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Studies on some aspects of chemistry of Isoxazole derivatives

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Abstract: Isoxazole 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-resistent antibiotics such as cloxacillin, dicloxacillin and flucloxacillin. The synthetic androgenic steroid danazol also has an isoxazole ring. Synthesis of hybrid natural products has gained momentum in recent year.

Key words**:** *Isoxazole, anti-flammatory, antibacterial, beta-lactumase-resistent antibiotics.*

Introduction: Isoxazole are five membered heterocyclic ring containing adjacent one oxygen and one nitrogen atom on ring and consitituent an important family of heterocyclic chemistry.

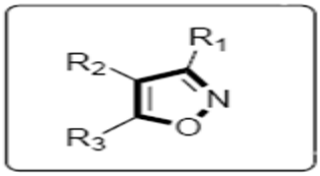
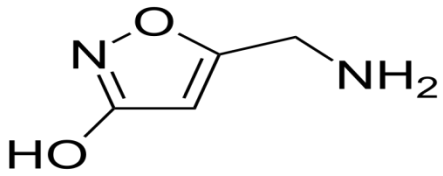
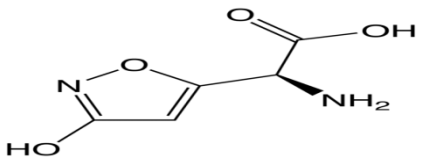


Fig. 1 Structure of isoxazole

The other systematic name of isoxazole is 1, 2- azole. 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. The naturally occurring antibiotic cycloserine, the monamine oxidase inhibitor isocarboxazide, isoxazole steroids, ibotenic acid, muscimol isolated from Amanita muscaria are potential isoxazole derivatives. They have been also used as selective COX-2 inhabitors such as valdecoxib and β_2-selective agonists such as Broxterol. Broxaterol is a potential bronchodialatory agent in the therapy of asthma.

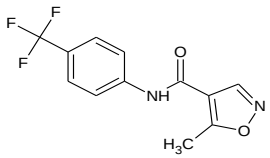
Isoxazole occurs from natural products like Ibotenic acid, they also exhibit potential therapeutic and many pharmacological activities. and Muscimol isolated from Amanita Muscaria.





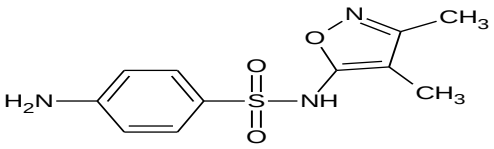
Muscimol **Ibotenic acid**

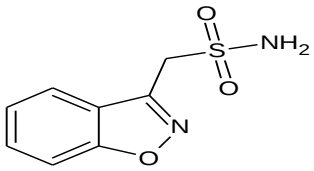
Drugs containing isoxazole: Leflunomide is used in active moderate to severe rheumatoid arthritis and psoriatic arthritis.



Leflunomide

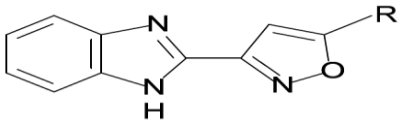
Sulfafurazole and Zonisamide are used as sulfonamide antibacterial antiepileptic respectively.



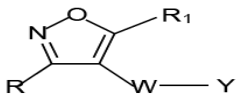


Sulfafurazole Zonisamide

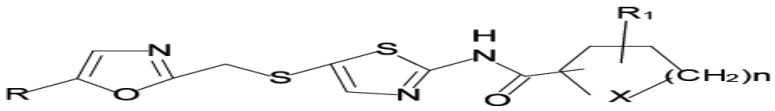
THERAPEUTIC IMPORTANCE OF ISOXAZOLE: Isoxazole derivatives shows various bio-activities such as Antimicrobial, Antiviral, Antibacterial, Anti-inflammatory, Fungicidal, Insectisidal, Herbicidal, Hypoglycergic, Musscle relaxant. Anti-inflammatory activity of some newly synthesized isoxazole have been reported by A. Ando. Teley Hand co-workers and Mishra et al. synthesized isoxazole and reported their activities. Inai, Masato Shi et al. also prepared isoxazole derivative possessing analgesic activity. Vekariya , N.A. et al. synthesized isoxazoles and tested their anticancer activity. Burk Robert M.et al. have prepared isoxazoles as antagonists.



