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Synthesis and Antimicrobial Studies of Isoxazole Derivatives

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ABSTRACT

The substituted isoxazoles are considered to be important synthons due to their versatility towards chemical transformations to useful synthetic intermediates. The isoxazoles are five membered heterocyclic ring containing two hetero atoms. They have broad spectrum of biological and pharmacological activities. Such as Antibacterial, antitumor, anti-flammatory, anti-fungal etc. Isoxazoles were synthesized by reaction of chalcones derivatives with hydroxylamine. The final product have been characterized by IR, ^{1}H NMR and ^{13}C NMR spectra. These compounds were also screened for their antibacterial and anti- fungal activities. Growth of S. aureus (gram positive), E.Coli (gram negative) bacteria and *Candida albicans, Aspergillus niger* are inhibit by the action of synthesized compounds.

Keywords: Chalcone, Isoxazole. 1H NMR, 13C NMR, of S. aureus. E.Coli, Candida albicans, Aspergillus niger

INTRODUCTION:

Isoxazole are five membered heterocyclic ring containing adjacent one oxygen and one nitrogen atom on ring and constituent an important family of heterocyclic chemistry. The formula of isoxazole is C_3H_3NO.[1-3]

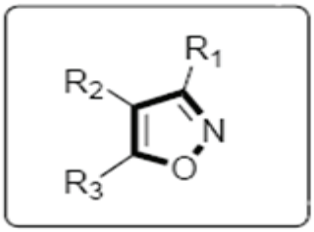
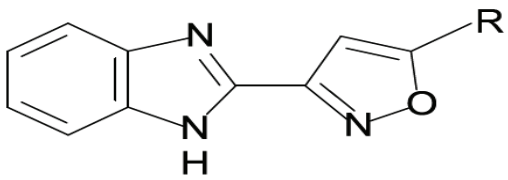
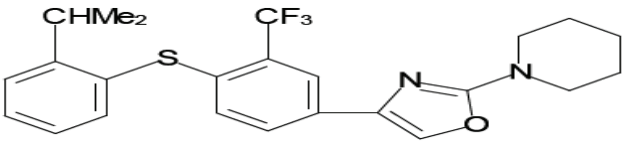


Fig. 1 Structure of isoxazole

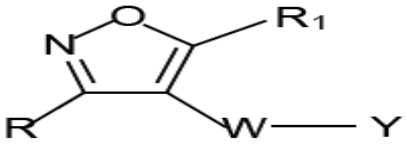
The other systematic name of isoxazole is 1, 2-azole. Isoxazolyl is the univalent radical derived from isoxazole. The substituted isoxazoles are also considered to be important synthons due to their versatility towards chemical transformations to useful synthetic intermediates. Isoxazole are present in structure of many natural products and pharmaceutical agents. Isoxazoles have long been used in organic synthesis due to broad spectrum of their biological and pharmacological activities which include analgesic, anti-flammatory, antibacterial and anti tumor activities. Isoxazole also form the basis for a number of drugs. Isoxazolyl group is found in many beta-lactumase-resistant antibiotics such as cloxacillin, dicloxacillin and flucloxacillin. Isoxazole derivatives shows various bio-activities such as Antimicrobial, Antiviral, Antibacterial, Anti-inflammatory, Fungicidal, Insecticidal, Herbicidal, Hypoglycergic, Musscle relaxant.Anti-inflammatory activity[4.5].Veeraswamy B. et. al. synthesized isoxazoles and tested their anticancer activity[6]. Denmark SE.et al. have prepared isoxazoles as antagonists.[7]



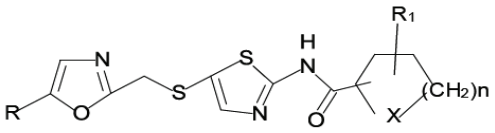
Wang, Gary, synthesized aryl phenyl heterocycles sulfide derivatives (II) as cell adhesion inhabiting, anti-inflammatory and immunosuppressive activity.[8]



