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Design, Synthesis and Evaluation for Anti-bacterial Activity for Coumarin and its Derivatives

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Abstract : A series of coumarin derivatives were synthesized using coumarin as the starting material, which was prepared from resorcinol and H2SO4. The obtained products were characterized by FTIR spectroscopy to identify the functional groups present. The antibacterial activity of the synthesized coumarin derivatives was evaluated against standard bacterial strains using the disk diffusion method. Amikacin was used as the standard drug for comparison. The results showed that the coumarin derivatives exhibited significant antibacterial activity, with zone of inhibition values comparable to that of the standard drug. These findings suggest that coumarin derivatives may be potential candidates for the development of new antibacterial agents.

Keywords: Coumarin derivatives, synthesis, FTIR, antibacterial activity, amikacin.

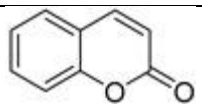
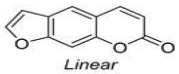
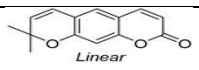
INTRODUCTION:-

Synthesis of large number of natural products from plants is called secondary metabolites. Secondary metabolites have important ecological functions, they have protective activity against microbes and herbivores and participate in the attraction of pollinators etc. these secondary metabolites are used by humans in many ways like as a source of drugs, flavouring agents etc. Natural compound like coumarins have contains considerable phytochemical and pharmacological exploration in the last decades. From the last three years, over 400 coumarins have been described in scientific publication. Coumarins are present naturally in a large number of plants, considerably in high concentration in Coumarouna odorata (tonka bean) (Fabaceae/leguinosae). It is also present in high amount in vanilla grass (Anthoxanthum odoratum) sweet clover (genus melilotus), cassia cinnamon (Cinnamomum cassia), in the extraction of Justicia pectoralis, and in many of the cherry blossom tree. Coumarin contents are mostly rich in apiaceae family. Many plants contain different concentration of coumarin. Tonka beans, liquorice, and cassia cinnamon have a high concentration of natural coumarin. Some cherry blossom strawberry and apricot contain coumarin in smaller quantities. Despite its sweet smell, animals tend to avoid plants that contains coumarin due to their bitter taste. Coumarin are natural bioactive coumarin recognized for their anti-inflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, anti-hypertensive, anti-tuberculous and antihyperglycemic pharmacological activity, as well as its antioxidant and neuroprotective action over 1300 coumarin have been identified from plant source.

Synthetic source:

Coumarins belong to the benzopyrone family commonly found in medicinal plants. Their structure consists of two six membered rings with lactone carbonyl group. Most coumarin compounds are not thermally liable and have optical activity. The coumarin biosynthesis takes part in involving multiple P450 enzyme. Ortho hydroxylation is a key point for the biosynthesis of natural coumarin in plants.

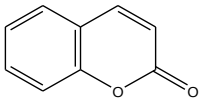
Classification of coumarin:

S.NO	CLASS	GENERAL STRUCTURE	EXAMPLE
1	Simple coumarin		Osthole(neuroprotective, osteogenic,Immunomodulatory,anticancer, Hepatoprotective,Cardiovascular protective and antimicrobial)
2	Furanocoumarin		Psoralen (antifungal), Antoghenol (anti-bacterial) Columbianedin(anti-inflammatory)
3	Pyranocoumarin		Grandivittin (anti bacterial), InophyllumA,B,C,E,P,G1 and 2 (antiviral)

DRUG PROFILE:-

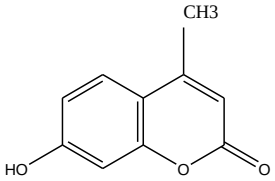
COUMARIN: -

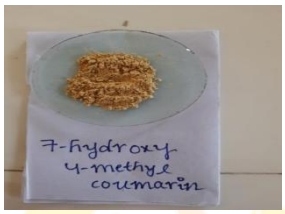
Take 150ml of concentrated H2SO4 in 500ml beaker was stirred with external ice water cooling until the temperature of acid become 50c-100c.Then 37gm of resorcinol was added to 45ml of ethyl acetoacetate until a complete solution was obtained. Then this solution was added slowly to H2SO4.In such that the temperature does not rise about 100c and stirring was continued for half an hour. Then the mixture is poured into the ice water and the solid product is separated, filtered out and dried then the coumarin was obtained**.**



7-HYDROXY-4-METHYL COUMARIN:

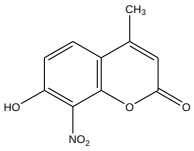
Take the crude coumarin then it was washed with the cold water and it undergoes recrystallisation by using ethanol to obtain pure 7-hydroxy-4-methyl coumarin**.**





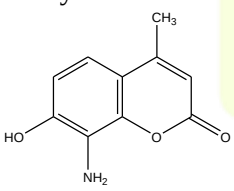
8-NITRO-7-HYDROXY-4-METHYL COUMARIN: -

In a conical flask take 12gm of 7-hydroxy-4-methyl coumarin dissolve it in concentrated H2SO4 and keep the flask in a ice bath. When the temperature inside the flask below 10c then add 20ml of nitrating mixture (15ml of concentrated H2SO4, 5ml of concentrated nitric acid) . Remove the flask from ice bath and keep it at room temperature for 1 hour. Then shake the flask and pour into a beaker containing crushed ice and transfer the crude mixture into a conical flask containing ethanol and boil then the residue is 6-nitro-4-methyl-7-hydroxy coumarin. Then concentrate the filtrate and cool in an ice bath. Recrystallize from ethanol and collect 8- nitro-4-methyl-7-hydroxy coumarin.