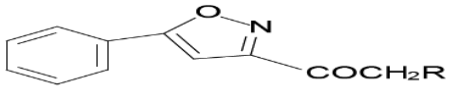
Dyke, Hazel Joun et al. have prepared quinazolinedione derivatives as inosine 5-monophosphate dehydrogenase (IMPDH) inhibitors for use in pharmaceutical compositions. Momose, Yu Mackawa et al. reported isoxazoles derivatives for prevention and treatment of diabetes.



Diana, Guy D. et al. documented some isoxazole derivatives as antipicor-navirus as agents. Misra, Raj, N et al. prepared isoxazoles as inhitors of cyclin dependent kinases.

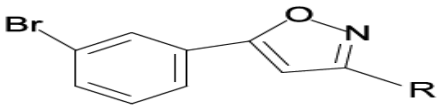


Shionogi et al. synthesized and tested isoxazole derivatives as antipyretic, analgesic, antiflammatory and anticough activity.



G. Daidone, D. Roffa et al. synthesized some noval isoxazole derivatives and tested for their analgesic and antiflammatory activities as well as for their acute toxicity and ulcerogenic effect.

Joshi et al. synthesized some isoxazole derivatives as antitubercular and antimicrobial agents. Antitumor activity of isoxazole derivatives have been reported by S. Rung and M. Scobie.



Experimental work:

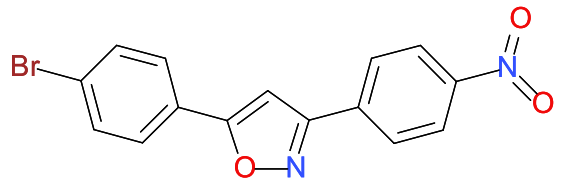
Techniques Used: Techniques used for the analysis of complexes are following. Given below shows the various instruments used during project.

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.

General Procedure for 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.



5-(4-bromophenyl)-3-(4-nitrophenyl)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.

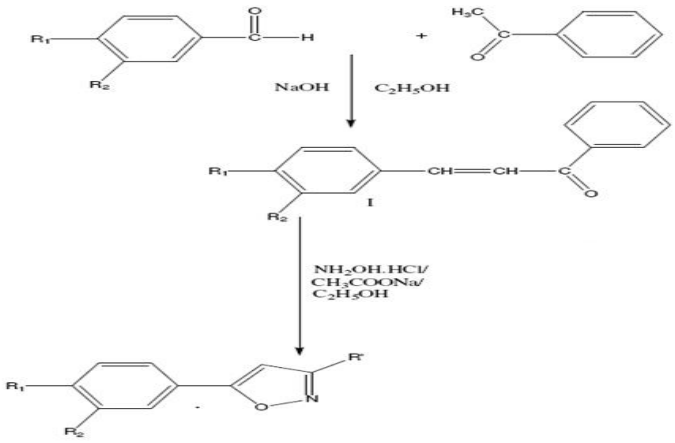
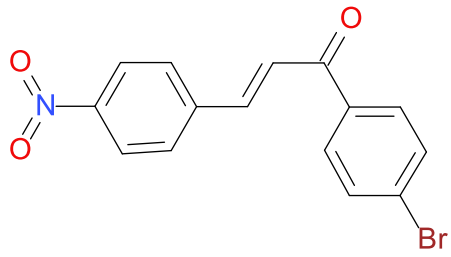
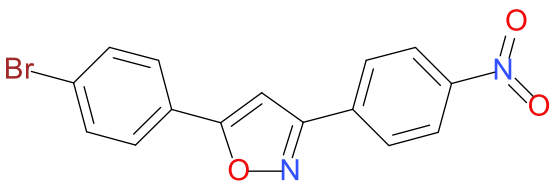
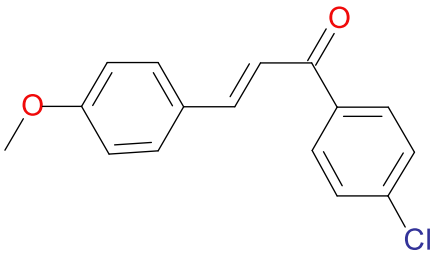
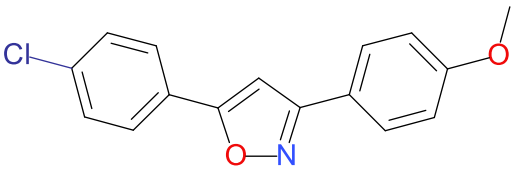
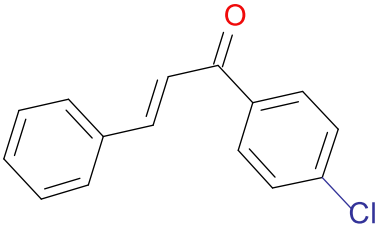
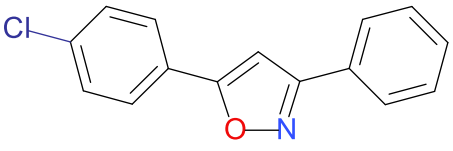