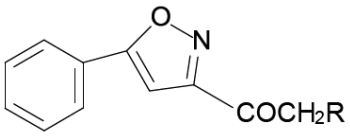
Lee Courtland M et. al. have prepared quinazolinedione derivatives as inosine 5- monophosphate dehydrogenase (IMPDH) inhibitors for use in pharmaceutical compositions.[9] Michael L et. al. reported isoxazoles derivatives for prevention and treatment of diabetes.[10]



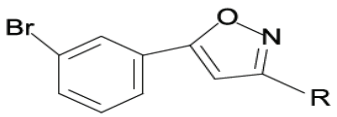
Suvitha S et. al. documented some isoxazole derivatives as antipicorna virus as agents.[11]Gucma M et al. prepared isoxazoles as inhibitors of cyclin dependent kinases.[12]



Maczynski M et. al. synthesized and tested isoxazole derivatives as antipyretic, analgesic, antiflammatory and anticough activity.[13]



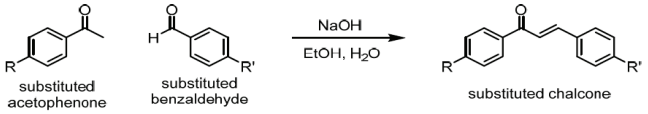
Narlawat R et. al. synthesized some novalisoxazole derivatives and tested for their analgesic and antiflammatory activities as well as for their acute toxicity and ulcerogenic effect.[14]. Joshi et al. synthesized some isoxazole derivatives as antitubercular and antimicrobial agents.[15] Antitumor activity of isoxazole derivatives have been reported by Bhatt B. A.[16]



EXPERIMENTAL

Synthesis of Isoxazole From Chalcones:-

General Introduction of chalcones: Chalcones have been used as intermediate for the preparations of isoxazole derivatives. Chalcone can be prepared by the aldol condensation of an aromatic ketone and an aldehyde. In chalcone two aromatic rings are joined by three carbon α,β-unsaturated carbonyl system. Chemically name of chalcones are 1,3-diaryl-2-propen-1-ones. Chalcones are a subset of compounds known as flavonoids. Flavonoids are produced naturally in plants and are responsible for the colour of leaves and flowers. Many naturally occurring flavonoids have been shown to anti-oxidant and anti-inflammatory properties and synthetic chalcones have potential application in medicine as well as other possible use.[17] Chalcones are prepared by Claisen-Schmidt condensation of arylaldehydes and acetophenones. The reactions are generally base catalyzed, but acid catalysed, solid and resin supported and microwave assisted versions also reported. These reaction methodologies are associated with drawbacks such as low yields.

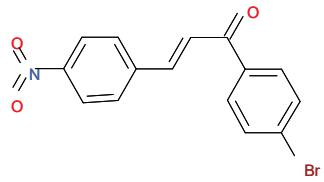


Synthesis Of Chalcones

Techniques Used: Techniques used for the analysis of complexes are following. Table 1 given below shows the various instruments used during project.

- **Analytical:** The elemental chemical analysis for carbon, hydrogen and nitrogen were performed at SAIF, CIL Punjab University, Chandigarh using elemental analyser series-Flash 2000 Thermo Fischer Scientific(USA).
- **FTIR Spectroscopy:** FTIR spectra were recorded using Perkin-Elmer 400 spectrometer (Germany) at SAIF, CIL Punjab University, Chandigarh in range 4000-5000 cm^{-1}.
- **NMR Spectroscopy:** C^{13} NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer at SAIF, CIL Punjab University Chandigarh, using tetramethylsilane (TMS) as internal reference.

