of potato seed from tissue culture, known as microtubers, is utilized to address issues with transferring plantlets from "in vitro" to "in vivo" environments. In vitro produced microtubers and plantlets obtained through meristem culture can be used by farmers to obtain disease-free plants, especially virus-free planting materials (Borna et al., 2019). Because of their small size and low weight, microtubers have several benefits for mechanization, storage, and transportation. Microtubers serve dual purposes in germplasm exchange and conservation, as they can either be utilized in greenhouses to yield minitubers or directly deployed in the field. They may be grown at any time of year and planted directly in the ground. The in vitro production of microtubers plays a crucial role in generating and preserving valuable seed potato stocks. Potato microtubers obtained by "in vitro" culture from single-node cuttings are convenient for handling, storage and exchange of a healthy germplasm, representing an important component, along with plantlets and minitubers, for seed potato production programs (Rozu et al., 2004).

This study aimed to create an effective procedure for in vitro propagation of potato cultivars and microtuber formation employing a liquid culture system. An evaluation of the microtuber development process' performance was done by compared the semi-solid and liquid culture systems. To produce the microtubers, different sucrose

concentrations and plant growth regulators have been used. The effect of microtuber storage conditions and size were also evaluated for plantlets' development and acclimatization.

Materials and Methods

Plant Materials and in Vitro Culture Initiation

Different types of potatoes (*Solanum tuberosum* L.) Varieties like– Kufri Pukhraj, Kufri Badshah, and Kufri Leema - were collected from the Potato Research Station, Gujarat, India (Fig. 1). Potatoes were refrigerated at 4°C for 6-8 weeks. In vitro cultures were started using potato bud sprouts as explants (Figure 1B). Surface sterilization of bud sprouts involved a 5-minute treatment with 0.1% HgCl2, followed by a 5-minute exposure to 8% Sodium hypochlorite. Subsequently, the sprouts underwent a thorough rinsing process with autoclaved deionized water, repeating the wash four times, with each rinse lasting 3 minutes. The bud sprouts were grown on a semi-solid MS (Murashige and Skoog) [8] basal medium supplemented with 0.8 g of Agar and 3% sucrose after sterilization. Before the medium was autoclaved for 20 minutes, its pH was adjusted to 5.70. at 118 kPa and 121 °C.

The in vitro cultures were sustained on MS (Murashige and Skoog, 1962) medium with an addition of 3% sucrose, following standard culture conditions of a 16-hour light and 8-hour dark photoperiod. The light was provided by cool white fluorescent lamps with a light intensity of 40 μmolm-2s-1. The optimization of shoot multiplication medium involved utilizing both liquid and semi-solid MS medium, with the addition of varying concentrations of Benzyl aminopurine (0, 0.2, 0.5, 1.0 mg/L) and Gibberellic acid (0, 0.1 mg/L). For the purpose of developing roots, micro shoots that have been taken from the cultures used in shoot multiplication tests were placed in basal media.

Microtubers formation and storage:

For the microtuberization experiments, four-week-old in vitro shoots with five to six internodes were utilized. For the goal of developing microtubers, different concentrations of sucrose (3, 6, 8, 10%) were utilized in both the liquid and semi-solid media. The culture vessels containing liquid medium were consistently placed on a rotary shaker at a constant speed of 100 rpm. After a 6-week cultivation period, the total number of microtubers was noted. Microtubers measuring over 0.5 cm were collected and stored in a dark environment at a temperature of 4°C in the refrigerator. The assessment of shoot development from microtubers was conducted both in vitro and in greenhouse conditions following storage periods of 0, 1, and 2 weeks. Rooted shoots, originating from both microtubers and directly from nodal explants, were transplanted into trays filled with a soil mixture and covered with plastic for a duration of 10 days. Every experiment was run twice, requiring a minimum of three replications. Tukey's test was used to compare the means, and values with $p < 0.05$ were deemed statistically significant.

Results and discussion

Potato cultivation faces significant challenges due to the limited accessibility of region-specific potato varieties and their seed tubers, as well as issues related to transportation and storage losses. The objective of this current research was to develop an economical method for producing microtubers and to examine the impact of liquid culture systems, nutrient mediums, plant growth regulators, and the number of sub-culture cycles on plant quality, as well as the quantity and size of tubers during the stages of potato shoot multiplication and tuber induction. Generally, protocol is genotype specific, the optimized protocol will be evaluated based on three potato cultivars to make the most common protocol.