Table: 4 Detail of Synthesized Compounds

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

Spectral studies of isoxazole derivatives

IR Spectra of 5-(4-bromophenyl)-3-(4-nitrophenyl)-isoxazole: The IR spectra of compound 5-(4-bromophenyl)-3-(4-nitrophenyl)-isoxazole exhibit characterstics band at 3052 cm^{-1} attributing to Ar-H stretching vibrations. The band due to stretching vibration of C=N group was observed at 1665 cm^{-1}.The band due to stretching vibration of N-O and C-O groups was observed at 1274cm^{-1} and 1174cm^{-1} respectively. The band of Ar-NO_2 group was observed at 1585cm^{-1}. The band of C-Br group was observed at 673 cm^{-1}.

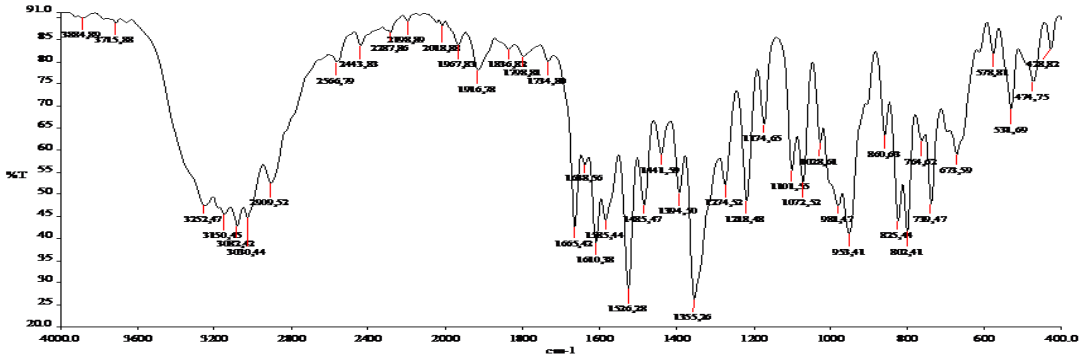


Fig 1: IR spectra of 5-(4-bromophenyl)-3-(4-nitrophenyl)-isoxazole

^{13}C NMR spectral analysis of isoxazole:

^{13}C NMR spectra of 5-(4-chlorophenyl)-3-(4-methoxyphenyl)-isoxazole: In ^{13}C NMR spectra of isoxazole compound was observed at 157.10ppm for –C=N-O group. The peak was observed at 114.82ppm for –C=C-O and 159.7ppm for -C-O groups in isoxazole ring. The peak of Methoxy group (–OCH_3) observed at 55.43ppm. The range of aromatic carbon was lies between 100-160ppm. This downfield shift is due to resonance of aromatic and electronegative group.

