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DESIGN & SYNTHESIS OF ISOXAZOLE DERIVATIVE FROM CHALCONES FOR ANTI MICROBIAL ACTIVITY

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ABSTRACT

The increasing emergence of drug-resistant microorganisms poses a significant challenge to public health worldwide. Therefore, there is a pressing need to develop novel antimicrobial agents with improved efficacy. The Isoxazole scaffold has shown promise as a potential antimicrobial agent. This study aims to synthesize a series of Isoxazole analogues and evaluate their antimicrobial activity.

OBJECTIVE: Find out novelty by using software Scifinder. Synthesis and characterize the molecules which shows significant in- silico results. Assess the toxicity of the synthesized compounds. Evaluate biological efficacy of synthesized compounds for anti-bacterial activity using suitable in-vitro methods.

METHODS: Ethyl acetoacetate and ammonium acetate was mixed with certain amount of corresponding aldehydes in methanol. The resulting mixture was left under reflux for 6 hours and the solid product formed was separated by filtration, purified by recrystallization from ethanol, washed with ethanol, and then dried. The compounds were further evaluated for their anti-bacterial activity.

RESULTS: , 4-dihydro pyridine analogues were prepared by the reaction of ethyl acetoacetate was mixed with certain amount of corresponding aldehydes in of methanol. The resulting mixture was left under reflux for 6 hrs, and the solid product formed was separated by filtration, purified by recrystallization from ethanol, washed with ethanol and then dried. The compounds were tested for anti-bacterial activity against (staphylococcus aureus (+ ve), Escherichia coli (- ve), klebsiella pneumonia (- ve) and bacillus (+ ve). The synthesized compounds tested for anti-bac erial activity by the microbiological assay method. The compounds AB 1, AB 3, AB6 shown maximum zone of inhibition than the standard drug.

CONCLUSION: In this work we have synthesized Isoxazole and our aim is to inspect these compounds against some kind of bacteria we have concluded that the synthesized compounds AB 1, AB 3, AB 6 have shown higher anti-bacterial activity compared to standard drug. This study aims to explore the antimicrobial potential of Isoxazole analogues. The results obtained from the synthesis and biological evaluation will contribute to the understanding of the structure-activity relationship and identify promising compounds for further development as antimicrobial agents. The development of novel and effective antimicrobial agents is crucial in combating the rising threat of drug-resistant microorganisms and improving global public health.

KEY WORDS: *Isoxazole Derivatives, Chalcones, Claisen– Schmidt Condensation, Cyclization, Hydroxylamine Hydrochloride, Thin Layer Chromatography (TLC), Heterocyclic Compounds, Synthetic Chemistry.*

1. INTRODUCTION

Heterocyclic compounds occupy a prominent position in medicinal chemistry owing to their remarkable structural diversity and extensive biological activities. These compounds are characterized by the presence of one or more heteroatoms such as nitrogen, oxygen, or sulfur within a cyclic ring system. The incorporation of heteroatoms into cyclic structures significantly influences their physicochemical properties, chemical reactivity, and biological behavior. Studies on the synthesis and biological evaluation of isoxazole derivatives have demonstrated that structural modifications significantly influence antimicrobial activity. In recent years, antimicrobial resistance has emerged as a major global health concern[1].

Chalcones represent another important class of compounds that have received considerable attention in medicinal chemistry. Chemically, chalcones are α, β-unsaturated ketones consisting of two aromatic rings connected through a three-carbon enone system. They are considered open-chain flavonoids and serve as key intermediates in the biosynthesis of flavonoids and isoflavonoids in plants. α, β-unsaturated carbonyl moiety present in chalcones is primarily responsible for their diverse biological activities[10].

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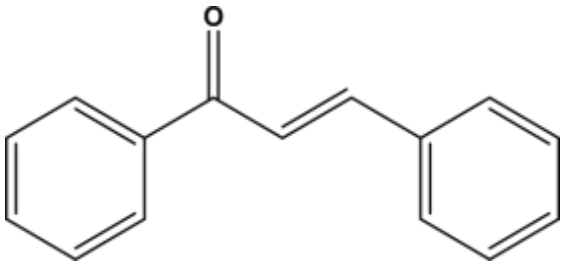

THE STRUCTURE OF CHALCONE

Chalcones are widely distributed in nature and occur in numerous fruits, vegetables, spices, and medicinal plants. They exhibit a broad range of pharmacological activities including antimicrobial, antifungal, antiviral, anti- inflammatory, antioxidant, anticancer, antimalarial, antitubercular, anti-HIV, and antidiabetic properties. Their biological significance, combined with ease of synthesis and structural flexibility, has made chalcones attractive targets for medicinal chemistry research. The Claisen–Schmidt condensation is a base-catalyzed crossed aldol condensation involving an aromatic aldehyde and an aromatic ketone.

In addition, Mass Spectrometry is employed to determine molecular weight and fragmentation patterns, thereby confirming the identity of synthesized compounds. Antimicrobial screening is an essential step in evaluating the biological efficacy of newly synthesized compounds. Various in vitro methods such as agar diffusion, cup plate, broth dilution, and minimum inhibitory concentration (MIC) determination are commonly used to assess antimicrobial activity. The synthesized compounds are generally tested against bacterial strains such as Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and Pseudomonas aeruginosa, as well as fungal strains including Candida albicans and Aspergillus Niger.

Structure–Activity Relationship (SAR) studies play an important role in understanding the relationship between chemical structure and biological activity. SAR investigations help identify structural features responsible for enhanced antimicrobial activity and provide guidance for the design of more potent compounds.

The present investigation is significant because it combines two biologically active pharmacophores, namely chalcones and isoxazoles, into a single molecular entity. The study is also expected to provide valuable information regarding the influence of different substituents on antimicrobial activity. Understanding these relationships may facilitate the rational design of more potent antimicrobial agents in future studies.

Furthermore, synthetic procedures and readily available starting materials make chalcone-derived isoxazole compounds attractive candidates for further pharmaceutical development[15]. The increasing prevalence of microbial resistance against existing antibiotics has become a global health concern. Pathogenic microorganisms have developed resistance to many conventional antimicrobial agents, resulting in reduced therapeutic effectiveness and increased treatment costs. Therefore, the discovery and development of new antimicrobial agents with improved efficacy and safety profiles remain an important area of pharmaceutical research[16].