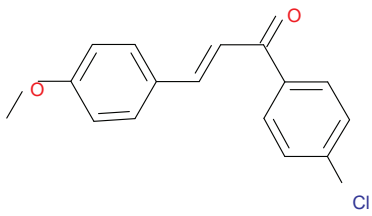
Synthesis of 1-(4-bromophenyl)-3-(4-nitrophenyl)-prop-2-en-1-one: Equimolar quantities of 4-nitrobenzaldehyde (0.01ml) and 1-(4-bromophenyl)-enone (0.01ml) were dissolved in minimum amount of ethanol at room temperature. Sodium hydroxide solution was added slowly and the mixture stirred for 12 hours until entire mixture becomes very cloud. Then the mixture was poured slowly into 400ml water with constant stirring and kept in refrigerator for 24 hours. The precipitate obtained was filtered. Washed and recrystallized from ethanol.



(E)-1-(4-bromophenyl)-3-(4-nitrophenyl)prop-2-en-1-one

Synthesis of 1-(4-chlorophenyl)-3-(4-methoxyphenyl)-prop-2-en-1-one:-

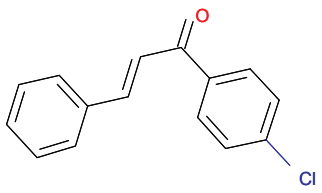
Equimolar quantities of Anisaldehyde (4-methoxybenzaldehyde) (0.01ml) and p-chloroacetophenone (0.01ml) were dissolved in minimum amount of alcohol at room temperature. Sodium hydroxide solution (0.02mol) was added slowly and the mixture stirred for 12 hours until the entire mixture becomes very cloud. Then the mixture was poured slowly into 400ml of water with constant stirring and kept in refrigerator for 24 hours. The light yellow coloured precipitate was obtained. The obtained precipitate was filtered. Washed and recrystallized from ethanol.



1-(4-chlorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one

Synthesis of 1-(4-chlorophenyl)-3-phenyl-prop-2-en-1-one:-

Equimolar quantities of 4-chloroacetophenone and benzaldehyde (0.01ml) were dissolved in minimum amount of alcohol at room temperature. Sodium hydroxide (0.02ml) was added slowly and mixture stirred for 12 hours until the entire mixture becomes very cloud. Then the mixture was poured slowly into 400ml water with constant stirring and kept in refrigerator for 24 hours. The precipitate obtained was filtered. Washed and recrystallized from ethanol.



(E)-1-(4-chlorophenyl)-3-phenyl-prop-2-en-1-one

General Procedure of Recrystallized Of Product:

Recrystallization is a procedure for purifying compounds. First take washed beaker and dry it. Add 20ml solvent in beaker with small amount of compound. Mixture is heated when it was completely dissolved and mixture was filtered with help of funnel. The solution is allowed to cool over night and crystal was obtained. Weigh your crystals. Submit the purified product in a properly labeled sample vial.

General Procedure for synthesis of Isoxazole derivatives:

2.5.1 Synthesis of 5-(4-bromophenyl)-3-(4-nitrophenyl)-isoxazole: 1-(4-bromophenyl)-3-(4-nitrophenyl)-prop-2-en-1-one (0.01mol) react with hydroxylamine hydrochloride (0.01mol) in presence of ethanol (25ml) was refluxed for 6 hours. The mixture was concentrated by distilling out the solvent under reduced pressure and poured into ice water. The precipitate obtained was filtered, washed and recrystallized. The pale white colour precipitate of isoxazole was obtained.

Synthesis of 5-(4-chlorophenyl)-3-(4-methoxyphenyl)-isoxazole: 1-(4-chlorophenyl)-3-(4methoxyphenyl)-prop-2-en-1-one (0.01mol) react with hydroxylamine hydrochloride (0.01mol) in presence of ethanol (25ml) was refluxed for 6-8 hours. The mixture was concentrated by distilling out the solvent under reduced pressure and poured into ice water. The precipitate obtained was filtered, washed and recrystallized. This product was sticky and pet ether was used to remove stickiness.

Synthesis of 5-(4-chlorophenyl)-3-phenyl-isoxazole: 5-(4-chlorophenyl-3-phenyl-prop-2-en-1-one (0.01mol) react with hydroxylamine hydrochloride (0.01mol) in presence of ethanol (25ml) was refluxed for 6 hours. The mixture was concentrated by distilling out the solvent under reduced pressure and poured into ice water. The precipitate obtained was filtered, washed and recrystallized.

