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Synthesis, Characterization and Antimicrobial Activity of Isoxazoline Derivatives

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ABSTRACT:

In present study isoxazoline derivatives were synthesized and antimicrobial activity of different derivatives were checked with various microbial stain. Various intermediates was synthesized and characterized in between time to time by chromatography using TLC. An isoxazoline series of compounds were synthesized by condensation reaction of phenyl ethyl acetate with substituted benzaldehyde in presence of alcoholic sodium hydroxide to get intermediate phenethyl cinnamate (chalcones), which were further treated with hydroxylamine hydrochloride in presence of sodium hydroxide to get isoxazoline derivatives. The synthesized compounds were characterized on the basis of their spectral (IR) data and evaluated for the antimicrobial activity by using Zone of Inhibition by cup plate method.

Keywords: Isoxazoline, Antimicrobial activity, Zone of Inhibition.

1. INTRODUCTION

Isoxazolines are an important class of nitrogen and oxygen containing heterocycles that belong to the azoles family, and have gained great importance in the field of medicinal chemistry as anticancer agents. They are also reported to possess good antimicrobial, analgesic, anti-inflammatory activities.

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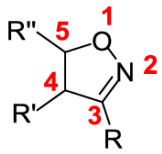
The isoxazoles are five membered rings containing a nitrogen and an oxygen atom adjacent to each other. Dihydro derivatives of isoxazoles are called as isoxazolines. Isoxazolines are generally synthesized from chalcones represents a class of compounds of great biological importance. Isoxazoline possess a broad spectrum of biological activity. Isoxazoline derivatives have been reported in the literature to possess antifungal, antibacterial, anticonvulsant, anti-inflammatory, antiviral analgesic activity and antitubercular. It serves as an important building block for the synthesis of biologically active molecules. The isoxazoline ring was named in-line with the Hantzsch-Widman nomenclature.



Isoxazoline Ring

2. MATERIAL AND METHODS

Synthesis of Phenethyl cinnamate:-Phenethyl acetate (0.1mol) was taken in 100 ml conical flask and dissolved in 25 ml of absolute ethanol. Ethanolic NaOH (0.1mol) was then added and stirred for 30 min. After 30 min. to this solution appropriately substituted benzaldehyde (0.1mol) was added and stirred for about 12 hour. After 12 hour precipitated product phenethyl cinnamate (Compound A) was obtained. This precipitated product was filtered, dried and recrystallized from ethanol. It is off-white crystalline powder, and melting point was found between 54-56^{o}C, R_f value 0.60, Molecular Weight-252.

Synthesis of 3-phenethoxy-5-phenyl-4,5-dihydroisoxazole:- The isoxazoline were prepared by reacting a mixture of purified phenethyl cinnamate (0.1mol) Compound A, hydroxylamine hydrochloride (0.03mol) and a solution of NaOH (0.1mol) in dry distilled ethanol by refluxing for 14-16 hour. After completion of the reaction, an excess of the solvent was removed by distillation and the resultant mass was poured into ice water with vigorous stirring. The solution was acidified with dilute HCl. It was kept overnight in cool condition. The resultant solid product (Compound B) was filtered, washed with sufficient cold water,

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dried and purified by recrystallization from ethanol. R_f value is 0.45. Melting Point-170-172^oC, Molecular Weight- 265. Newly formed compounds colour is off-white and Infrared Spectral Features (cm^{-1})2853 (-CH_2), 1162 (C-O).

Synthesis of 3-phenethoxy-5-(*p*-tolyl)-4,5-dihydroisoazole:-First took 100 ml conical flask Phenethyl acetate (0.1mol) was added in it and dissolved in 25 ml of absolute ethanol. Ethanolic NaOH (0.1mol) was then added and stirred for 30 min. After 30 min. to this solution appropriately substituted methylbenzaldehyde (0.1mol) was added and stirred for about 12 hour. After 12 hour precipitated product was obtained. This precipitated product was filtered, dried and recrystallized from ethanol. The isoxazoline were prepared by reacting a mixture of purified chalcones (0.1mol), hydroxylamine hydrochloride (0.3mol) and a solution of NaOH (0.1mol) in dry distilled ethanol by refluxing for 12-13 hour. After completion of the reaction, an excess of the solvent was removed by distillation and the resultant mass was poured into ice water with vigorous stirring. The solution was acidified with dilute HCl. It was kept overnight in cool condition. The resultant solid product was filtered, washed with sufficient cold water, dried and purified by recrystallization from ethanol. The compound with Meltin Point109-111^oC, Molecular weight- 279 , R_f value- 0.42, solid state and Infrared Spectrum Features (cm^{-1})2936(CH3), 1024 &1108(C-O), 1424(-CH2).