Particularly because of their broad spectrum of biological activities, are of great interest in medicinal chemistry. In recent years, antimicrobial resistance has emerged as a major global health concern. The resistance of pathogenic microorganisms to antibiotics has created an urgent need for the development of new antimicrobial agents. Heterocyclic compounds, particularly isoxazole derivatives, have shown promising antimicrobial activity. The name of the systematic isoxazole is 1, 2-oxazole. Considered an important synthon because of its ability to undergo various chemical transformations leading to useful synthetic products. Isoxazole derivatives are present in the structures of many natural products and pharmaceutical agents. They have long been used in organic synthesis due to their broad spectrum of biological and pharmacological activities. Belonging to the azole family, containing adjacent oxygen and nitrogen atoms and one double bond in the ring. The weak nitrogen–oxygen bond present in isoxazolines contributes to their unique chemical reactivity[17].

Chalcones are an important class of naturally occurring and synthetic compounds characterized by the presence of an α, β-unsaturated carbonyl. System connecting two aromatic rings. Chalcone derivatives possess a wide range of biological properties, including antioxidant, antimalarial, and anticancer activities. The reactive double bond present in chalcones makes them valuable intermediates for the synthesis of numerous heterocyclic compounds, including isoxazole and pyrazole derivatives. The synthesis of isoxazole derivatives from chalcones generally involves cyclization reactions using hydroxylamine hydrochloride or related reagents. These reactions provide an efficient route for constructing the isoxazole ring system while retaining important structural features

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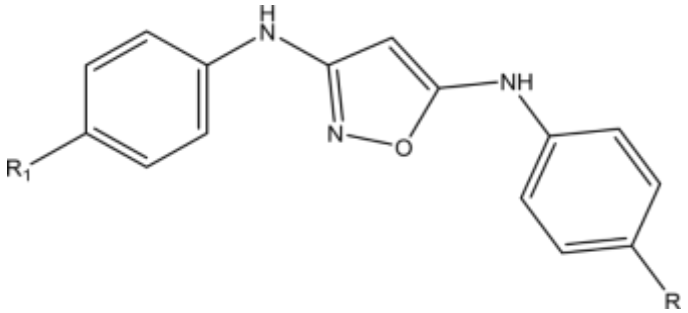
responsible for biological activities[18]. Pathogenic microorganisms continuously develop resistance mechanisms that reduce the effectiveness of existing drugs. Methicillin-resistant Staphylococcus aureus (MRSA), multidrug-resistant Escherichia coli, and several fungal pathogens pose significant challenges to public health.[19] Heterocyclic compounds occupy a central position in medicinal chemistry because of their wide range of biological activities and therapeutic applications. The increasing prevalence of microbial resistance against existing antibiotics has become a global health concern. Pathogenic microorganisms have developed resistance to many conventional antimicrobial agents, resulting in reduced therapeutic effectiveness and increased treatment costs. Therefore, the discovery and development of new antimicrobial agents with improved efficacy and safety profiles remain an important area of pharmaceutical research[20].

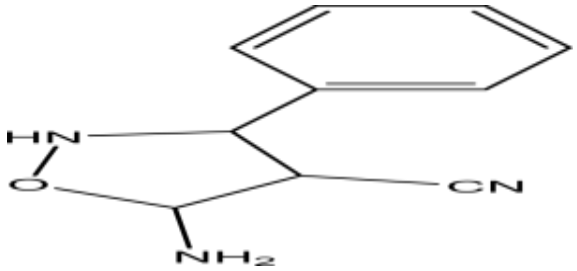
These derivatives have demonstrated promising antimicrobial activities against a variety of Gram-positive bacteria, Gram-negative bacteria, and fungal species. The antimicrobial activity of these compounds is attributed to their ability to interfere with vital cellular processes, including protein synthesis, enzyme activity, and cell wall biosynthesis. Structural modifications in the oxazole framework can significantly influence antimicrobial potency, and the synthesis of multi-substituted isoxazole derivatives from various aldehydes under mild reaction conditions. The study demonstrated that several synthesized oxazole derivatives exhibited significant antimicrobial activity, highlighting the potential of the oxazole scaffold in the development of new antimicrobial agents.

2. LITERATURE REVIEW

- **Patel Riddhip et al.** was Synthesis and Antibacterial Screening of Some Novel Isoxazole Derivatives



- **Hamid Beyzai et al.** was Green Multicomponent Synthesis, Antimicrobial and Antioxidant Evaluation of Novel 5-Amino-4-Isoxazole- 4- Carbonitriles



- **Asma Alshamari et al.** was Synthesis and Antimicrobial and Antioxidant Activities of 2-Isoxazoline Derivatives

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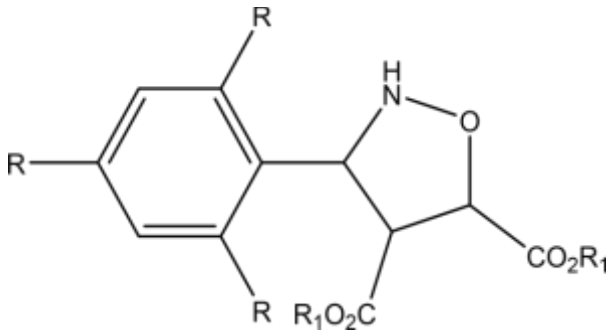
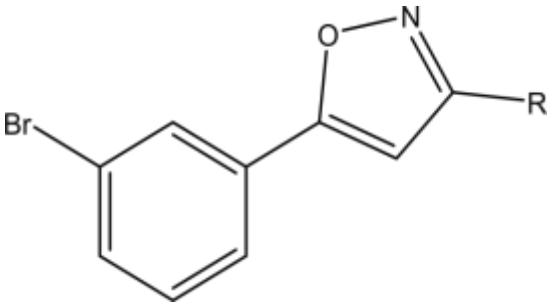
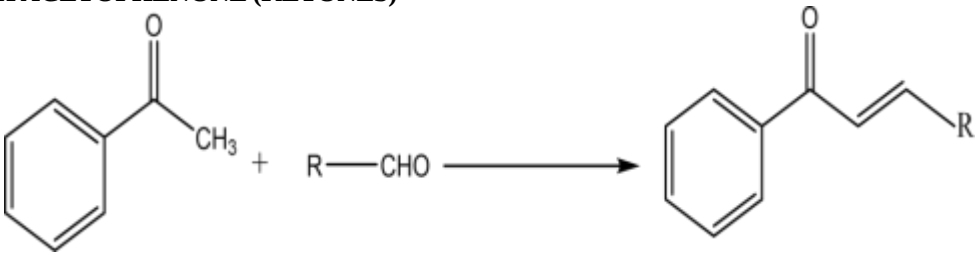


➢ **Archana Pathak et al.** was Synthesis and Antimicrobial Study of Isoxazole Derivatives