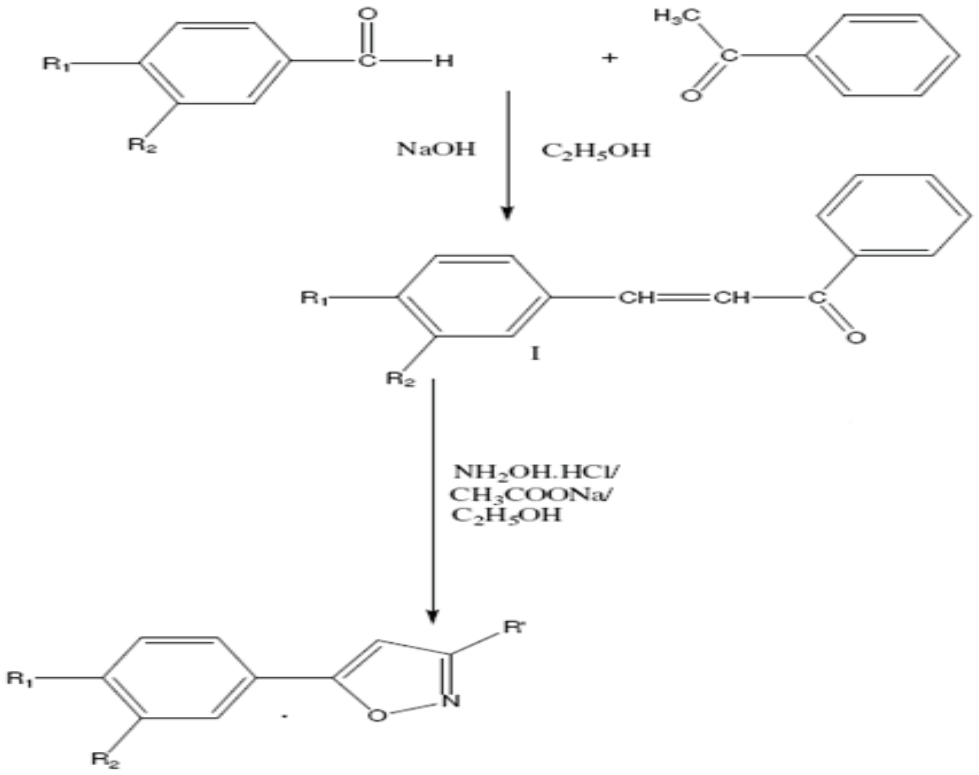
Chemical Reaction Of Isoxazole:-

STEP 1st:

Substituted acetophenone and substituted benzaldehyde react with each other in presence of ethanol. Sodium hydroxide added slowly in this mixture and water was removed from it. Then chalcone was prepared.

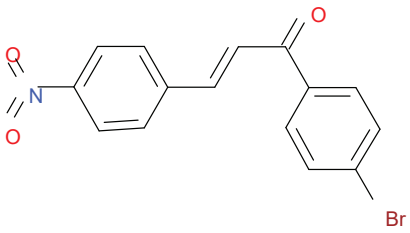
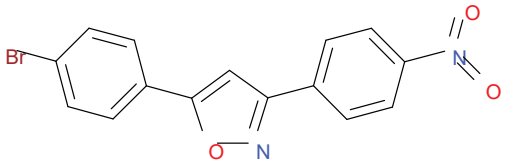
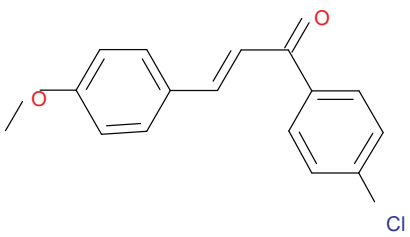
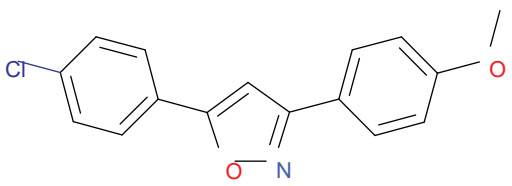
STEP 2nd:

