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EXTRACTION OF COUMARIN FROM CINNAMON AND EXAMINATION OF ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT:

Coumarin and its derivatives have showed impressive interest because of potential in human wellbeing. Coumarin and its simple are generally utilized in fragrances and makeup industry and they showed critical adaptable scope of organic exercises, for example, antibacterial, anti-inflammatory, antioxidant, antiviral, anticonvulsant, anticancer, antifungal and so on coumarin and its derivatives are normally happening as an optional metabolites present in seeds, barks, leaves and roots. These writing showed that cinnamomum cassia has a place with family lauraceae contain follow sum coumarin and these coumarin separated vitro against gram positive microscopic organism's staphylococcus aureus and gram negative microbes E.coli of fluid concentrate, ethanolic remove and 70% liquor however ethanolic concentrate of show most noteworthy action against gram positive microorganism's staphylococcus aureus and gram negative microorganisms E.coli. These article feature the antibacterial action normally extricated coumarin and makes them fascinating particle for various fields of examination.

KEY WORDS:

Coumarin , antibacterial activity , S.aureus, E.coli, cassia cinnamon.

INTRODUCTION:

The point of this study was to look at the impacts of coumarin derivatives with their isolation, physical and substance evaluation. coumarins show a momentous exhibit of biochemical and pharmacological action. Coumarins comprising of intertwined benzene and α-pyrone rings are available in critical sums in plants, and more than 1300 coumarins have been identified from natural source. Derivatives of coumarins normally happen as auxiliary metabolites present in seeds, roots, and leaves of many plant species. Coumarins have an assortment of significant natural exercises, for example, anti-inflammatory, antioxidant, antiviral, antimicrobial and anti-cancer. Coumarins are shown to build focal sensory system activity.[1,2,3,4,5] Coumarins are phenolic compounds

broadly circulated in the plant kingdom. The isolation of coumarin was first detailed by Vogel in 1820 [6,7]. The name coumarin starts from a Caribbean word coumarou for the Tonka tree (Dipteryx odorata Wild, Leguminosae), what shares the trademark smell of these mixtures and was referred to organically at one time as Coumarouna odorata Aubl. Naturally happening coumarins, which are grouped by their benzopyran-2-one core, have been disconnected from various plants, especially individuals from the Apiaceae, Rutaceae, and Fucaceae, just as from certain genera of Leguminosae.

The coumarins analogs (engineered or regular) are a fundamental part in the beauty care products and scents industry [8]. Organically dynamic regular items have coumarin skeleton and utilized as a middle for the combination of bioactive heterocyclic mixtures which uncovered its antimicrobial, antifungal, mitigating, hostile to malignant growth, against tubercular, cell reinforcement, and anticoagulant properties. Hardly any manufactured mixtures with a coumarin ring have been accounted for to be dynamic against multi-drug safe (MDR) bacteria.[9,10,11,12]

Cinnamon a native flavor, having a place with the Lauracea family, is found in pretty much every family. Utilized chiefly as a seasoning specialist, it has been a significant constituent of our food since quite a while. For quite a while, our predecessors have been involving it as a solution for respiratory and stomach related diseases. Nonetheless, less is referred to about its advantageous impact as a cell reinforcement, calming, antilipidemic, antidiabetic, antimicrobial, and anticancer agent.[13] the trial study show that cassia cinnamon contain follow measure of coumarin.[14]

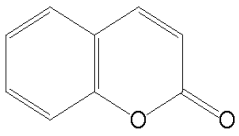
Writing survey uncovers that coumarin (2-oxo-2H-chromene) and its subordinates address one of the most dynamic classes of heterocyclic mixtures which have a wide range of organic exercises: antitumor, antibacterial, antifungal, anticoagulant, cell reinforcement, and hostile to inflammatory.[15,16,17,18]

Coumarin (1,2-benzopyrone), a fragrant oxygen-containing heterocyclic compound, has different natural capacities. Past investigations have shown that coumarin and its subsidiaries display antibacterial movement against gram positive species S.aureus and gram negative species E.coli.