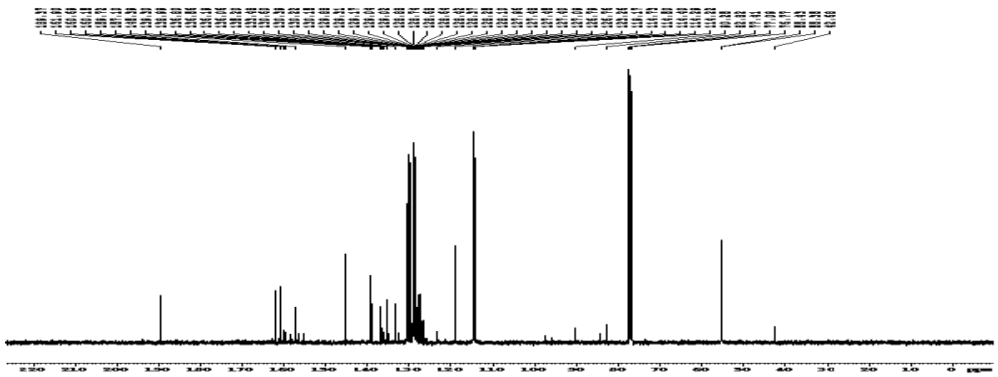


Fig: 2 ^{13}C NMR spectra of 5-(4-chlorophenyl)-3-(4-methoxyphenyl)-isoxazole

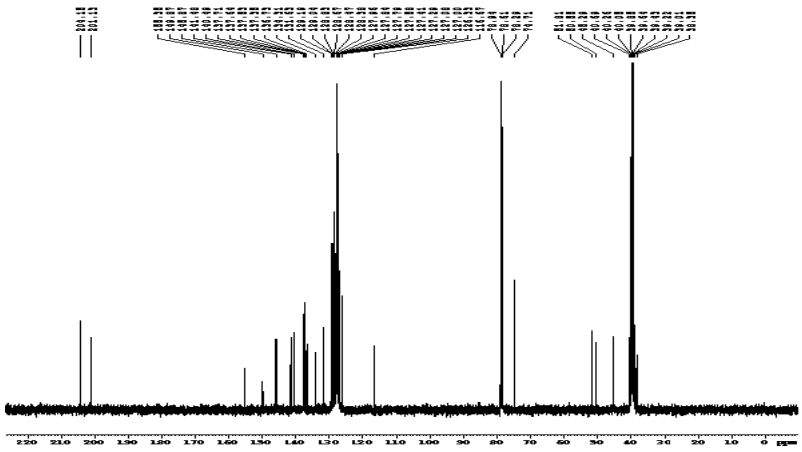


Fig 3: ^{13}C NMR spectra of 5-(4-chlorophenyl)-3-phenyl-isoxazole

^{1}H NMR spectra of 5-(4-chlorophenyl)-3-phenyl-isoxazole: In ^{1}H NMR spectra of isoxazole compound a complicated pattern was observed at δ 6.84-8.10ppm for eight aromaticprotons.

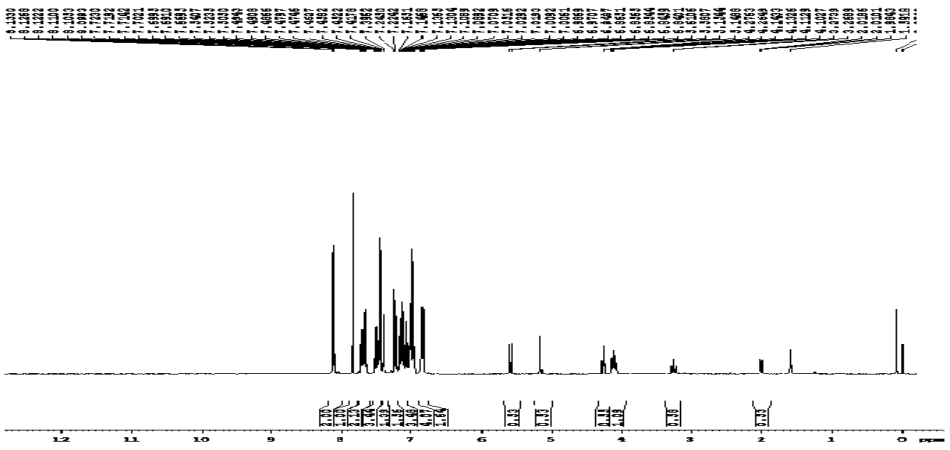


Fig: 4 ^{1}H NMR spectra of 5-(4-chlorophenyl)-3phenyl-isoxazole

Antimicrobial Study: Method used → Through Agar well diffusion method with strain Staphylococcusaureus

MTCC-737, E.coli MTCC-1687, Candida albicans MTCC- 227& Aspergillus niger MTCC-282.

Standard drug used → Ampicillin trihydrate ISFAL/WS/A22 & Ketoconazole ISFAL/WS/K02

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.

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.

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.