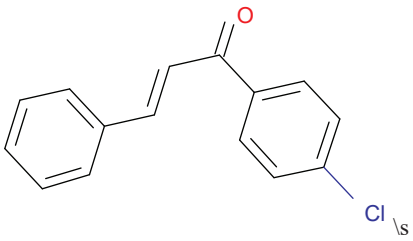
Chalcones react with hydroxylamine hydrochloride in presence of ethanol.



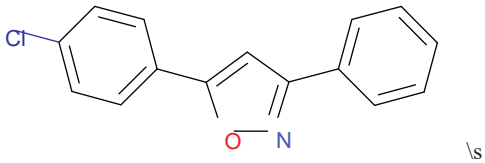
3. RESULTS:

Table-1: Detail Of Synthesized Compounds

Sr. No.	Reactant		Product
1.		$NH_2OH.HCL/C_2H_5OH$	
2.		$NH_2OH.HCL/C_2H_5OH$	

3. 

$NH_2OH.HCL/C_2H_5OH$



3.1 Spectral Studies of Isoxazole Derivatives

Table-2: IR and NMR Spectral Data:

Sr. No.	Sample	IR(KBr)	^{1}H NMR ($CDCl_3$)	^{13}C NMR
1.	**5-(4-bromophenyl)-3-(4-nitrophenyl)-isoxazole**	3052cm^{-1}(Ar-H), 1665cm^{-1}(C=N), 1274cm^{-1}(N-O), 1174cm^{-1}(C-O), 1585cm^{-1}(Ar-NO_2), 673cm^{-1}(C-Br).	6.9-8.12 ppm multiplet,	-
2.	**5-(4-chlorophenyl)-3-(4-methoxyphenyl)-isoxazole**	3050cm^{-1}(Ar-H), 1663cm^{-1}(C=N), 1270cm^{-1}(N-O), 1175cm^{-1}(C-O), 710cm-1(C-Cl). -	6.6-8.8ppm multiplet (8H),1.1ppm singlet (3H)	157.10ppm (-C=N-O), 114.82ppm (-C=C-O), 159.7ppm(-C-O), 55.43(-OCH_3), 100-160ppm(Ar-C)
3.	**5-(4-chlorophenyl)-3-phenyl-isoxazole**	3055cm^{-1}(Ar-H), 1660cm^{-1}(C=N), 1275cm^{-1}(N-O), 1175cm^{-1}(C-O), 1585cm-1 705cm-1(C-Cl). -	6.84-8.10ppm multiplet (8H)	155.35ppm(-C-O-N), 116.67ppm(-C=C-O), 149.87ppm(-C=N-O), 136.7ppm(-C-C=N), 134.31ppm(-C-C-O)

Antimicrobial Study:

Methodology

The evaluation of the anti microbial effect of Synthesized Isoxazole was done by using Agar Well Diffusion method in the Department of microbiology, ISFAL (A unit of ISF College), Moga Punjab (INDIA). Through Agar well diffusion method with strain Staphylococcus aureus MTCC-737, E.coli MTCC-1687, Candida albicans MTCC- 227&Aspergillus niger MTCC-282.

3.2.2 Standard Drug Used: Ampicillin trihydrate ISFAL/WS/A22 & Ketoconazole ISFAL/WS/K02

3.2.3 Preparation Of Media And Media Plates: Antibiotic Assay Medium No 11 (30.5 gm/1000ml of distilled water) was dissolved and added in a conical flask. Then the flask was plugged with cotton and autoclaved for complete sterilization. The sterilized media was poured in sterile petri dishes aseptically in a laminar flow. After solidifying of Agar plates (nearly about 15 to 20 minutes) they were kept inverted in incubator at 35±2°C for overnight for checking any contamination. The ready Agar plates then transferred in zip seal plastic cover and kept in a cold room.