Writing features the incorporated coumarin subsidiaries applied meaningful movement against for their in-vitro antibacterial action against four Gram-negative (E. coli, S. flexneri, P. aeruginosa and S. typhi) and two Gram-positive (B. subtilis and S. aureus) bacterial strains.[19]

PROPERTIES OF COUMARIN:

- Coumarin is found in high concentrations in certain types of cinnamon. The Cassia cinnamon contains high amounts of coumarins, also found in sweet clover, lavender oil, and Tonka beans.
- It is slightly soluble in water and very soluble in ether, chloroform
- Its melting point is 71^0 c.
- It has vanilla caramel taste.
- It has a sweet aromatic odour.
- It is weakly acidic in nature.
- Molecular Formula: C9H6O2
- Molecular Weight: 146.145 g/mol



SAR:

At C-3 position

- Presence of ethylene moiety with carboxylic or tertiary amine group, alkoxyimino group, carbonyl group, hydroxamate group.
- Furthermore, benzyl group with hydroxyl or halide group replacement is viable.
- Coumarins substituted with 3-ketone,3-ester &3-alkoxyimino derivatives were viewed as amazingly active against the gram positive and gram negative strains along with Staphylococci and Enterococcus species.
- The benzene rings subbed at methylene-bis position were estimated for their proficiency as antibacterial agents, mainly to counter gram positive and gram negative microscopic organisms, for example, staphylococcus aureus and E coli individually.
- As t-butyl substituted at para position showed most reduced antibacterial action.
- Methoxy bunch substituted at methylene-bis position had most minimal inhibitory action against gram positive and gram negative microbes like S. aureus and E. coli.

At C-4 position

- When contrasted with the unsubstituted methyl or ethyl substituted derivatives, methyl substituted compounds have intense inhibitory movement for staphylococcus aureus.
- Non-appearance of methyl group from the C-4 place of Coumarins moiety seriously less the intensity.
- -CH3 bunch, halogen substituted iodinated aryloxymethyl bunch, thiotriazo subordinate are more effective.

AT C-5 position

- Nitro group is active against H.pylori than the lead compound at C-5 position

At C-7 position

- Carbonyl group, complex alkoxy group substituted with electron withdrawing group, biphenyl group is significant against S.aureus

At C-8 position

- Methyl group & ethyl group at C-8 position is more effective.[20]

Various Activity Of Coumarin Ring:

- ***Anticonvulsant activity:***
  Synthesis various coumarin derivatives of heteroaryl semicarbazones by the reaction of heteroaryl hydrazine carboxamide with aryl aldehydes or ketones.[21] The analog was tried for their anticonvulsant movement utilizing pentylenetetrazole actuated seizure, and maximal electroshock seizure tests at 30, 100, and 300 mg/kg portion levels alongside the investigation of neurotoxicological signs. Three mixtures having 3,4-Cl.C6, H3, 2-OCH3, C6 H4, and 4-Br, C6H4 addressed most noteworthy anticonvulsant action at a portion of 30 mg/kg, as contrasted and phenytoin.[22]

- ***Anticancer activity:***

  Hostile to malignant growth drugs have customarily been designated to harm the deviantly partitioning cell by intruding on the cell division process. These medications are successful, and cytotoxic, and consequently display extreme aftereffects, especially on ordinary multiplying tissues like the hematopoietic framework (Kokron et al, 1991, Marshall et al, 1991, Bosland, 1991).Often mix treatments, by which a few cytotoxic specialists are joined in the treatment system, offer better outcomes with less poisonous secondary effects, as they are painstakingly controlled to permit recuperation of typical, yet not harmful cells, from drug openness. At present, chemotherapy, radiotherapy and medical procedure joined proposition the best results for malignant growth patients and therapy blends have been effectively applied to specific disease types, for instance, Hodgkin's lymphoma, testicular disease and different leukemia. Coumarins can be utilized not exclusively to treat malignant growth however to treat the secondary effects brought about by radiotherapy (Marshall et al, 1990). The outcomes recommend that coumarin/troxerutin have a positive impact in the treatment of radiogenic sialadentis and mucositis . The interest in coumarin and 7-hydroxycoumarin as against disease specialists emerged from reports that these specialists had accomplished objective reactions in certain patients with cutting edge malignancies.[23,24,25]

