

Synthesis of coumarin derivatives and antimicrobial activities against E-Coli.

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ABSTRACT

Due to exceptional reactivity of coumarin, the synthesis of new coumarin derivatives type has been carried out. The newly synthesized derivatives contain different functional groups, and as such they could exhibit microbiological activity. Therefore, the microbiological activity of these derivatives on E-Coli is tested. The tested compounds have shown different activity in terms of growth inhibition of microorganisms.

Keywords: Coumarin derivatives, Disc diffusion technique, antibacterial activity, E-Coli, minimum inhibitory concentrations.

INTRODUCTION

The coumarin derivatives are quite interesting objects for both synthesis and pharmacological screening. A variety of synthesized coumarin derivatives have been experimentally shown to exert pharmacological activities including anticoagulant, antitumor, antiproliferative, anti-inflammatory, antioxidative as well as antiviral activities. The recent discovery of coumarins having weak estrogenic activity resulted in the use of such derivatives as therapeutic agents in preventing the emergence of menopause-related diseases, such as osteoporosis, increased risk of cardiovascular disease and cognitive deficiencies.

Materials and Methods

Solvents and reagents used for the synthesis were of reagent grade and were purified by standard methods. The reaction progress and purity of all prepared compounds was followed by TLC in the system benzene/ethyl acetate and benzene/pet ether visualizing spots with UV lamp and iodine vapour. Laminar airflow Cabinet (Kemi), autoclave (Osworld, "Autoclave Steam Sterilizer" JRIC-39) and incubator (Genuine) were used for microbial activity.

I. Preparation of coumarins (1A)

1. Preparation of 4- hydroxycoumarin (1A)

A mixture of phenol (4.5g), malonic acid (5.2 g), fused zinc chloride (18.5 g) and phosphorus oxychloride (15 ml) was irradiated in a microwave oven for 1 1/2 hours. The progress of reaction was monitored by TLC. The product was cooled and decomposed with ice water and allowed to stand. The resulting 4-hydroxy coumarin was dissolved in 10% sodium carbonate and acidified. At about neutral

point some oily by product separated out and was remove. Acidification of the remaining solution gave 4-hydroxy coumarin. On recrystallisation from dilute alcohol pure 4-hydroxy coumarin was obtained.

2. Preparation of 7-hydroxy-4-methyl coumarin (2A)

A Solution of resorcinol (11 g) in ethyl acetoacetate (13g) is added to cold concentrated sulphuric acid (100 ml) with stirring. The reaction mixture was kept for 20 hours at room temperature and poured with vigorous stirring onto crushed ice. The separated product was filtered, washed with water and crystallized from dilute alcohol.

3. Preparation of 4, 7-dihydroxycoumarin (3A)

A mixture of dry resorcinol (5g), malonic acid (5.2g), fused zinc chloride (18.5 g) and phosphorus oxychloride (15 ml) was irradiated in a microwave oven for 11/2 hours. After the reaction was complete as monitored by TLC, the crude product obtained from the reaction was recrystallised from hot water, as pale yellow crystals.

4. Preparation of 4-methyl-7, 8-dihydroxycoumarin (4A)

A mixture of phenolic substrate (trihydroxybenzaldehyde) (20ml) and ketoester (21 ml) was heated at 70°C in the presence of concentrated sulphuric acid. After completion of the reaction, the reaction mixture was cooled at room temperature and poured onto crushed ice (50 g).The solid product obtained was filtered off, washed with ice-cold water and recrystallised from hot ethanol, to obtain he pure product.

5. Preparation of 4, 7-dimethylcoumarin (5A)

A mixture of p-cresol (21ml) and ethyl acetoacetate (26 ml) in sulphuric acid (100 ml) was heated on a water bath for 5 hours. The reaction mixture was then cooled and poured into ice water. The product was filtered, washed with water, dried and then crystallized from dilute alcohol to give white crystals.

6. Preparation of 7-hydroxycoumarin (6A)

An intimate mixture of resorcinol (3g), malonic acid (2.4 g) and 60ml of concentrated sulphuric acid were irradiated in a microwave oven for 2 ½ hours until the effervescence ceased. It was cooled and treated with excess of crushed ice. The mixture became clear in 1½ hours and the heating was stopped. The crude product thus obtained was recrystallised from ethanol as dark red powder.

Antimicrobial activity of compounds.

The synthesized coumarin derivatives were tested for their antibacterial activity and activity was compared.

Preparation of culture media for antibacterial studies.

Preparation of Nutrient agar medium

Three grams of beef extract, 50g of peptone of NaCl and 15g of agar were taken in a beaker and then distilled water (1000ml) was added. The mixture was boiled and mixed thoroughly with a glass rod. After complete dissolution of agar the medium it was dispensed into several conical flask of 250ml volume. The conical flasks were closed with cotton plug and rapped with aluminum foil. It was there

auto calved for 15 minutes at 121°C and 15 psi. After autoclaving; the medicine was used for culturing different micro organism.