3.2.4 Procurement Of Cultures: The pathogenic strains of different species of E.coli (MTCC-1687) and Staphylococcus aureus (MTCC-737) bacteria and Aspergillus niger (MTCC-282), Candida albicans MTCC- 227 Fungus were procured from Department of Microbiology I.S.F. college of Pharmacy, Moga The cultures were in freeze dried form (i.e. in dormant state). So, their revival was necessary. For this 100 ml nutrient broth medium was made and transferred in five small conical flasks (of quantity 100ml) 20ml each. The flasks were capped with cotton plug and autoclaved at 121°C for 15 minutes at 15 lb pressure per square inch.**4.5 Spreading:** For isolation of micro- organisms in pure form without contamination, streaking was done on solid media plates by applying a microbial culture with a loop to the surface of Agar in a petri plate and spreading them with a sterile spreader. Already prepared solid media plates were

used for streaking process. A drop of previously made broth cultures of E.coli, Staphylococcus aureus Candida albicans and Aspergillus niger respectively was added at one edge of the two sets each of four agar plates and the spreading of cultures was done with sterilised spreader. Each time the spreader was sterilised on the burner flame and cooled in to the edge of agar in the respective plate. The spread of 16 culture plates, each set of 4 loaded each of E.coli, Staphylococcus aureus Candida albicans and Aspergillus niger respectively.

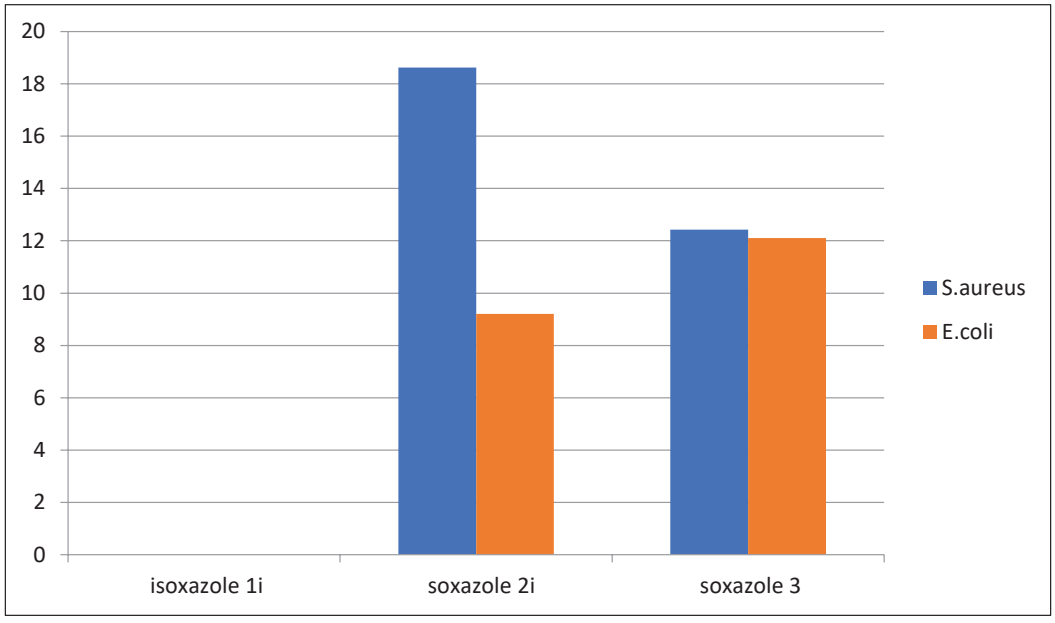
Loading Of The Plates And Measurement Of Zone Of Inhibition:

By using sterile cavity cork borer of 8mm size, wells were made in the centre of each of incubated culture plate to enable the introduction of the test sample and standard control. With the help of micropipette 100µl of concerned sample of aqueous were introduced into well of each plate streaked with different bacterial and fungal stains of E.coli, Staphylococcus aureus Candida albicans and Aspergillus niger respectively. For comparison one plate each for E.coli and Staphylococcus aures was loaded with Ampicillin trihydrate and for Aspergillus niger, Candida albicans with Ketoconazole. Then the plates were allowed to stand by for 30 minutes and were incubated for a time period of 24 hrs at the temperature of 37°C. The zone of inhibition was examined and measured with the help of antibiotic zone reader.