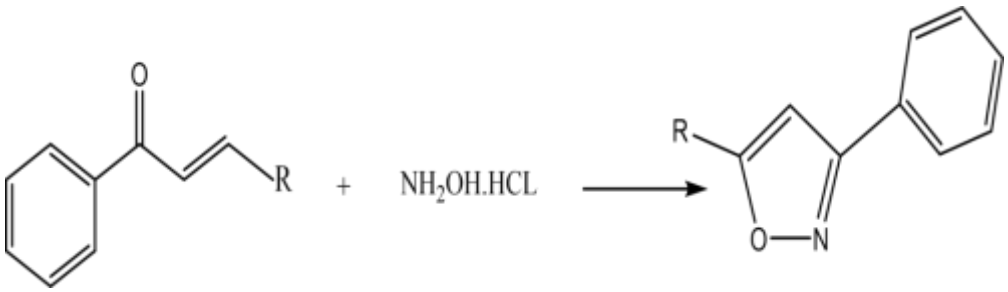


3. SCHEME OF WORK

STEP I: SYNTHESIS OF CHALCONES DERIVATIVES FROM DIFFERENT ALDEHYDES WITH ACETOPHENONE (KETONES)



STEP II: SYNTHESIS OF ISOXAZOLE DERIVATIVES FROM CHALCONES



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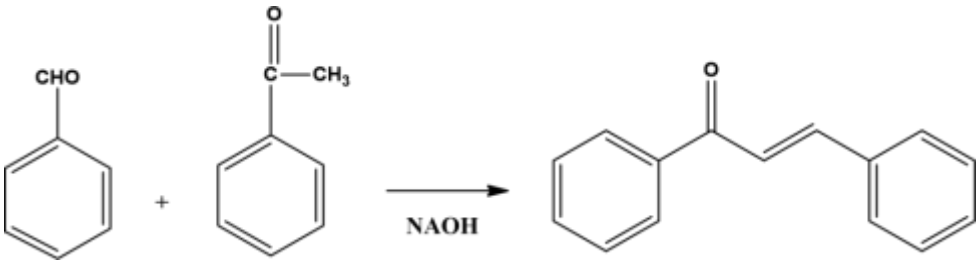
4. EXPERIMENTAL WORK

PREPARATION OF CHALCONE

- **COMPOUND AB-1**



BENZALDEHYDE ACETOPHENONE 3, 5-DIPHENYL-1, 2-OXAZOLE

STEP-1: METHOD OF PREPARATION:

Take a conical flask of 250m add ethanol (15ml) and add 10% of sodium hydroxide solutions (25ml), now slowly add acetophenone solution (5ml), add benzaldehyde solution (4.5ml). Dissolve the mixture properly then the formation of yellow- coloure we see. Now place the mixture on heating mantle/ in water bath (Lukewarm). After about 40min later the oil is separated from the mixture. Now place the conical flask on ice bath and stir them properly until we get the crude precipitate which is forms as yellow coloured solid or oily mass which solidifies on cooling.

STEP-2: PREPARATION OF MOBILE PHASE

Take a beaker and add N-hexane (3ml) and ethyl acetate (2ml) at a ratio of 3:2

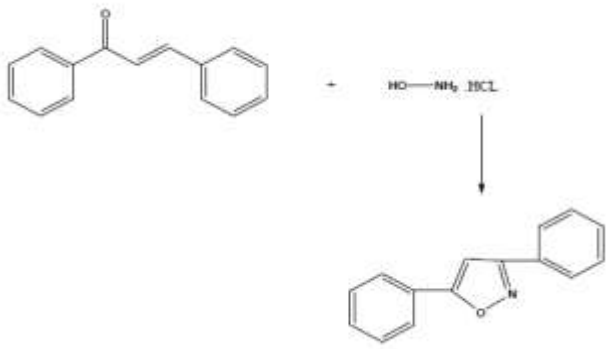
STEP-3: PRERATION OF TLC PLATE:

Take TLC plate cut the TLC plate with required length of 6 cm and to this plate make a mark of 1cm and draw a line.

STEP-4: Place the reaction on TLC plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate completely, spray the ninhydrin solution on TLC plate is placed under the UV cabinet and observe the spots presents on TLC plate.

STEP 5: After TLC cool the reaction mixture in an ice bath to enhance precipitation. Filter the precipitate using filter paper and dry the obtained product.

.PREPARATION OF ISOXAZOL FOR COMPOUND AB- 1



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3, 5-DIPHENYL-1, 2-OXAZOLE+ HYDROXYLAMINE HYDROCHLORIDE ⟶
3, 5-DIPHENYL ISOXAZOLE.

STEP 1: Dissolve 1. 46gm (0.01m) of 1- phenyl- but -2- en- 1- one in 20ml of ethanol in 100ml RBF. In a separate beaker, dissolve 0. 83g (0.012m) of hydroxylamine hydrochloride in 5ml of distilled water. Add 0.98g (0.012m) of sodium acetate. Transfer this solution to the flask containing the Chalcone derivative with constant stirring. Attach a condenser and heat the mixture under reflux at 70- 80 foe 4- 5hrs.

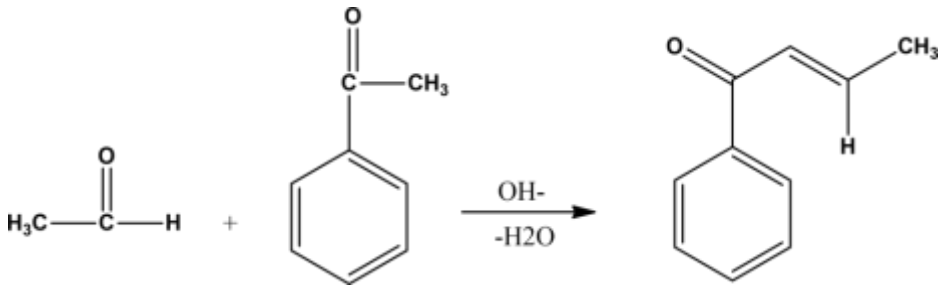
STEP 2: PREPARATION OF MOBILE PHASE: Take a beaker and add N-hexane (3ml) and ethyl acetate (2ml) at a ratio of 3:2

STEP-3: PRERATION OF TLC PLATE: Take TLC plate cut the TLC plate with required length of 6 cm and to this plate make a mark of 1cm and draw a line.