Synthesis of 3-phenethoxy-5-phenyl-4,5-dihydroisoxazole:- Phenethyl acetate (0.1mol) was taken in 100 ml conical flask and dissolved in 25 ml ethanol. Ethanolic NaOH (0.1mol) was then added and stirred for 30 min. After 30 min. to this solution appropriately substituted benzaldehyde (0.1mol) was added and stirred for about 12 hour. After 12 hour precipitated product was obtained. This precipitated product was filtered, dried and recrystallized from ethanol. The isoxazoline were prepared by reacting a mixture of purified compound A (0.1mol), hydroxylamine hydrochloride (0.3mol) and a solution of NaOH (0.1mol) in dry distilled ethanol by refluxing for 14-16 hour. After completion of the reaction, an excess of the solvent was removed by distillation and the resultant mass was poured into ice water with vigorous stirring. The solution was acidified with dilute HCl. It was kept overnight in cool

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condition. The resultant solid product was filtered, washed with sufficient cold water, dried and purified by recrystallization from ethanol. The compound with Rf value is 0.50. Molecular Weight-265, Melting Point- 142-145^{o}C, solid state. Infrared spectrum Features (cm^{-1})2926 & 2946(C-H), 1236(C-O), 1135(-CH_2).

Synthesis of 5-(4-chlorophenyl)-3-phenethoxy-4,5-dihydroisoxazole:- Phenethyl acetate (0.1mol) was taken in 100 ml conical flask and dissolved in 25 ml of absolute ethanol. Ethanolic NaOH (0.1mol) was then added and stirred for 30 min. After 30 min. to this solution appropriately substituted chlorobenzaldehyde (0.1mol) was added and stirred for about 12 hour. After 12 hour precipitated product was obtained. This precipitated product was filtered, dried and recrystallized from ethanol. The isoxazoline were prepared by reacting a mixture of purified compound A (0.1mol), hydroxylamine hydrochloride (0.03mol) and a solution of NaOH (0.1mol) in dry distilled ethanol by refluxing for 14-16 hour. After completion of the reaction, an excess of the solvent was removed by distillation and the resultant mass was poured into ice water with vigorous stirring. The solution was acidified with dilute HCl. It was kept overnight in cool condition. The resultant solid product compound B was filtered, washed with sufficient cold water, dried and purified by recrystallization from ethanol. The compound with Melting Point of 135-138^{o}C, Solid State, Molecular Weight-299, Solubility in Ethanol. Infrared Spectrum Features (cm^{-1}) 815(-Cl), 1121(C-O), 2973(C-H).

Synthesis of 5-(4-methoxyphenyl)-3-phenethoxy-4,5-dihydroisoxazole:-To take Phenethyl acetate (0.1mol) was added in 100 ml conical flask and dissolved in 25 ml of ethanol. Ethanolic NaOH (0.1mol) was then added and stirred for 30 min. After 30 min. to this solution appropriately substituted methoxybenzaldehyde (0.1mol) was added and stirred for about 12 hour. After 12 hour precipitated product was obtained. This precipitated product was filtered, dried and recrystallized from ethanol. The isoxazoline were prepared by reacting a mixture of purified compound A (0.01mol), hydroxylamine hydrochloride (0.3mol) and a solution of NaOH (0.1mol) in dry distilled ethanol by refluxing for 13-14 hour. After completion of the reaction, an excess of the solvent was removed by distillation and the

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resultant mass was poured into ice water with vigorous stirring. The solution was acidified with dilute HCl. It was kept overnight in cool condition. The resultant solid product compound B was filtered, washed with sufficient cold water, dried and purified by recrystallization from ethanol. The compound with Melting Point of 170-172^{o}C, Rf value- 0.42, Molecular weight- 295, Solid state. Infrared Spectrum Features (cm^{-1})852(O-CH3), 1323(C-O), 1425(C-H).