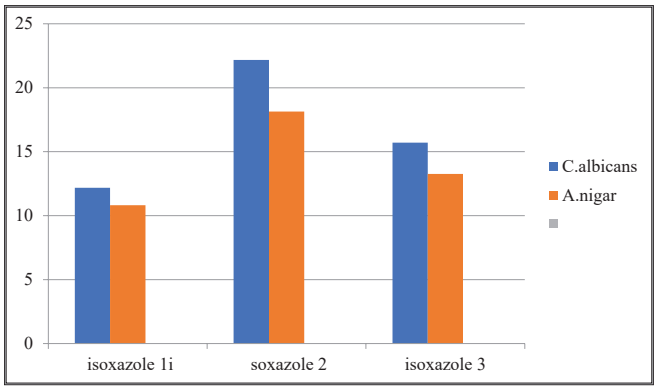
Table: 3 Zone Of Inhibition

S. No.	Compound 100mcg/ml	Zone of inhibition (mm)			
		Gram positive	Gram negative	Anti Fungal	
		S. aureus	E.coli	C. albicans	A. nigar
1.	5-(4-bromophenyl)-3-(4-nitrophenyl)-isoxazole	-	-	12.18	10.82
2.	5-(4-chlorophenyl)-3-(4-methoxyphenyl)-isoxazole	18.63	9.21	22.17	18.14
3.	5-(4-chlorophenyl)-3-phenyl-isoxazole	12.43	12.11	15.71	13.26
4.	Standard drug	23.76	19.24	25.48	21.34
5.	Solvent control	_	_	_	_

Graph: 1 Comparative Study Of Anti-bacterial Activity



Graph: 2 Comparative Study Of Anti-Fungal Activity



Where

i) Isoxazole 1 is 5-(4-bromophenyl)-3-(4-nitrophenyl)-isoxazole.
ii) Isoxazole 2 is 5-(4-chlorophenyl)-3-(4-methoxyphenyl)-isoxazole.
iii) Isoxazole 3 is 5-(4-chlorophenyl)-3-phenyl-isoxazole.

DISCUSSION:

In this experiment, For anti-bacterial activity the value of gram +ve of S. aureus species for isoxazole 2 is more as compared to isoxazole 1 and isoxazole 3 w.r.t standard drug. Value of gram –ve of E.coli for isoxazole 3 is higher than other two isoxazoles w.r.t standard drug. Similarly for anti-fungal activity the value of C.albicans and A.nigar species for isoxazole2 is more than other two isoxazoles with respect to standard drug. Isoxazoles 1, 2, 3 are used as a inhibitor and destructor of microbes. It have strong agent to kill all the microbes and inhibit the growth of microbes.

CONCLUSION:

Isoxazole derivatives occupy a distinct and unique place in our life because it is an essential constituent of all living cells. This heterocyclic moiety has great biological and medicinal significance. The various pharmacological activities discussed here proves biological importance of pyrazole moiety. So it can be seen from the literature review that Isoxazole ring containing heterocyclic system has wide medicinal applications. A vast literature has been accumulated over the years and chemistry of Isoxazole continues to be a blossoming field. The biological profiles of this new generation of Isoxazole represent much progress with regard to the older compounds. The derivatives of Isoxazole are used as inhibitors and destructor of microbes, they have strong agent to kill all the microbes and inhibit the growth of microbes. We hope that in the future many new biological profiles will be added to it and more investigations must be carried out to evaluate more activities of Isoxazole for many diseases whose treatment are challenging in the field of medical sciences. The versatile synthetic applicability and biological activity of these heterocycles will help the medicinal chemists to plan, organize and implement new approaches towards discovery of novel drugs.