- ***Antifungal activity:***

  The antifungal action was thought about in contrast to various contagious strains like Aspergillus Niger (NCIM No. 1196), Penicillium Chrysogenum (NCIM No. 723) and Curvularia Lunata (NCIM No. 1131). Fluconazole, miconazole and amphotericin B were utilized as standard medications for the examination of antifungal action. The plates were brooded at 37 C for all microorganisms; absorbance at 410 nm was recorded to evaluate the restraint of cell development after 48 h. The most minimal focus repressing development of the creatures was recorded as the MIC. DMSO was utilized as a dissolvable or negative control. To explain any impact of DMSO on the natural screening, separate examinations were done with arrangements alone of DMSO and showed no action against any microbial strains. The mixtures which are showing promising antifungal action were chosen for least inhibitory focus studies. The still up in the air by measuring at 128, 64, 32, 16, 8, 4, 2, 1 and 0.5 lg/mL focuses alongside principles at the equivalent concentrations.[26]

- ***Antibacterial activity:***

Coumarin itself has exceptionally low antibacterial movement, while coumarin items, which have long hydrocarbon replacements, for example, ammoresinol and ostruthin, are viewed as the best medication against Gram-positive microbes, for example, Bacillus megaterium, staphylococcus aureus.[27]

The antimicrobial susceptibility testing of recently integrated mixtures was acted in vitro against bacterial strains viz. gram-positive Staphylococcus Aureus (ATCC No. 29737), Micrococcus Luteus (ATCC No. 398), Bacillus Cereus (ATCC No. 6630) and gram-negative Escherichia Coli (NCIM No. 2256), Pseudomonas Fluorescens (NCIM No. 2173) and Flavobacterium Devorans (ATCC No. 10829), separately, to discover least inhibitory focus (MIC). Sequential twofold weakening's of all examples were ready in three-fold in microtiter plates and vaccinated Med Chem Res 123 with appropriately pre-arranged cell suspension to accomplish the necessary introductory focus. Sequential weakenings were ready for screening. Dimethylsulfoxide (DMSO) was utilized as dissolvable control. Ampicillin, kanamycin and chloramphenicol were utilized as a standard antibacterial medication. The focus scope of tried mixtures and standard was 128-0.5 lg/mL. The plates were brooded at 37 C for all microorganisms; absorbance at 595 nm was recorded to survey the hindrance of cell development after 24 h. The mixtures which are showing promising antibacterial movement were chosen for least inhibitory focus studies. The still up in the air by measuring at 128, 64, 32, 16, 8, 4, 2, 1 and 0.5 lg/mL fixations alongside norms at the equivalent concentrations.[26]

BACKGROUND:

Coumarin is a member categorized under compounds called benzopyrones which consist of fused benzene and α-pyrone rings. Coumarin is used in the cosmetic, as perfumery and household products industry due of its pleasant bitter-sweet odour (Egan et al., 1990). In view of the established low toxicity, relative cheapness, and presence in the diet, coumarins and their derivatives have been found to exhibit a wide range of biological and pharmacological activities (Hoult and Paya´, 1996).

The antibacterial activity of coumarins was investigated by employing a microdilution method against bacterial species including gram positive S.aureus and gram negative E.coli.[28]

MATERIALS AND METHODS:

Extraction of coumarin from cinnamon:

Prepared 25 gram powdered cinnamon & mixed with 50 ml ethanol vigorously stirred for 10- 15 minutes followed by small amount anhydrous magnesium sulphate powder to remove any fine or traces or water. Filtered the solution, collected the filtrate and evaporated the content on hot plate to get dry powdered as residue.[29]

As the extraction was performed the color of the extract was found to be wine red. This coloration is may be due to pigment of cinnamon containing coumarin.