STEP-4: Place the reaction on TLC plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate completely, spray the ninhydrin solution on TLC plate is placed under the UV cabinet and observe the spots presents on TLC plate

STEP- 5: Upon completion, the mixture was allowed to cool to room temperature and then poured in to ice cold water with continuous stirring. The resulting precipitate was collected by filtration, washed thoroughly with cold water, and dried. The crude product was purified by recrystallization from ethanol to obtain the corresponding isoxazole derivative

- **COMPOUND AB-2**



ACETALDEHYDE ACETOPHENONE CROTONOPHENONE

STEP-1: METHOD OF PREPARATION: Take a conical flask of 250m add ethanol (15ml) and add 10% of sodium hydroxide solutions (25ml), now slowly add acetophenone solution (5ml), add acetaldehyde solution (4.5ml). Dissolve the mixture properly then the formation of yellow- color we see. Now place the mixture on heating mantle/ in water bath (Lukewarm). After about 40min later the oil is separated from the mixture. Now place the conical flask on ice bath and stir them properly until we get the crude precipitate which is forms as yellow coloured solid or oily mass which solidifies on cooling.

STEP-2: PREPARATION OF MOBILE PHASE: Take a beaker and add N-hexane (3ml) and ethyl acetate (2ml) at a ratio of 3:2

STEP-3: PRERATION OF TLC PLATE: Take TLC plate cut the TLC plate with required length of 6 cm and to this plate make a mark of 1cm and draw a line.

STEP-4: Place the reaction on TLC plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate completely, spray the ninhydrin solution on TLC plate is placed under the UV cabinet and observe the spots presents on TLC plate.

STEP 5: After TLC cool the reaction mixture in an ice bath to enhance precipitation. Filter the precipitate using filter paper and dry the obtained product

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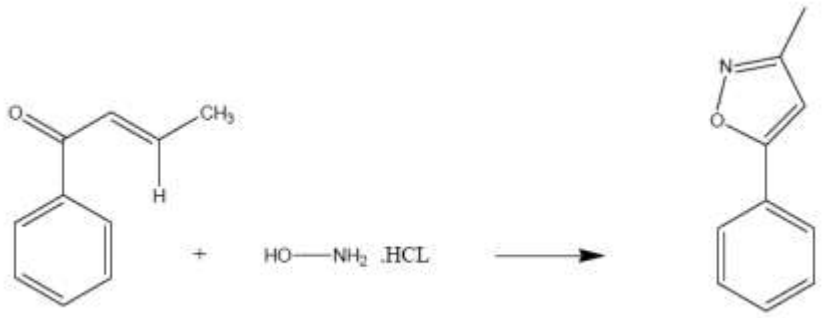
PREPARATION OF ISOXAZOLE FOR COMPUND AB- 2



CROTONOPHENONE+ HYDROXYLAMINE HYDROCHLORIDE → 3-METHYL-5-PHENYLISOXAZOLE

STEP 1: Dissolve 1. 46gm (0.01m) of 1- phenyl- but -2- en- 1- one in 20ml of ethanol in 100ml RBF. In a separate beaker, dissolve 0. 83g (0.012m) of hydroxylamine hydrochloride in 5ml of distilled water. Add 0.98g (0.012m) of sodium acetate. Transfer this solution to the flask containing the Chalcone derivative with constant stirring. Attach a condenser and heat the mixture under reflux at 70- 80 for 4- 5hrs.

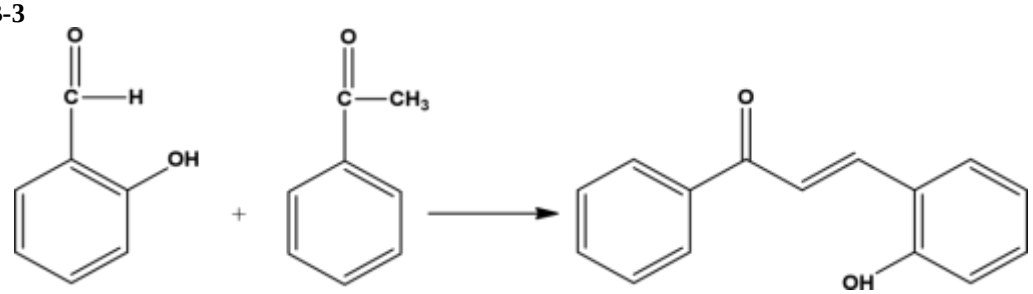
STEP 2: PREPARATION OF MOBILE PHASE: Take a beaker and add N-hexane (3ml) and ethyl acetate (2ml) at a ratio of 3:2

STEP-3: PRERATION OF TLC PLATE: Take TLC plate cut the TLC plate with required length of 6 cm and to this plate make a mark of 1cm and draw a line.

STEP-4: Place the reaction on TLC plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate completely, spray the ninhydrin solution on TLC plate is placed under the UV cabinet and observe the spots presents on TLC plate

STEP- 5: Upon completion, the mixture was allowed to cool to room temperature and then poured in to ice cold water with continuous stirring. The resulting precipitate was collected by filtration, washed thoroughly with cold water, and dried. The crude product was purified by recrystallization from ethanol to obtain the corresponding isoxazole derivative.

- **COMPOUND AB-3**



SALCYLALDEHYDE ACETOPHENONE 2- HYDROXIDE CHALCONE

STEP-1: METHOD OF PREPARATION: Take a conical flask of 250m add ethanol (15ml) and add 10% of sodium hydroxide solutions (25ml), now slowly add acetophenone solution (5ml), add salicylaldehyde solution (4.5ml). Dissolve the mixture properly then the formation of yellow- color we see. Now place the mixture on heating mantle/ in water bath (Lukewarm). After about 40min later the oil is separated from the mixture. Now place the conical flask on ice bath and stir them properly until we get the crude precipitate which is forms as yellow coloured solid or oily mass which solidifies on cooling. Filter the product using whatmann filter paper.

STEP-2: PREPARATION OF MOBILE PHASE: Take a beaker and add N-hexane (3ml) and ethyl acetate (2ml) at a ratio of 3: 2.

STEP-3: PRERATION OF TLC PLATE: Take TLC plate cut the TLC plate with required length of 6 cm and to this plate make a mark of 1cm and draw a line.