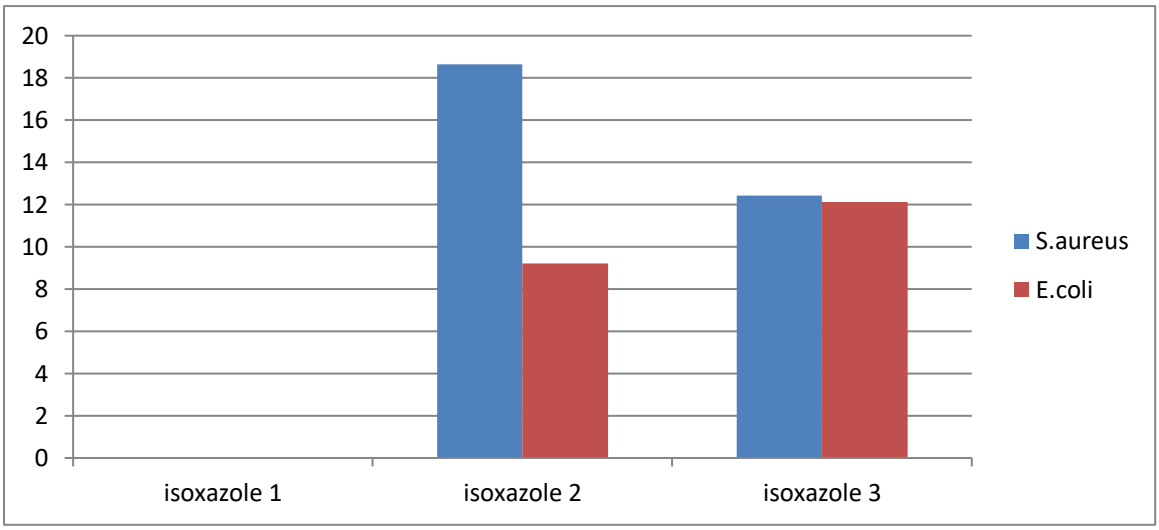
Loading of the plates and measurement of zone of inhibition: By using sterile cavity cork borer of 8mm size, wells were made in the centre of each of incubated culture plate to enable the introduction of the test sample and standard control. With the help of micropipette 100µl of concerned sample of 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: 6 Measurement of Zone of inhibition

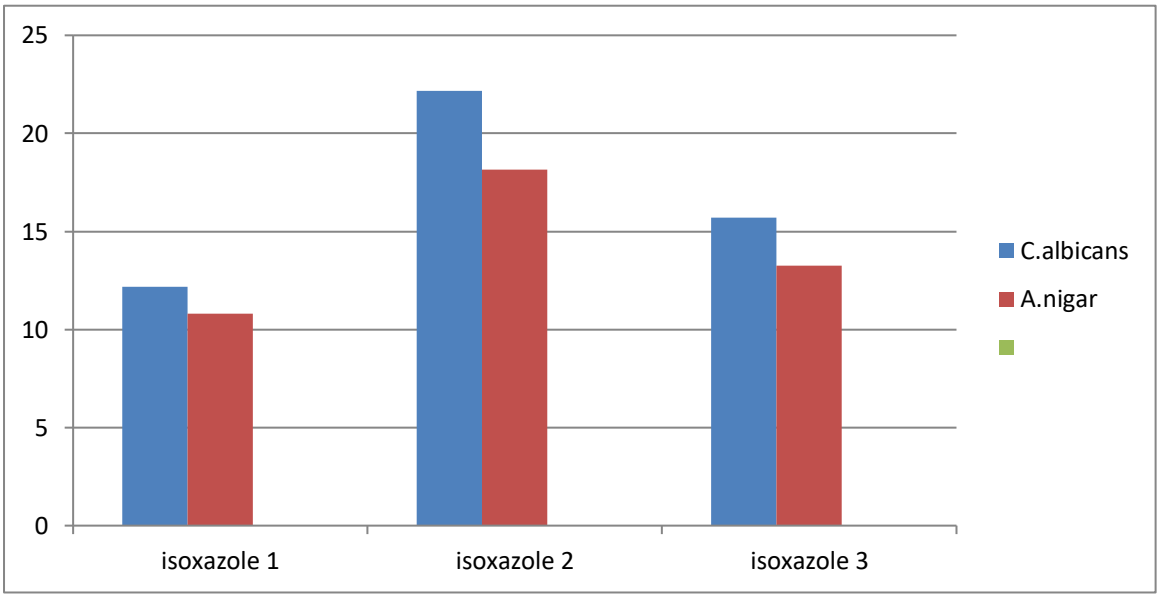
S. No.	Compound 100mcg/ml	Zone of inhibition (mm)			
		Gram positive	**Gram negative**	**Anti Fungal**	
		S. aureus	E.coli	C. albicans	A. nigar
1.	**5-(4-bromophenyl)-3-(4-nitrophenyl)-isoxazole**	-	-	12.18	10.82
2.	**5-(4-chlorophenyl)-3-(4-methoxyphenyl)-isoxazole**	18.63	9.21	22.17	18.14
3.	**5-(4-chlorophenyl)-3-phenyl-isoxazole**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

Result: 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 isoxazole 2 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.

Graph: 1 Readings for anti-bacterial activity



Graph: 2 Reading for 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.

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