A sterile culture of three diverse potato cultivars has been initiated using bud sprouts as the explant (refer to Figure 1C, D). Every bud sprout exhibited a positive response to the MS basal medium for shoot development; however, approximately 22% experienced contamination within a 10-day period. Shoot multiplication and development were carried out using individual nodal segments. The Kufri Badshah cultivar demonstrated the greatest shoot height (8.6 cm) and number of internodes per shoot (6) when grown in a semi-solid medium with MS supplemented with BA (1.0 mg/L) and GA3 (0.1 mg/L), outperforming other levels (Table: -1).

Table:1 Effect of different concentration of BAP+GA on shoot multiplication in MS basal media

Name of Varieties	Hormones name and concentration (mg/l) (Mean±SE)							
	Control		BAP(0.2)+GA(0.1)		BAP(0.5)+GA(0.1)		BAP(1.0)+GA(0.1)	
	Shoot length	No. of shoots /nodes	Shoot length	No. of shoots /node	Shoot length	No. of shoots /node	Shoot length	No. of shoots /node
Kufri Pukhraj	1.73±0.23	1±0.57	3.86±0.26	3±0.57	5.9±0.17	3.33±0.33	7.6±0.26	4.33±-.88
Kufri Badshah	1.9±0.23	2.6±0.33	4.76±0.29	4±0.57	7.3±0.32	5±0.57	8.6±0.18	6±0.57
Kufri Leema	1.73±0.26	2.6±0.88	4.23±0.24	2.33±0.33	6.7±0.20	2.66±0.33	7.4±0.26	4±0.57

A higher number of microtubers (4 microtubers per shoot) was noted when the MS medium was enriched with 8% sucrose, a significant increase compared to varying sucrose concentrations in *Kufri Badshah* varieties. Sucrose, serving as the carbon

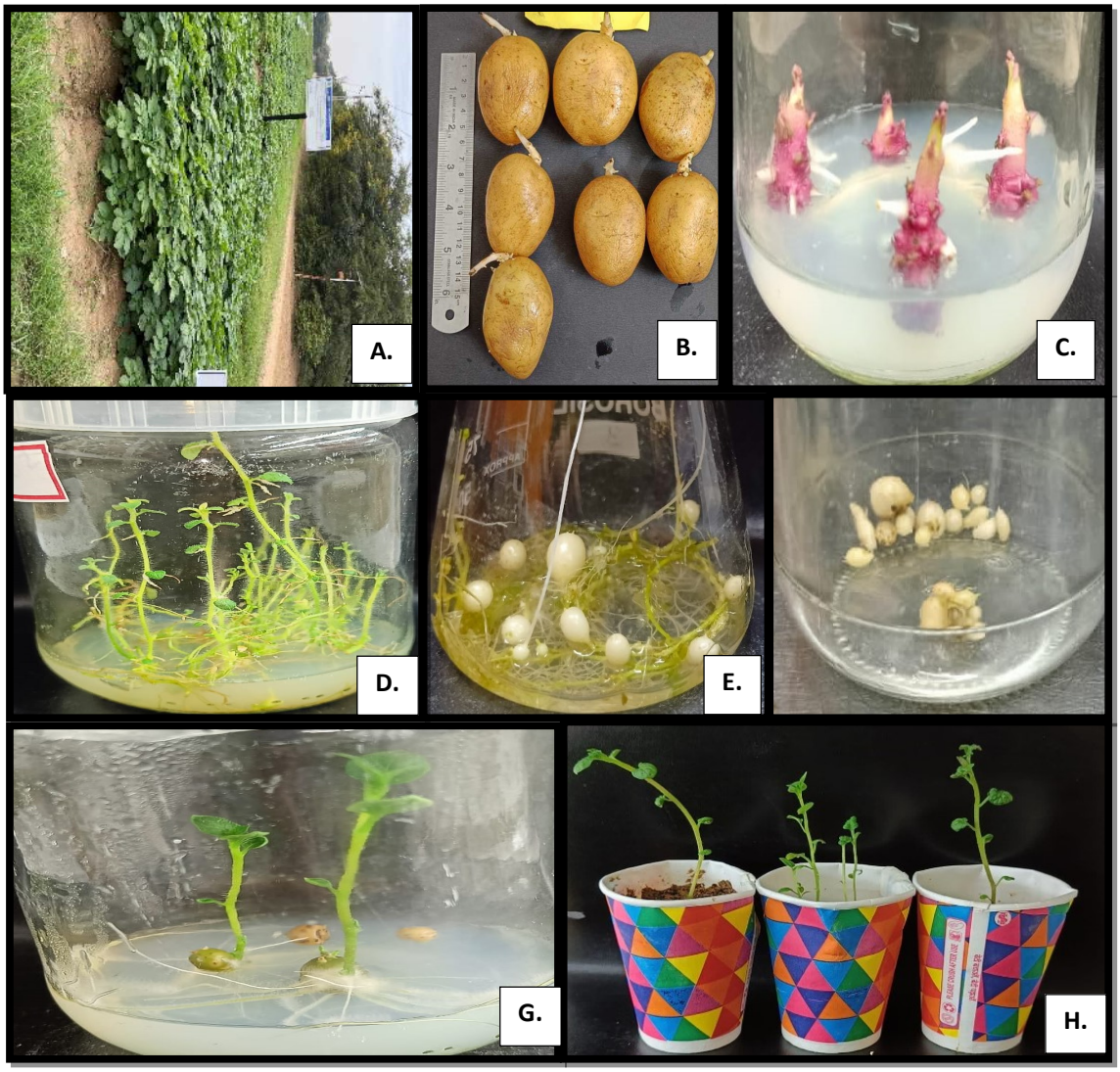
source, plays a pivotal role in the medium for the development of microtubers (Kauri and moorby, 1995, Yu. *et al.,* 2000). One of the Higher numbers of microtubers (4) were observed in liquid culture system. A liquid culture system based on temporary immersion has been documented for the development of microtubers (Sarkar and naik 1998). The size and numbers of the tubers varied by cultivars; however, the cultivar response was not significantly different. Similar observations were recorded in the study conducted by Akita and Takayama 1994. In the present study, an average of 15 microtubers per flask was harvested after 6 weeks of culture. (Table:2)