ACKNOWLEDGEMENT:

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Declaration: *We also declare that all ethical guidelines have been followed during this work and there is no conflict of interest among authors.*

REFERENCES:

Kalirajan R., Sivakumar S.U., Jubie S.,. Gowramma B and. Suresh B (**2009**). Synthesis and Biological evaluation of some heterocyclic derivatives of Chalcones Ind .Jou.of chem. 0974-4290; 27-31.

Rajput S.S, ..Patel S.N, Nilesh B. (**2015**). Isoxazole- A Basic Aromatic Heterocycle, *Org. Biomol. Chem.* 0974-4290; 297-306.

Mohmad J, Hussiania. A (**2012**). Synthesis of some Heterocyclic Compounds derived from Chalcones. Int.J.Chem.Sci 2277-2669; 103-106.

LI Yang, XU Liangyu, GAO Wentao (**2015**). Facile synthesis of 5-bromotropono[c]-fused pyrazole and isoxazole, Turk J Chem, 2014; 38:470-476.S.S.Rajputet al / Int.J. Chem Tech Res. 8(7), pp 297-317. 317.

Soliman HA, Morsy EMH, Sayad HH, Abd Elwahed N. (**2013**). Synthesis and reactions of isoxazole Derivative for Biological evaluation., OJOC, 1(3):33-40.

Veeraswamy B , Kurumurthy C, Santosh Kumar G (2012). Synthesis of novel 5-substituted isoxazole -3-carboxamide derivative and cytotoxicity studies on lung cancer cell line,Ind .Jou.of chem., 51(B);1369-1375

Denmark SE , Kallemeyn JM . (**2005**). Synthesis of 3, 4, 5-Trisubstituted Isoxazoles via sequential [3+2] cycloaddition / Silicon based cross-coupling reactions, Jou. of Chem., 70:2839-2842.

Wang XZ , Jia J, Zhang Y , Xu WR, Liu W , . (**2014).**

Concise Synthesis and antimicrobial activities of new substituted 5-isoxazol penicillins, J.Chin.chem. SOC., 54; 643-652**.**

Lee Courtland , Robert E. (**2011**). Malononitriles And Cyanoacetamides Containing Isoxazoles. Pp;1-9**.**

Michael L . Edwards H, Stemerick D, and Prasad S, Synthesis of Chalcones:A new class of Antimitotic agents, *J. Med. Chem.***33**,1948-54.

Suvitha S, Ibrahim Abdelwahab S, Synthesis and antibacterial activity of substituted isoxazole derivatives, Scientific Reviews & Chemical Communication,.1420-3049; 6179-6182:**2012.**

Gucma M, Golebiewski WM, Michalczyk A, (2010). Synthesis and fungicidal activity of substituted isoxazolecarboxamides. , pesticides, (1-4):21-31

Maczynski M, Ryng S , Artym J , Kocieba M, Zimecki M. (**2014**). New lead structure in the isoxazole system: Relationship between quantum chemical parameters and immunological activity., APPDR. 71(1):71-83.

Narlawar R, Pickhardt M, Leuchtenberger S, Baumann K, Dyrks T, . (**2018**). Curcumin – Derived pyrazoles and isoxazoles: Swiss Army Knives or Blunt tools for Alzheimer's Disease, Chem Med Chem, 3:165-172.

Joshi VD, Kshirsagar MD., Singhal S. (**2012).** Synthesis and Biological evaluation of some novel isoxazoles and Benzodiazepines, JOCPR, 4(6);3234-3238**.**

Bhatt B A,. Dhar KL., Saxena. AK, (**2015**). Synthesis and biological evaluation of Chalcones and their derived Pyrazoles as potential cytotoxic agents, *Bio org. Med. Chem. Lett.,* **15 (3)**, 177-3180.

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