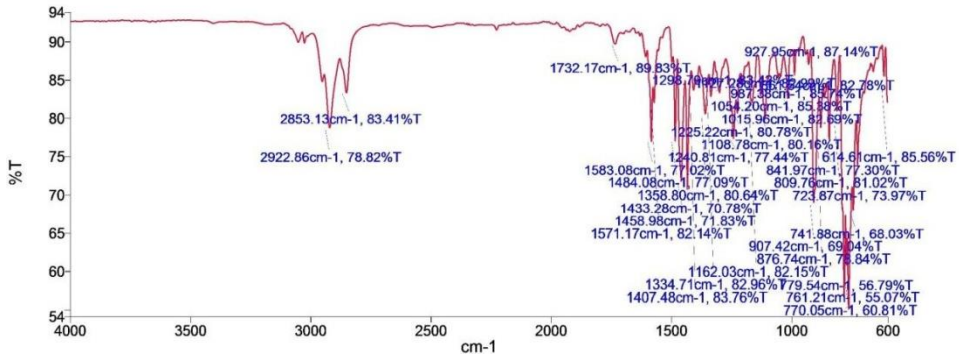

Fig.No. 1: IR Spectra of 3-phenethoxy-5-phenyl-4,5-dihydroisoxazole

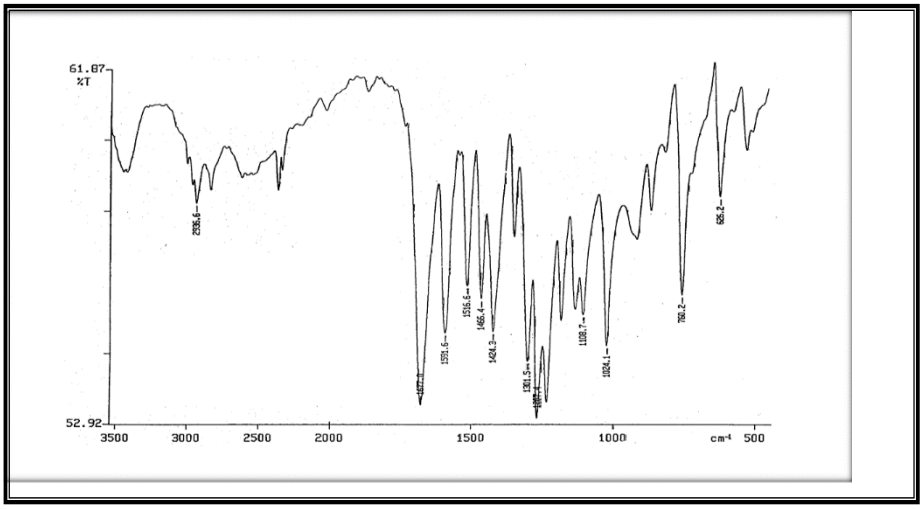

Fig.No. 2: IR Spectra of3-phenethoxy-5-(*p*-tolyl)-4,5-dihydroisoazole

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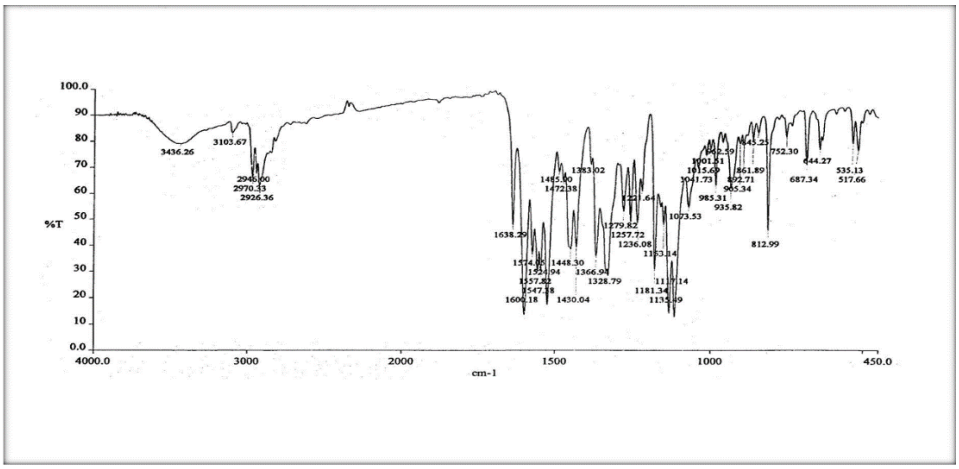