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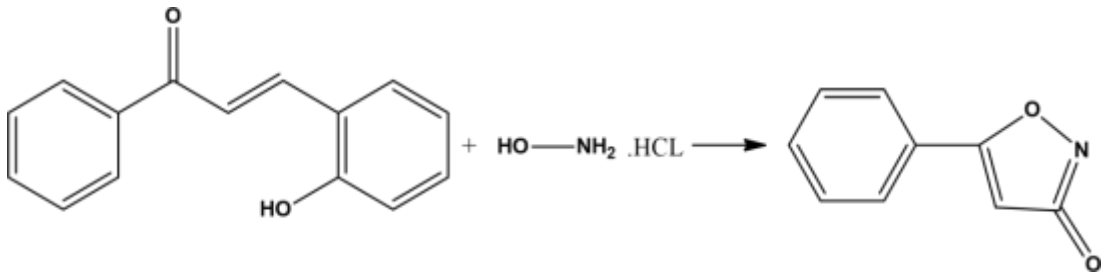
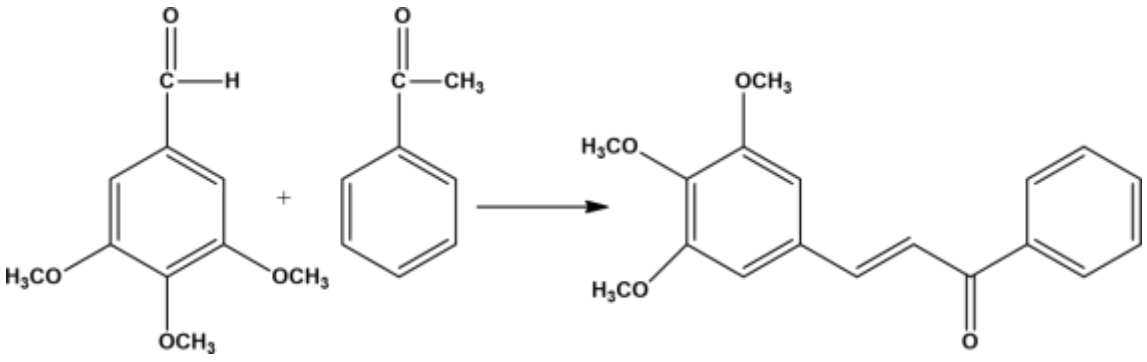
STEP-4: Place the reaction on TLC plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate completely, spray the ninhydrin solution on TLC plate is placed under the UV cabinet and observe the spots presents on TLC plate.
STEP 5: After TLC cool the reaction mixture in an ice bath to enhance precipitation. Filter the precipitate using filter paper and dry the obtained product

PREPARATION OF ISOXAZOLE FOR COMPOUND AB- 3



2- HYDROXIDE CHALCONE+ HYDROXYLAMINE HYDROCHLORIDE ⟶ 5-PHENYL-1, 2-OXAZOL-3(2H)-ONE
STEP 1: Dissolve 1. 46gm (0.01m) of 1- phenyl- but -2- en- 1- one in 20ml of ethanol in 100ml RBF. In a separate beaker, dissolve 0. 83g (0.012m) of hydroxylamine hydrochloride in 5ml of distilled water. Add 0.98g (0.012m) of sodium acetate. Transfer this solution to the flask containing the Chalcone derivative with constant stirring. Attach a condenser and heat the mixture under reflux at 70- 80 foe 4- 5hrs.
STEP 2: PREPARATION OF MOBILE PHASE: Take a beaker and add N-hexane (3ml) and ethyl acetate (2ml) at a ratio of 3:2
STEP-3: PRERATION OF TLC PLATE: Take TLC plate cut the TLC plate with required length of 6 cm and to this plate make a mark of 1cm and draw a line.
STEP-4: Place the reaction on TLC plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate completely, spray the ninhydrin solution on TLC plate is placed under the UV cabinet and observe the spots presents on TLC plate
STEP- 5: Upon completion, the mixture was allowed to cool to room temperature and then poured in to ice cold water with continuous stirring. The resulting precipitate was collected by filtration, washed thoroughly with cold water, and dried. The crude product was purified by recrystallization from ethanol to obtain the corresponding isoxazole derivative.

COMPOUND AB 4



3, 4, 5 TRIMETHOXY BENZALDEHYDE+ ACETOPHENONE⟶ 5-(3, 4, 5-TRIMETHOXYPHENYL)-1,2-OXAZOLE

STEP-1: METHOD OF PREPARATION: Take a conical flask of 250m add ethanol (15ml) and add 10% of sodium hydroxide solutions (25ml), now slowly add acetophenone solution (5ml), add 3, 4, 5 trimethoxy benzaldehyde solution (4.5ml). Dissolve the

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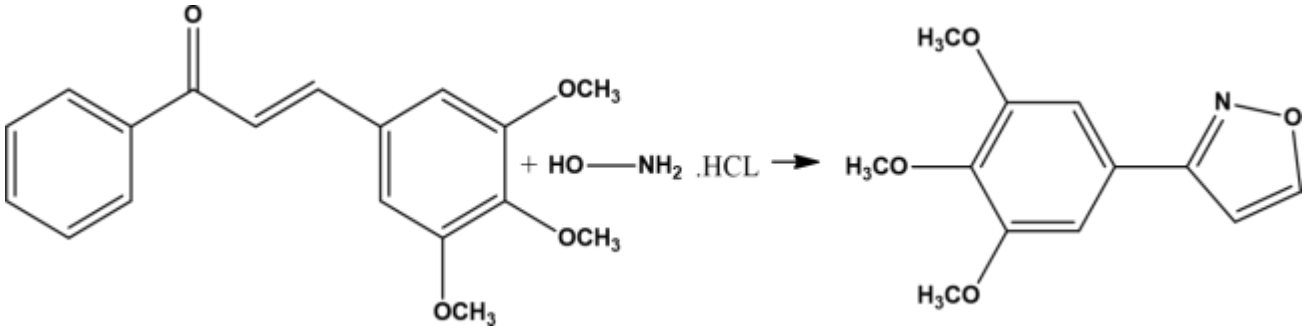
mixture properly then the formation of yellow- color we see. Now place the mixture on heating mantle/ in water bath (Lukewarm). After about 40min later the oil is separated from the mixture. Now place the conical flask on ice bath and stir them properly until we get the crude precipitate which is forms as yellow coloured solid or oily mass which solidifies on cooling.

STEP-2: PREPARATION OF MOBILE PHASE: Take a beaker and add N-hexane (3ml) and ethyl acetate (2ml) at a ratio of 3:2
STEP-3: PRERATION OF TLC PLATE: Take TLC plate cut the TLC plate with required length of 6 cm and to this plate make a mark of 1cm and draw a line.
STEP-4: Place the reaction on TLC plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate completely, spray the ninhydrin solution on TLC plate is placed under the UV cabinet and observe the spots presents on TLC plate.
STEP 5: After TLC cool the reaction mixture in an ice bath to enhance precipitation. Filter the precipitate using filter paper and dry the obtained product

PREPARATION OF ISOXAZOLE OF COMPOUND AB- 4



5-(3,4,5-TRIMETHOXYPHENYL)-1,2-OXAZOLE+ HYDROXYLAMINE HCL →
3- (3, 4, 5- TRIMETHOXYPHENYL) ISOXAZOLE.