Table:2Effect of different concentration of sucrose on microtuberization in MS liquid media

Name of Varieties		Sucrose concentration (%) (Mean±SE)			
	No. of microtubers/Nodes				
	Control	Sucrose (3%)	Sucrose (6%)	Sucrose (8%)	Sucrose (10%)
Kufri Pukhraj	0.33±0.33	1.66±0.33	2.66±0.33	3±0.57	2±0.57
Kufri Badshah	1±0.57	2.66±0.33	2.33±0.66	4±0.57	2.66±0.33
Kufri Leema	0.66±0.33	1.33±0.33	1.66±0.33	3.33±0.33	2±0.57

Microtubers with a size greater than 0.5 cm were collected and stored in a dark environment at 4 °C in a refrigerator. Our observations revealed that all microtubers successfully produced shoots both under in vitro conditions and in the greenhouse following 18 days of storage. The uniformity of microtubers and size were important factors for shoot development Donnelly *et al.,* 2003. All shoots from in vitro microtubers survived in the greenhouse conditions. In 10 days, rooting was observed in all shoots from the microtubers and nodal explants when transferred to half strength MS basal medium. Three-weeks-old rooted shoots from nodal explants and microtubers acclimatized successfully, with a survival rate of more than 90%.

The major issue of the lack of healthy seed tubers of tolerant cultivars can be resolved by mass producing microtubers and supplying them at low cost. The optimized protocol can be used to mass-produce disease-free microtubers at commercial scale. Various potential germplasms, as well as their microtubers, can be maintained under in vitro conditions using the standardized protocol, showing potential for farmers and



breeders.

Figure:1 In vitro shoot multiplication and microtubers development in various potato cultivars. Potatoes of various cultivars were collected from Potato Research Station, Gujarat, India (**A**) and bud sprouts (**B**) were used to initiate the in vitro cultures (**C**,). In vitro shoots were multiplied on optimal Murashige and Skoog basal medium (**D**) and individual shoots with 5–6 internodes were used for microtuber development on liquid (**E**) medium. The microtubers were collected and stored in the refrigerator at 4 °C (**F**) and all microtubers developed healthy shoots (**G**) after 18 days. All the shoots developed from microtubers were transferred to the greenhouse (**H**) with more than 90% survival rate.