8-AMINO-7-HYDROXY-4-METHYL COUMARIN: -

8gm of iron powder was added portion wise with stirring to a hot mixture of 8-nitro-7-hydroxy- 4-methyl coumarin(4.4gm, 0.02moles) in 20ml of ethyl alcohol and 30ml of concentrated HCl at reflux temperature after completion the addition, the refluxing was continued for 6 hours. Upon. cooling a black precipitate was formed, which was filtered off , washed with cold water and dried and then recrystallized with ethanol then 8-amino-7-hydroxy-4-methyl coumarin was obtained.



MECHANISM OF ACTION OF COUMARIN:

Inhibition of bacterial cell wall synthesis:

1.Inhibition of MurA and MurB enzymes:

Coumarin inhibits the activity of MurA and MurB enzymes, which are essential for the synthesis of peptidoglycan, a critical component of the bacterial cell wall.

2.disruption of cell wall integrity:

By inhibiting peptidoglycan synthesis, coumarin disrupts the integrity of the bacterial cell wall, ultimately leading to lysis and death.

MECHANISM OF ACTION OF COUMARIN DERIVATIVES: -

Antimicrobial Mechanism:

1. Inhibition of bacterial DNA gyrase: 7-Hydroxy-4-Methyl Coumarin inhibits the activity of bacterial DNA gyrase, an enzyme essential for DNA replication and transcription .

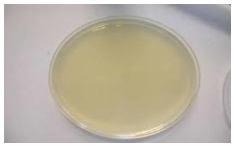
2. Disruption of bacterial cell membrane: The compound disrupts the bacterial cell membrane, leading to changes in membrane permeability and ultimately, cell death.

PREPARATION OF NUTRIENT BROTH:

Weigh all ingredients separately by physical balance. Take a 1000ml conical flask to this add 200ml distilled water and weigh amount of beef extract, peptone, sodium chloride. Heat the mixture and agitate the mixture to dissolve the ingredients and add the distilled water and makeup the final volume. Adjust the pH of the medium to 7.Pour 5ml of the medium in each test tube and plug the tubes with cotton. These medium containing tubes are sterilized in an autoclave at 121ºC under `15lb pressure for 15 minutes. Allow the autoclave to cool and remove the broth tubes.

PREPARATION OF NUTRIENT AGAR:

Weigh all ingredients separately by physical balance. Take a 1000ml conical flask to this add appropriate volume of distilled water and weigh amount of ingredients accept agar.Adjust the pH of the medium to 7. Heat the mixture and add agar to the flask and is heated until the agar completely dissolve and makeup the volume upto required quantity with distilled water.this medium is pour in to petri plate.



STANDARD DRUG DILUTIONS OF AMIKACIN SULPHATE

Inoculate a previously liquefied medium appropriate to assay with requisite quantity of suspension of the microorganism. Add the suspension to the medium at a temperature between 40º and 50º and immediately pour the inoculated medium into the petri dishes, to give a depth of 3 to 4mm, put the plate on a level surface. Store the prepared dishes to manner so as to ensure that no significant growth of the test organism occurs before the dishes or plates are used and the surface of the agar layer is dry at the time of use. Prepare solution of known concentration of the standard preparation and solution of corresponding concentration of antibiotic to be examined in an appropriate buffer solution. Add solutions of antibiotic in the cavities prepared in the agar medium in a sufficient volume almost to fill the holes. Arrange the solution of the standard preparation and the antibiotic under examination on each dish so that they alternate around the dish, henceforth the highest concentration of standard and test preparation are not adjacent. Leave the dishes for 1 to 4 hours at room temperature at 4ºc as appropriate as a period of pre incubation diffusion to minimize the effects of variation in time between the applications of the different solutions. Incubate them for 18 hours at the temperature of 37ºc. accurately measure the diameters or areas of circular inhibition zones and calculate the results.