STEP 1: Dissolve 1. 46gm (0.01m) of 1- phenyl- but -2- en- 1- one in 20ml of ethanol in 100ml RBF. In a separate beaker, dissolve 0. 83g (0.012m) of hydroxylamine hydrochloride in 5ml of distilled water. Add 0.98g (0.012m) of sodium acetate. Transfer this solution to the flask containing the Chalcone derivative with constant stirring. Attach a condenser and heat the mixture under reflux at 70- 80 foe 4- 5hrs.
STEP 2: PREPARATION OF MOBILE PHASE: Take a beaker and add N-hexane (3ml) and ethyl acetate (2ml) at a ratio of 3:2
STEP-3: PRERATION OF TLC PLATE: Take TLC plate cut the TLC plate with required length of 6 cm and to this plate make a mark of 1cm and draw a line.
STEP-4: Place the reaction on TLC plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate completely, spray the ninhydrin solution on TLC plate is placed under the UV cabinet and observe the spots presents on TLC plate
STEP- 5: Upon completion, the mixture was allowed to cool to room temperature and then poured in to ice cold water with continuous stirring. The resulting precipitate was collected by filtration, washed thoroughly with cold water, and dried. The crude product was purified by recrystallization from ethanol to obtain the corresponding isoxazole derivative

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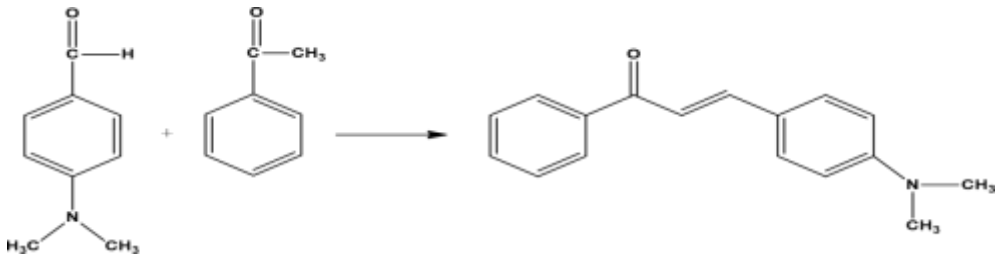
- **COMPOUND AB 5**



P-DIMETHYLAMINOBENZALDEHYDE+ ACETOPHENONE⟶
4-DIMETHYLAMINO CHALCONE

STEP-1: METHOD OF PREPARATION: Take a conical flask of 250m add ethanol (15ml) and add 10% of sodium hydroxide solutions (25ml), now slowly add acetophenone solution (5ml), add p- dimethylamino benzaldehyde solution (4.5ml). Dissolve the mixture properly then the formation of yellow- color we see. Now place the mixture on heating mantle/ in water bath (Lukewarm). After about 40min later the oil is separated from the mixture. Now place the conical flask on ice bath and stir them properly until we get the crude precipitate which is forms as yellow coloured solid or oily mass that solidifies on cooling.

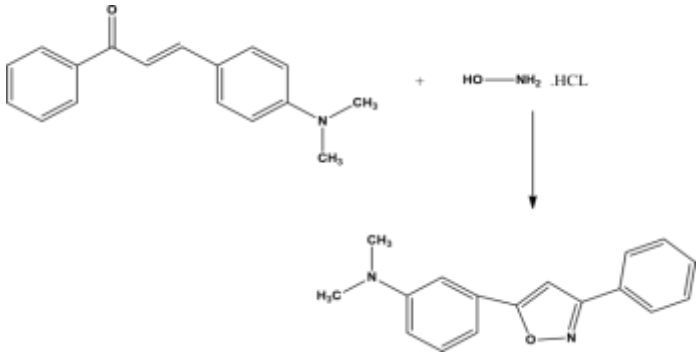
STEP-2: PREPARATION OF MOBILE PHASE: Take a beaker and add N-hexane (3ml) and ethyl acetate (2ml) at a ratio of 3:2

STEP-3: PRERATION OF TLC PLATE: Take TLC plate cut the TLC plate with required length of 6 cm and to this plate make a mark of 1cm and draw a line.

STEP-4: Place the reaction on TLC plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate completely, spray the ninhydrin solution on TLC plate is placed under the UV cabinet and observe the spots presents on TLC plate.

STEP 5: After TLC cool the reaction mixture in an ice bath to enhance precipitation. Filter the precipitate using filter paper and dry the obtained product

PREPARATION OF ISOXAZOLE FOR COMPOUND AB- 5



4- DIMETHYLAMINO CHALCONE+ HYDROXYLAMINE HCL ⟶
3- (4- DIMETHYLAMINOPHENYL) – 5- PHENYL- 5 OXAZOLINE

STEP-1: METHOD OF PREPARATION: Take a conical flask of 250m add ethanol (15ml) and add 10% of sodium hydroxide solutions (25ml), now slowly add acetophenone solution (5ml), add 3, 4, 5 trimethoxy benzaldehyde solution (4.5ml). Dissolve the mixture properly then the formation of yellow- color we see. Now place the mixture on heating mantle/ in water bath (Lukewarm). After about 40min later the oil is separated from the mixture. Now place the conical flask on ice bath and stir them properly until we get the crude precipitate which is forms as yellow coloured solid or oily mass which solidifies on cooling. Filter the product using whatmann filter paper.

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STEP-2: PREPARATION OF MOBILE PHASE: Take a beaker and add N-hexane (3ml) and ethyl acetate (2ml) at a ratio of 3:2.

STEP-3: PRERATION OF TLC PLATE: Take TLC plate cut the TLC plate with required length of 6 cm and to this plate make a mark of 1cm and draw a line.
STEP-4: Place the reaction on TLC plate above the line by using capillary tube, place the TLC plate in the mobile phase solution, after the TLC plate completely, spray the ninhydrin solution on TLC plate is placed under the UV cabinet and observe the spots presents on TLC plate.
STEP 5: Upon completion, the mixture was allowed to cool to room temperature and then poured in to ice cold water with continuous stirring. The resulting precipitate was collected by filtration, washed thoroughly with cold water, and dried. The crude product was purified by recrystallization from ethanol to obtain the corresponding isoxazole derivative.

5. PHARMACOLOGICAL EVALUTION

Biological activity:

Anti-bacterial activity: To study the anti-bacterial evaluation of the synthesized compounds. The following drugs, chemicals, and micro-organisms were used for the study.

Materials required: Petri plates, Beakers, Normal saline (0.9% w/v), Nutrient agar, Cork borers, Acetone, L-shaped glass rod, Inoculating loop, Auto clave.

Bacteria used for anti-bacterial assay: Escherichia coli (gram -ve), Bacillus subtills (gram +ve), Klebsiella pneumonia (gram -ve), Staphylococcus aureus (gram +ve).