Conclusion

In conclusion, in vitro cultures of three different locally adapted potato cultivars were established, and the shoot multiplication protocol was standardized. Shoot

multiplication highest recorded in BAP (1.0) +GA (0.1) mg/l. The microtubers protocol was optimized using four-week-old shoots, and a mean of four microtubers per shoot was observed on the Murashige and Skoog medium supplemented with sucrose (8%). Harvested microtubers were used to evaluate storage conditions and shoot growth coming out from microtubers under in vitro as well as ex vitro conditions. This optimized protocol emphasizes the importance of living germplasm conservation under in vitro conditions

References

Akita, M.; Takayama, S. Stimulation of potato (Solanum tuberosum L.) tuberization by semicontinous liquid medium surface level control. Plant Cell Rep. 1994, 13, 184–187. [PubMed]

Arora, R.K.; Sharma, S.; Singh, B.P. Late blight disease of potato and its management. Potato J. 2014, 41, 16–40. 4.

Bamberg, J.B.; Del Rio, A. Conservation of genetic resources. In Genetic Improvement of Solanaceous Crops Vol. 1: Potato; Razdan, M.K., Mattoo, A.K., Eds.; Science Publishers, Inc.: Enfield, NH, USA, 2005; p. 451.

Borna, R. S., Hoque, M. I., & Sarker, R. H. (2019). In vitro microtuber induction and regeneration of plantlets from microtuber discs of cultivated potato (Solanum tuberosum L.). *Plant Tissue Culture and Biotechnology*, *29*(1), 63-72.

Donnelly, D.J.; Coleman, W.K.; Coleman, S.E. Potato microtuber production and performance: A review. Am. J. Potato Res. 2003, 80, 103–115. [CrossRef]

Khuri, S.; Moorby, J. Investigations into the role of sucrose in potato cv. Estima microtuber production in vitro. Ann. Bot. 1995, 75, 295–303. [CrossRef] 10.

Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 1962, 15, 473–497. [CrossRef]

Nistor, A. N. D. R. E. E. A., Campeanu, G. H. E. O. R. G. H. E., Atanasiu, N. I. C. O. L. A. E., Chiru, N. I. C. O. L. E. T. A., &Karacsonyi, D. I. A. N. A. (2010). Influence of potato genotypes on "in vitro" production of microtubers. *Romanian Biotechnological Letters*, *15*(3), 5317-5324.

Peerzada, S.; Viswanath, H.; Bhat, K. In vitro studies on effect of fungicides against mycelial growth and sporangial germination of Phytophthora infestans (Mont) de Bary) causing late blight of potato. Int. J. Chem. Stud. 2020, 8, 2069–2075. [CrossRef]

Rosu, R., Chiru, N., &Rolot, J. L. (2004). Researches on genotype influence on potato Microtuberization Anale, ICDCSZ, XXXI. In *Proceedings of EAPR agronomy section meeting, Mamaia, Romania, June* (pp. 120-128).

Sarkar, D.; Naik, P.S. Effect of inorganic nitrogen nutrition on cytokinin-induced potato microtuber production in vitro. Potato Res. 1998, 41, 211–217. [CrossRef]

Shukla, S. R., Zala, H. N., Solanki, S. D., & Ant, H. M. (2023, October). Optimizing Microtubers Production for Sustainable Potato Cultivation in Gujarat, India. In *Biology and Life Sciences Forum* (Vol. 27, No. 1, p. 2). MDPI.

Srivastava, A. K., Diengdoh, L. C., Rai, R., Bag, T. K., & Singh, B. P. (2012). In vitro micropropagation and micro-tuberization potential of selected potato varieties. *Indian Journal of Hill Farming*, *25*(2), 14-17.

Sundaresha S, Kumar S, Singh BP, Jeevalatha A, Rawat S, Mahota AK et al. Comparative genome analysis of Irish famine pathogen with Indian Phytophthora infestans isolate. In: 3rd International symposium on Phytophthora: Taxonomy, genomics, pathogenicity, resistance and disease management. 9th-12th September, 2015. Bengaluru, India, 2015, 24.

Yu, W.C.; Joyce, P.; Cameron, D. Sucrose utilization during potato microtuber growth in bioreactors. Plant Cell Rep. 2000, 19, 407–413. [CrossRef] [PubMed]