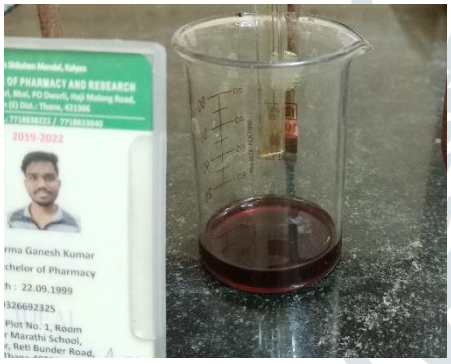


Figure 1 LIQUID FILTERATE OF COUMARIN

Figure 2 DRY EXTRACT OF CRUDE COUMARIN

Identification test for coumarin:

Test for phenols: Deep blue or black color appeared by mixing 5 % ferric chloride solution to the small amount of plant extract. Test for coumarins: 3 mL of 10 % NaOH was added to 2 mL aqueous plant extract and yellow color was observed in positive results.

Specific tests:[31]

(i) Ferric chloride test :

Concentrate alcoholic extract of drug sample + few drops of alcoholic FeCl3 solution →appearance of dark green color → turned to yellow after some time on addition of conc. HNO3 → indicates the presence of coumarins.

MICROORGANISM :[31]

The above mentioned compound was tested against a panel of microorganism including, the gram negative bacteria, E coli ATCC 25922 and gram positive bacteria staphylococcus aureus ATCC 25923.

ANTIMICROBIAL ACTIVITY OF CRUDE COUMARIN

The bacterial strains were obtained from *MICROBIO LABORATORY* vartak nagar thane, Maharashtra, India. Antibacterial activity was studied using two bacterial species. The two bacterial species were E Coli (Gram Negative) and Staphylococcus Aureus (Gram Positive).

To check and compare the activity of extracted coumarin, 3 different solutions were made. The solutions are as follows-:

- 1gm coumarin extract in 2 ml of distilled water
- 1gm coumarin extract in 2ml of 70% ethanol
- 2ml of 70% of ethanol

All the prepared solutions were used to check and compare the crude extract coumarin in different quadrant of bacterial media culture.

PREPARATION OF CULTURE MEDIA

Preparation of Soyabean Casein Digest[34]

We Suspended 30 grams of Soyabean Casein Digest Medium in 1000 ml of distilled water. Then Boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs. pressure (121 °C) for 15 minutes.

Components Item in (g/l) which are present in soyabean casein are Casein Enzymic Hydrolysate 17.00, Papaic Digest of Soyabean Meal 3.00, Sodium Chloride 5.00, Dipotassium Phosphate 2.50, Dextrose 2.50 and The agar used for bacterial strain was soyabean casein.

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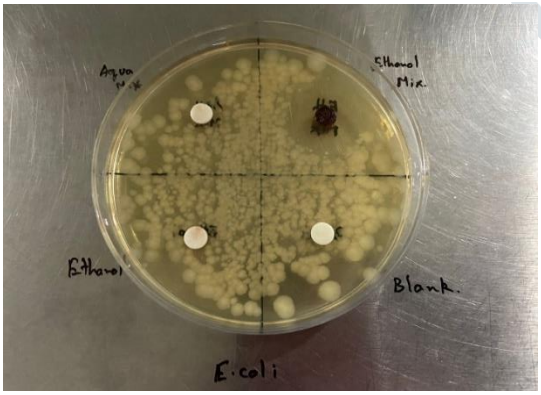

Figure 3 Antibacterial activity of crude coumarin against E.coli(gram negative)

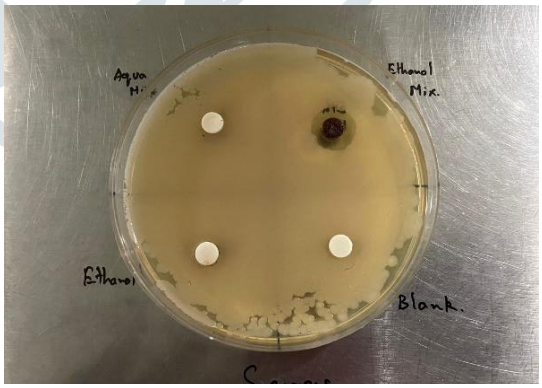

Figure 4 Antibacterial activity of crude coumarin against S. aureus(gram positive)