Nutrient agar media preparation: 7 grams of nutrient agar is dissolved in 250ml distilled water and heat to boiling and let the medium dissolve completely after dissolving the nutrient agar it is closed properly and sterilized in autoclave the agar is kept in autoclave for 25 min at 121 o c at 15 lbs pressure then the agar is stored in required conditions for further use 35.

Inoculation of bacteria: The prepared nutrient agar media was poured in the sterilized petri plates and stabilized until the media was solidified and were have taken Escherichia coli (gram -ve) and bacillus subtilis (gram-ve) which are taken in normal saline solution and inoculated into nutrient agar with the help of L-shaped glass rod and incubated for 24 hrs. at 30 degree Celsius. The standard drug is introduced at different concentrations (5mg and 10mg) and the unknown sample is also introduced with different concentrations as same as standard then the petri dishes are incubated at 27 o c for 24 hours and at the end of the procedure the zone of inhibition of both standardand test are measured and both were compared.

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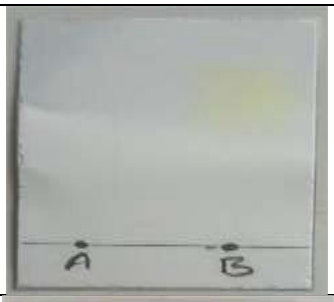
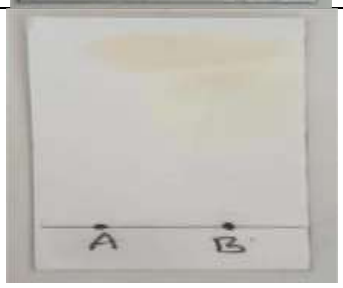
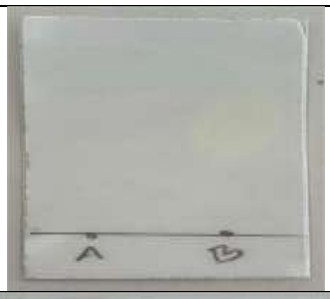
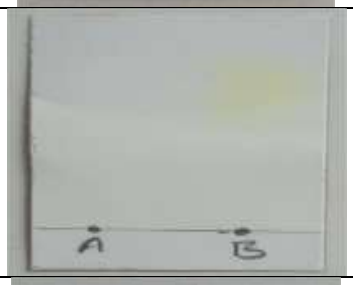
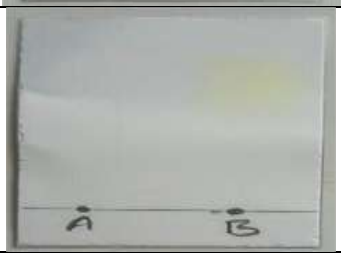
6. RESULTS AND DISCUSSION

MPOUND CODE	TLC PLATE	PRODUCT
AB-1		
AB-2		
AB-3		
AB- 4		
AB- 5		

Anti-bacterial activity results

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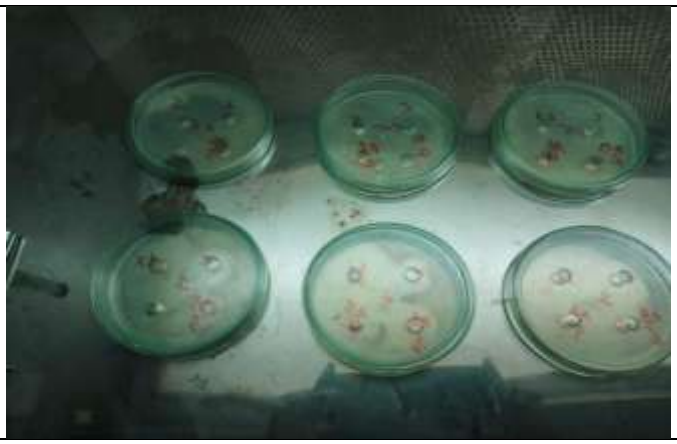
Zone of inhibition of synthesized compounds

-

Comp No	Mean zone of inhibition (*in mm*)											
	ANTI-BACTERIAL ACTIVITY											
	Bacillus Subtilis			*Staphylococcus Aureus*			*K. pneumonia*			*E. coli*		
	25 µg/ml	50 µg/ml	100 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
STD	12mm	18mm	17mm	14mm	15mm	16mm	15mm	16mm	16mm	13mm	17mm	17mm
AB 1	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm	1.5mm
AB 2	5mm	9mm	8mm	5mm	7mm	7mm	-	9mm	6-3mm	5mm	-	4.3mm
AB 3	1mm	1mm	1mm	-	1mm	1mm	1mm	1mm	1mm	1mm	1mm	4.8mm
AB 4	9mm	11mm	11mm	8mm	11mm	10mm	8mm	11mm	6.6mm	6mm	7mm	7.6mm
AB 5	8mm	10mm	10mm	7mm	9mm	9mm	9mm	10mm	10.3mm	7mm	9mm	9mm
CONT	6mm	8mm	7.6mm	6mm	7mm	7mm	7mm	11mm	10mm	7mm	9mm	8.6mm

Anti-bacterial activity of synthesized compounds on Escherichia coli

Compound : AB1- AB- 5
Concentration : 25, 50 and 100 microgram/ml
Control : DMSO
Standards : Ciprofloxacin



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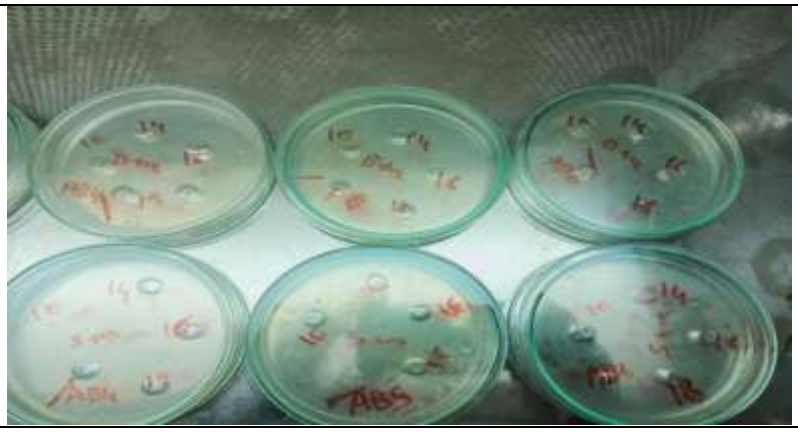
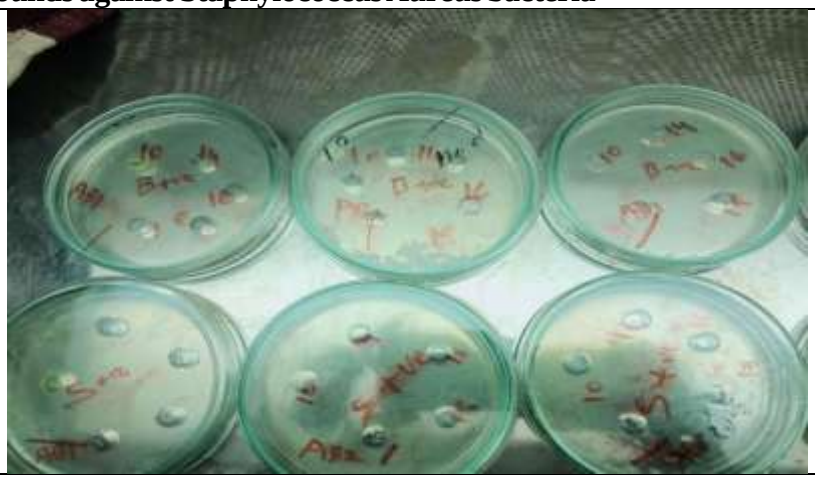
Anti-bacterial activity of synthesized compounds on Bacillas subtilis Zone of inhibition of synthesized compounds against Bacillas subtilis bacteria