Fig.No. 3: IR Spectra of3-phenethoxy-5-phenyl-4,5-dihydroisoxazole

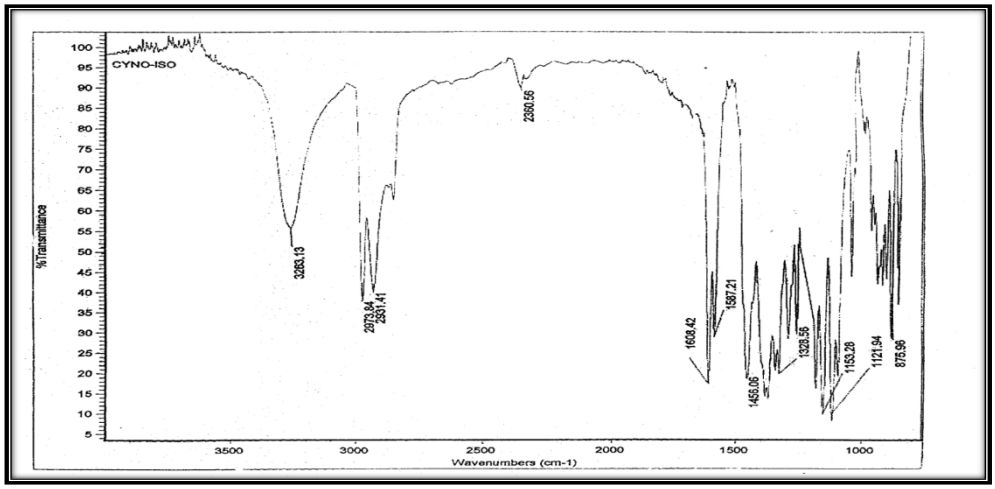

Fig.No. 4: IR Spectra of5-(4-chlorophenyl)-3-phenethoxy-4,5-dihydroisoxazole

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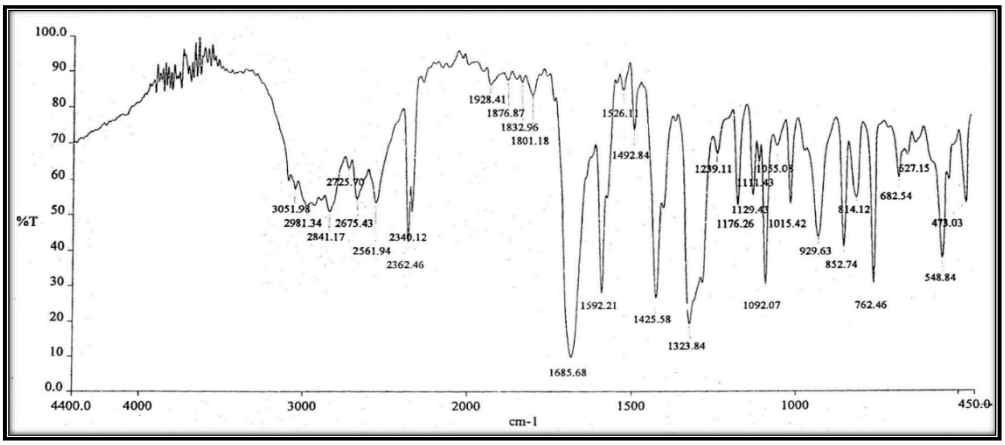
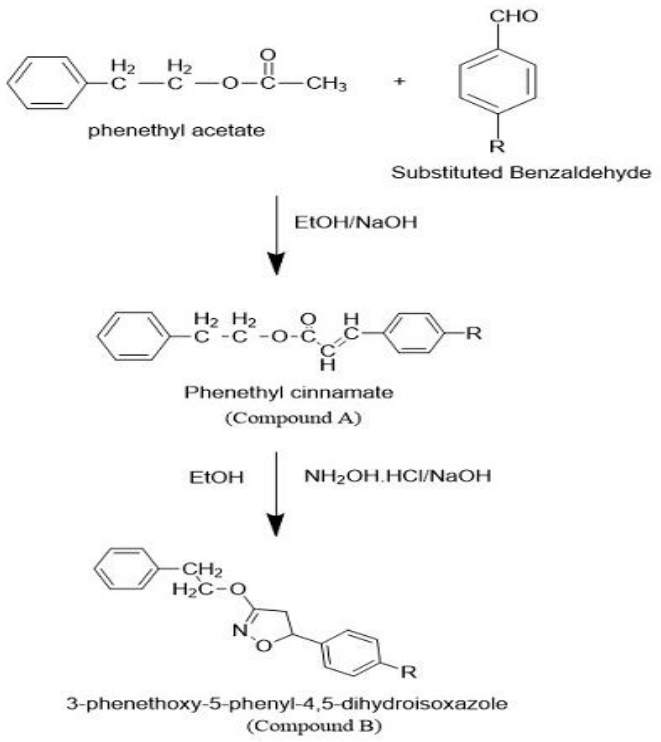