Disk diffusion method

Disk diffusion method was performed to the diffusion of an antimicrobial agent of a particular concentration from disks into the culture medium that has been putted with the chosen inoculum isolated in a pure culture. Disk

diffusion is based on the determination of an inhibition zone proportional to the bacterial susceptibility to the antibacteria present in the disk. The diffusion of the antibacterial agent into the seeded culture media results in a gradient of the antibacterial. When the concentration of the antibacterial becomes so diluted that it can no longer inhibit the growth of the test bacterium, the zone of inhibition is demarcated. The diameter of this zone of inhibition around the antibacterial disk is related to minimum inhibitory concentration (MIC) for that particular bacterium/antimicrobial combination; the zone of inhibition correlates inversely with the MIC of the test bacterium. Generally, the larger the zone of inhibition, the lower the concentration required to inhibit the growth of the organisms. Although, these things depend on the concentration of antibiotic in the disk and its diffusibility.

RESULT & DISCUSSION

In this study showed that coumarin is naturally present in cinnamon very well described in article ("Cassia Cinnamon as a Source of Coumarin" in ACS PUBLICATION)[33]. Reddish brown liquid extract of cinnamon powder was collected using ethanolic solution. 5.12 gm crude ethanolic extract coumarin was successfully prepared , using 100 gram of cinnamic powder. As the extraction was performed the color of the extract was found to be wine red. This coloration is may be due to pigment of cinnamon containing

The presence of coumarin was identified by performing ferric chloride test which showed change in color from deep green to yellow after adding nitric acid.

The study of Minimum inhibitory concentration was performed by disc diffusion method. In the present investigation, extracted crude coumarin from cinnamon sample was tested for antibacterial pattern against gram positive and gram negative bacteria. Agar disc diffusion assay was key the process to determine the antibacterial activity of extract prepared in ethanol solvent. Ethanolic form of crude coumarin extract was found more active as compared to aqueous extract. The highest activity in terms of zone of inhibition (22.32mm) was observed against gram negative bacteria E.coli. Other gram positive bacteria was also found susceptible to ethanolic extract and inhibition zone was noted as a 12.61mm.

In comparison with broad spectrum antibacterial activity, maximum antibacterial activity was shown by E.coli followed S. aureus.

FUTURE ASPECTS:

Traditional antibacterial therapy is going through a crisis due to the rapidly increasing development of resistance to existing agents therefore, interest in development and synthesis of new compounds and drugs are going on. Coumarins are naturally occurring compound having a versatile range of biological activity which helpful to researcher for continuous research. Coumarin and its derivatives are being extensively studied due to their broad array of biological activities, low toxicity, and lower drug resistance properties.

The biochemical properties and pharmacotherapeutic applications of crude coumarins depend on the pattern of substitution in basic coumarin moiety. Therefore, there is a need to conduct a careful study of the SAR of coumarins.[32]

Coumarin is a simple compound and many of its derivatives have been known for more than decades, it continues to maintain the interest of researchers being a plentiful source of potentially impactful drug because of their revealing therapeutic potential.

CONCLUSION

The extraction of crude coumarin from cassia cinnamon bark was successfully done along with its identification test with positive presence result. An assessment of the antibacterial activity of the crude coumarin revealed that it exhibited maximum activity against gram negative bacterial species (E.coli) taken up for the study. This observation could be useful in carrying out further studies on the coumarin and its derivatives particularly in clinical trials against various bacterial infections. Coumarin and its derivatives are known to be medicinally important by having different pharmacological activities including antibacterial activity. Coumarin serve as valuable source of lead compounds for the drug design and development of effective antibacterial therapy.

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