Compound : AB1- AB- 5
Concentration : 25, 50 and 100 microgram/ml
Control : DMSO
Standards : Ciprofloxacin



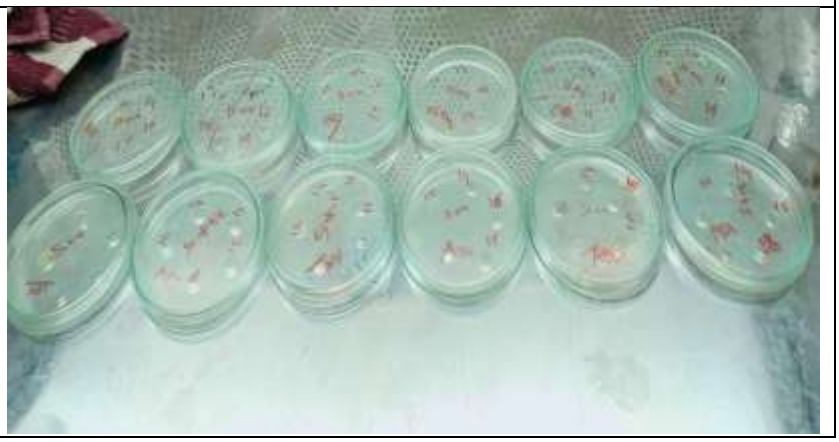
Anti-bacterial activity of synthesized compounds on Staphylococcus Aureus Zone of inhibitionof synthesized compounds against Staphylococcus Aureus bacteria

Compound : AB1- AB- 5
Concentration : 25, 50 and 100 microgram/ml
Control : DMSO
Standards : Ciprofloxacin



Anti-bacterial activity of synthesized compounds on Staphylococcus Aureus Zone of inhibitionof synthesized compounds against Staphylococcus Aureus bacteria.

Compound : AB1- AB- 5
Concentration : 25, 50 and 100 microgram/ml
Control : DMSO
Standards : Ciprofloxacin



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The synthesized compounds (AB1–AB5) were evaluated for their antibacterial activity using the **disc diffusion method** at concentrations of **25, 50, and 100 µg/mL**. The activity was tested against both **Gram-positive** and **Gram-negative** bacteria, including *Bacillus subtilis*, *Staphylococcus Aureus*, *Klebsiella pneumonia*, and *Escherichia coli*. **Ciprofloxacin** was used as the **standard drug**, and **DMSO** was used as the **negative control**. The **zone of inhibition** (in mm) was measured to assess the antibacterial effectiveness of each compound. **AB1 and AB3** showed **very weak activity**, with minimal inhibition zones (1–1.5 mm) across all test organisms and concentrations. **AB2** showed **moderate antibacterial activity**, especially against *Klebsiella pneumoniae* and *E. coli*, with inhibition zones ranging from **4.3 to 9 mm**. **AB4 and AB5** demonstrated **comparatively better activity**, particularly at higher concentrations (100 µg/mL), with inhibition zones ranging from **6 mm to 11 mm**, especially against *Bacillus subtilis* and *Staphylococcus aureus*.

The **standard drug ciprofloxacin** showed significantly higher zones of inhibition (**12–18 mm**) across all bacterial strains, indicating strong antibacterial activity. **Conclusion:** The results indicate that the synthesized compounds exhibited **mild to moderate antibacterial activity** in comparison to the standard. Among them: **AB4 and AB5** showed the **best activity**, especially at higher concentrations. **AB2** showed **selective moderate activity**. **AB1 and AB3** were the **least effective**.
Overall Activity Order: AB4 > AB5 > AB2 > AB1 ≈ AB3

7. SUMMARY & CONCLUSION

In the present research work, five chalcone derivatives (coded as AB1 to AB5) were successfully synthesized using the **Claisen-Schmidt condensation method**, which involved the reaction of various substituted aromatic aldehydes with acetophenone in ethanolic sodium hydroxide medium. The resulting compounds were purified through recrystallization and confirmed by **Thin Layer Chromatography (TLC)** and further characterized using spectral methods (IR, NMR), ensuring the correctness of the synthesized structures. The synthesized chalcone derivatives were screened for **antibacterial activity** against selected Gram-positive and Gram-negative bacterial strains, including *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*. The **disc diffusion method** was employed for antibacterial screening at concentrations of **25 µg/mL, 50 µg/mL, and 100 µg/mL**. **Ciprofloxacin** was used as the **standard drug**, and **DMSO** served as the negative control.
Observations*: AB4 and AB5** exhibited **notable antibacterial activity**, with maximum zones of inhibition observed at 100 µg/mL, especially against *Bacillus subtilis* and *Staphylococcus ureus*. **AB2** showed **moderate activity**, particularly against *Klebsiella pneumoniae* and *Escherichia coli*. **AB1 and AB3** displayed **negligible or very weak activity** against all tested bacterial strains. The standard drug ciprofloxacin showed higher zones of inhibition in all tested strains, confirming the sensitivity of the assay. ***Conclusion: From the results, it can be concluded that certain chalcone derivatives, especially **AB4 and AB5**, possess promising antibacterial properties and could serve as lead compounds for further development. The antibacterial activity varied depending on the nature and position of substituents on the aromatic rings, indicating a **structure-activity relationship (SAR)**. The findings support the potential of chalcone scaffolds in designing new antimicrobial agents, especially in the context of rising antibiotic resistance. Further research involving **mechanistic studies**, **SAR analysis**, and **chemical modification** may enhance the activity and therapeutic value of these derivatives.

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