Fig.No. 5: IR Spectra of5-(4-methoxyphenyl)-3-phenethoxy-4,5-dihydroisoxazole

3. SCHEME:-



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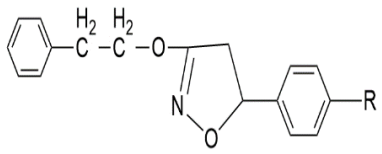

3-phenethoxy-5-phenyl-4,5-dihydroisoxazole

Compound Code	R
1R	$-CH_3$
2R	-H
3R	-Cl
4R	$-OCH_3$

4. ANTIMICROBIAL ACTIVITY

The antimicrobial activity of synthesized compound were evaluated by using Zone of Inhibition by cup plate method. This method is based on the diffusion of an antibiotic from a cavity, through the solidified agar layer of a petri-dish or plate, to an extent such that growth of the added microorganism is prevented entirely in a circular area or "zone" around the cavity containing a solution of the antibiotic.

❖ **Composition of Nutrient agar medium**

S. No.	Ingredients	Quantity
1.	Peptone	0.60g
2.	Yeast extract	0.30g
3.	NaCl	0.35 ml
4.	Agar	2.10g
5.	Distilled water	1000ml

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Materials:-

Microorganisms used:-

- Staphylococcus aureus (Gram positive)
- Bacillus subtilis (Gram positive)
- Escherichia coli (Gram negative)
- Pseudomonas aeruginosa (Gram negative)

Drug:

- Amoxicillin (Antibacterial)

Preparation of Drug Solution:-

The drug solutions were prepared by dissolving in Dimethyl sulfoxide (DMSO). The solution of test drug and standard drug (amoxicillin), were prepared at the concentration of 100µg/ml using DMSO as solvent.

Method:-

- A previously nutrient agar medium was inoculated with the required quantity of suspension of the microorganism.
- The suspension was added to the medium at a temperature between 40-45^{o}C and the inoculated medium was poured immediately into dried petri dish to occupy a depth of 3-4mm.
- The petri dishes were sterilized at 160-170^{o}C for 1 hour, before use.
- The holes about 8mm in diameter was cut in the medium with the help of a sterile cork borer.
- The test solution were placed on the holes (cups) of the medium.
- After addition of all the drugs, petri dishes were left standing for 1 to 4 hour at room temperature, as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications different solutions.
- All the Petri dishes were incubated for 24 hour at the required temperature, i.e. 37^{o}C for bacteria.
- After incubation, the diameters of the circular inhibition zones were measured.

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Antimicrobial Screening:-Antimicrobial test was carried out on four bacterial strains, namely Staphylococcus aureus (Gram positive), Bacillus subtillis (Gram positive), Escherichia coli (Gram negative), Pseudomonas aeruginosa (Gram negative). The results are given in below table.

Table No. 1 ZOI of Compounds

Microorganism	Zone of Inhibition (mm)					
	Compound B (100µg/ml)	Compound 1R (100µg/ml)	Compound 2R(100µg/ml)	Compound 3R(100µg/ml)	Compound 4R(100µg/ml)	Standard (100µg/ml)
Staphylococcus aureus	18	12	15	10	9	26
Bacillus subtillis	15	10	8	7	8	29
Pseudomonas aeruginosa	12	9	7	8	10	25
Escherichia coli	9	7	9	8	9	24

Standard drug- Amoxicillin

NA= No activity at this amount of test compound or standard

Table No. 2 : Physicochemical Properties of Compounds

S.No.	Name of compound	Molecular Formula	Molecular weight	Melting point	*R_f Value	% yield
1.	Phenethyl cinnamate	$C_{17}H_{16}O_2$	252	54-56^oC	0.60	65
2.	3-phenethoxy-5-phenyl-4,5-dihydroisoxazole	$C_{17}H_{15}O_2N$	265	170-172^oC	0.45	62