INSTRUMENTS USED:

AUTOCLAVE INCUBATOR

RESULTS AND DISCUSSION:

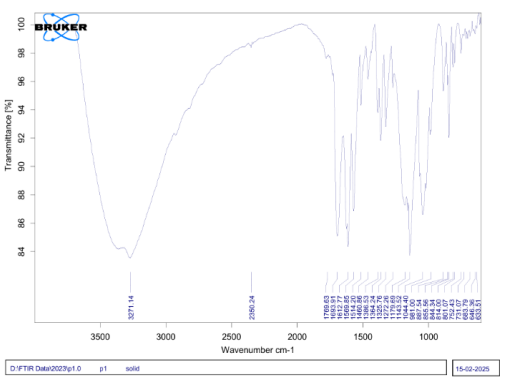


Fig1:FTIR Studies of coumarin

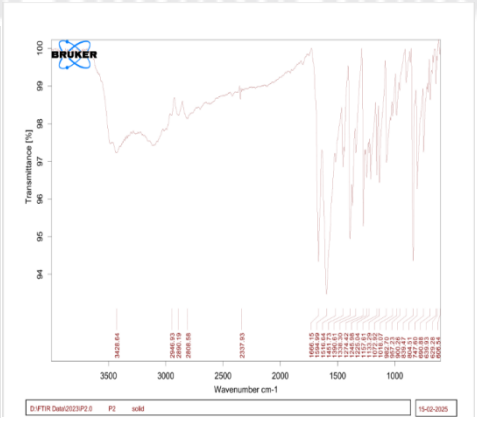


Fig 2:FTIR Studies of 7-hydroxy4-methyl Coumarin

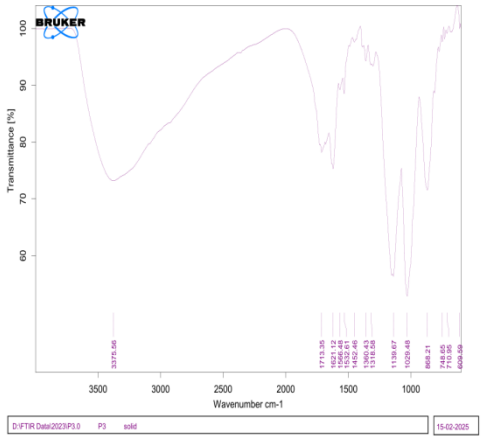


Fig 3: FTIR Studies of 8-nitro-7-Hydroxy-4-methyl coumarin

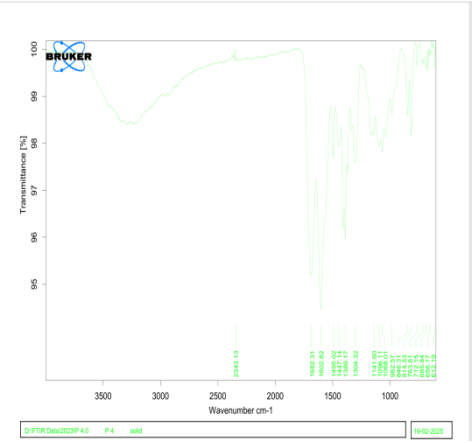
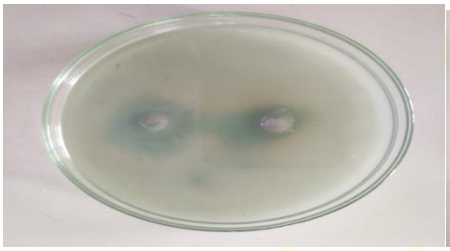
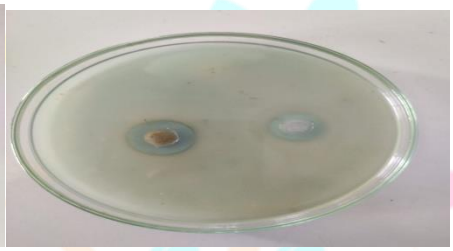
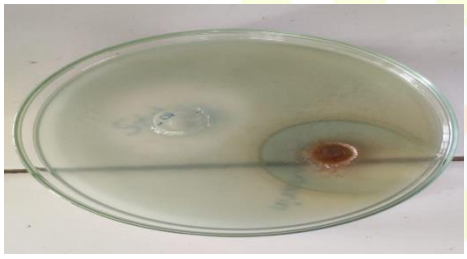


Fig 4: FTIR Studies of 8-Amino-7-hydroxy-4-methyl coumarin

ANTIBACTERIAL STUDIES:







S.NO	NAME OF THE SAMPLE	DIAMETER
1.	COUMARIN	32mm
2.	7-HYDROXY-4-METHYL COUMARIN	36mm
3.	8-NITRO-7-HYDROXY-4-METHYL COUMARIN	38mm
4.	8-AMINO-7-HYDROXY-4-METHYL COUMARIN	30mm

SUMMARY AND CONCLUSION

The coumarin and its derivatives has been drawn attention in the recent times because of their variety of its applications and its pharmacological activities [antibacterial and antifungal, antioxidant]. These coumarin molecules possesses some of the fundamental properties that ensures it an advisable role in the design of new biologically active derivatives. Coumarin are considered as ideal small molecules because they possess drug like properties such as high solubility, low molecular weight. The medicinal chemist continues to discover novel coumarin of natural and unnatural analogues to further improve the biological activity and uncover new medicinal uses.As the coumarin derivatives confirmed by FTIR and to the obtained derivatives we conducted the antibacterial studies by using amikacin as standard drug.Moreover, in this review we observed that the coumarin exhibits more antibacterial when it is compared with the standard drug amikacin.

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