Antimicrobial testing

Disc method for determination of zone of inhibition of antibacterial.

Paper discs of 4mm in diameter and glass Petri plates of 90mm in diameter were used throughout the experiment. Paper discs were sterilized in an autoclave and dried at 100°C in oven. Then the disc was soaked with test chemicals at the rate of 50g per disc for antibacterial analysis. One drop of bacterial suspension was taken in sterile Petri dish and then approximately 20ml of sterilized and melted NA (~45°C) was poured into the plate, and then mixed thoroughly. The paper discs after soaking with test chemicals were placed at the center of the inoculated pour plate. A control plate was also maintained in each case with alcohol. Firstly, the plates were kept for 4hrs at low temperature (4°C) the test chemicals diffused from disc to the surrounding medium by this time. The plates were then incubated at (35± 2) °C for growth of test organisms and were observed at 24 hours intervals for two days. The activity was expressed in terms of inhibition zone diameter in mm. Each experiment was repeated three times. The standard antibiotic, streptomycin and gentamycin was used as a positive control and compared with test chemicals under identical condition. The antimicrobial activities of the compounds were recorded.

The compounds synthesized will be designated as 1A, 2A, 3A, 4A, 5A, 6A, for the purpose of easy reference.

Result:

For the study 6 derivatives of coumarins have been prepared and their antimicrobial activities have been assessed.

The minimum inhibitory concentrations of synthesized compounds were tested against E-Coli. It was found that all the six compounds showed good inhibition at higher concentration. The results are graphically depicted in figure 1. and zone of inhibition s are depicted in table 1.

At 0.05µg/ml concentration, compound 3A showed good inhibition. At 0.1µg/ml concentration, compound 1A, 2A, 3A showed better inhibition. At 0.15µg/ml concentration, compound 1A, 2A, 4A showed better inhibition. However compound 3A, Showed maximum inhibition compared to all the compounds at all concentration,

Conclusion:

An assessment of the antibacterial activities of the coumarin derivatives revealed that all the coumarins exhibited maximum activity against all the bacterial species taken up for the study.

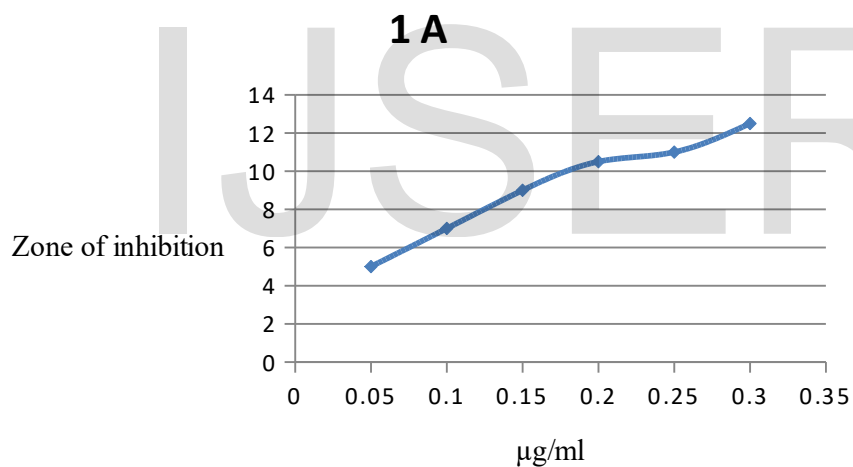
This observation could be useful in carrying out further studies on the coumarin derivatives particularly in clinical trials against various infections.

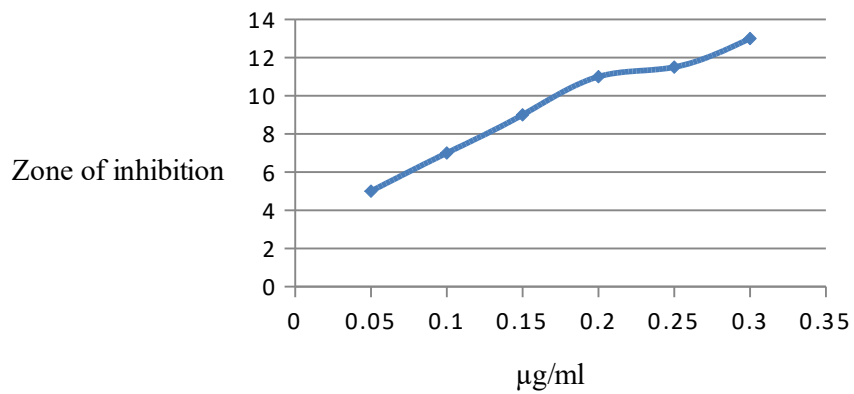
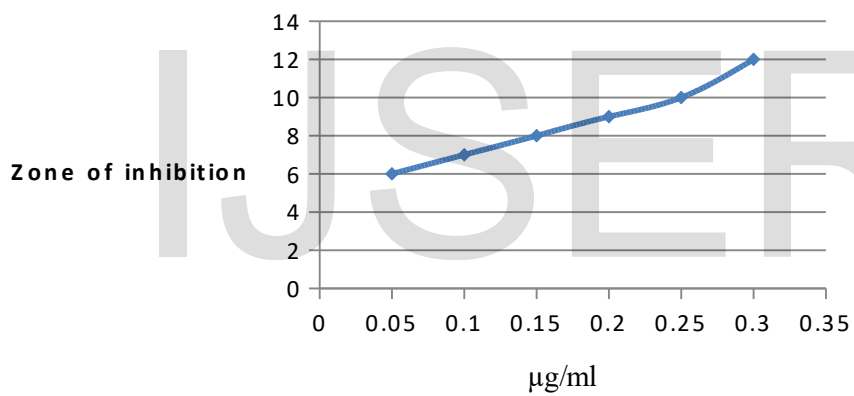
Table-1