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3.	3-phenethoxy-5-(*p*-tolyl)-4,5-dihydroisoazole	$C_{18}H_{17}O_2N$	279	109-111^oC	0.42	56
4.	3-phenethoxy-5-phenyl-4,5-dihydroisoxazole	$C_{17}H_{15}O_2N$	265	142-145^oC	0.50	75
5	5-(4-chlorophenyl)-3-phenethoxy-4,5-dihydroisoxazole	$C_{17}H_{14}O_2NCl$	299	135-138^oC	0.45	58
6.	5-(4-methoxyphenyl)-3-phenethoxy-4,5-dihydroisoxazole	$C_{18}H_{17}O_3N$	295	170-172^oC	0.42	66
*Mobile phase- Chloroform: Methanol (8:2) Recrystallization solvent: Ethanol						

5. RESULTS AND DISCUSSION

In the synthesis of Isoxazoline derivatives starting with phenyl ethyl acetate and benzaldehyde to form compound A and this compound is react with hydroxylamine hydrochloride to give compound B.

Compound B & its derivatives were synthesized and characterized physiochemically as well as biologically.Based on the results compound B showed better results compared to its derivatives which were synthesized and evaluated.

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6. CONCLUSION

This research work was oriented towards the finding of newer Isoxazoline derivative with antimicrobial activity. Isoxazoline are an important class of five-membered heterocyclic compound belongs to the azoles family with remarkable biological activities. Increased activity in the area of isoxazoline synthesis over the last few decades has been driven by growth in their application in both synthetic and medicinal chemistry.

The different substituted isoxazoline derivatives were synthesized followed by cycloaddition reaction. In the synthesis of isoxazoline derivatives, Starting with phenyl ethyl acetate and end product of compound B and their derivatives were synthesized and characterized physiochemically as well as biologically.

All reactions were carried out under prescribed laboratory conditions. Solvents and reagents used were of laboratory grade and were purified by distillation and crystallization techniques whenever necessary and their melting point were checked with the available literature. All the reactions requiring anhydrous condition were conducted in well dried apparatus.

The synthesized compounds were purified by recrystallization. Melting points of newly synthesized compounds were determined by open capillary method and were uncorrected. The final products were purified by recrystallization and purity was checked by TLC.

The IR spectra of the compounds were recorded on FTIR spectrometer. The formed compound B (3-phenethoxy-5-phenyl-4,5-dihydroisoxazole) with melting point of 170-172^{o}C, solid state, molecular weight-265, solubility in ethanol, DMSO.

The newly synthesized isoxazoline derivatives were evaluated for their antimicrobial activity. The antimicrobial activities of synthesized compounds were evaluated by the zone of inhibition method. This method is based on the diffusion of an antibiotic from a cavity, through the solidified agar layer of a petri-dish or plate, to an extent such that growth of the added microorganism is prevented entirely in a circular area or “zone” around the cavity containing a solution of the antibiotic used for the study. Growth of inoculated microorganism is inhibited entirely in a circular area zone around the cup of petri dish containing a solution of the antibiotic and test compounds. For antibacterial activity

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Staphylococcus aureus (Gram positive), Bacillus subtillis (Gram positive), Escherichia coli (Gram negative), Pseudomonas aeruginosa (Gram negative) microorganism used. Compound B showed potent activity against microorganism and Amoxicillin used as a standard drug. The synthesized compound B showed effective antimicrobial activity. Based on the results compound B showed better results compared to derivatives which were synthesized and evaluated.

The homogeneity and purity of all the synthesized compounds was confirmed by sharp melting range and thin layer chromatography (TLC).

The chemical structures of all the synthesized compounds were confirmed by infrared absorption (IR). The IR spectra of the synthesized compounds exhibited commonly the absorption peak at 1162 (C-O), 2853 (-CH_2).

These synthesized compounds exhibit significant antimicrobial activity. For antimicrobial activity used nutrient agar medium, microorganisms, prepared test solution and standard drug solution. For antimicrobial screening, gram-positive and gram-negative microorganism were used. And for antimicrobial activity studies, disc diffusion method was used. All the synthesized isoxazoline derivatives have shown moderate to weak antibacterial activity. In the antibacterial study, diameter of Zone (mm) show the potency of newly synthesized drugs against microorganism. These clearly indicate that new isoxazoline derivatives can be effectively synthesized by method mentioned in this study.

On performing the synthesis of Isoxazoline derivatives, it was concluded that the method used was simple, rapid, less-time consuming and cost-effective. In this synthesis, chemicals used which were easily available. It have good results.

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