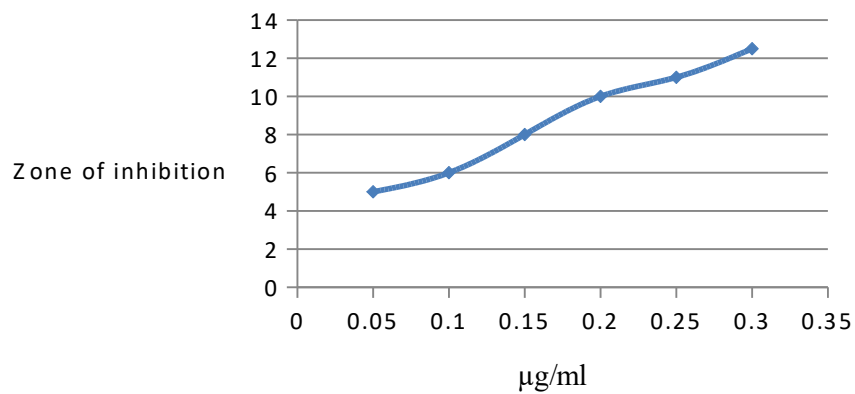
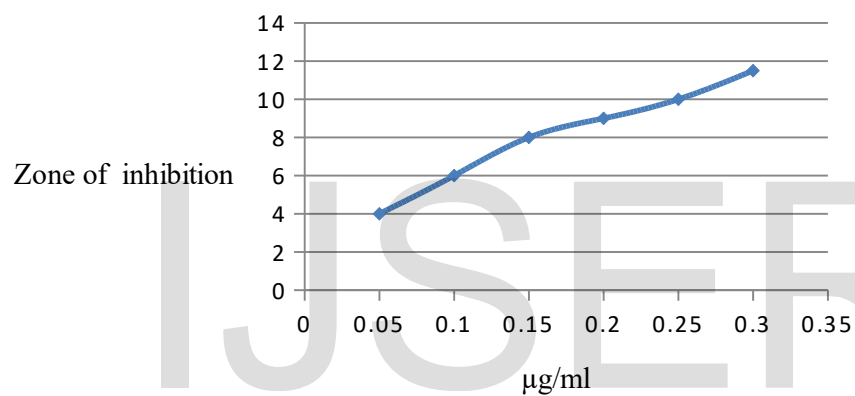
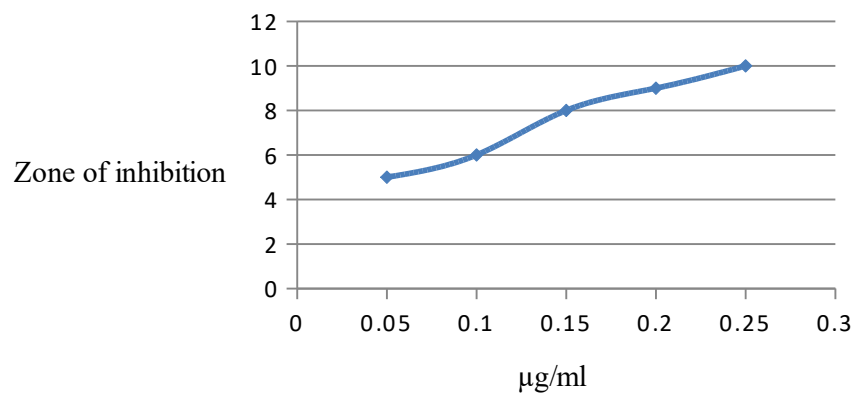
Minimum inhibitory concentration of compounds 1A, 2A, 3A, 4A, 5A, 6A against E-Coli.

Concentration ($\mu\text{g/ml}$)	Zone of inhibition of compounds (mm)					
	1A	2A	3A	4A	5A	6A
0.05	5	5	6	5	4	5
1	7	7	7	6	6	6
0.15	9	9	8	8	8	8
0.2	10.5	11	9	10	9	9
0.25	11	11.5	10	11	10	10
0.3	12.5	13	12	12.5	11.5	11.5

Figure-1



2 A**3 A**

4 A**5 A****6 A**

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