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CRUDE COUMARIN EXTRACT PREPARATION USING CASSIA CINNAMON POWDER AND ITS pH ANALYSIS WITH EXAMINATION OF ANTIMICROBIAL ACTIVITY

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ABSTRACT

Coumarinic compounds are a class of lactones, structurally constructed with fusion of a benzene ring and α-pyrone ring. Previous research studies have demonstrated that coumarin and its derivatives exhibit many pharmacological properties such as antitumor, antibacterial, antifungal, anticoagulant, antioxidant, anti-inflammatory and many others. In this study we had dealt with its antimicrobial activity followed by pH analysis. The aims of this study are to prepare and identify the crude coumarin extracted from cassia cinnamon and to study its antimicrobial activity. The antimicrobial activity of extracted crude coumarin from cinnamon sample was tested for antimicrobial pattern against gram positive, gram negative bacteria and fungi. The study of Minimum inhibitory concentration was performed by disc diffusion method. . The highest activity in terms of zone of inhibition (22.32mm) was observed against gram negative bacteria E.coli(shown in fig.6).Other gram positive bacteria staphylococcus aureus was also found susceptible to ethanolic extract and inhibition zone was noted as a 12.61mm (shown in fig.7). C. albicans fungi was also found susceptible to ethanolic extract and inhibitory zone was recorded Ambiguously in a wide range between 5-19 mm. However, there was no zone of inhibition found for aqueous extract form. In comparison with broad spectrum antimicrobial activity, maximum antibacterial activity was shown by E.coli followed S. aureus. The pH analysis was performed & it was found to be non-reactive with different acid & bases (Strong & Weak), So it cannot be used for titrimetric analysis although it has pigment but it can be effectively used as one of the active ingredient on skin without concerning about the change in pH of formulation.

KEYWORDS

Crude Coumarin, Candida albicans, E.coli, Antifungal activity, pH analysis, Cinnamon, Antibacterial activity.

INTRODUCTION

Coumarin is an aromatic compound contains oxygenated heterocyclic ring. Coumarinic compounds are a class of lactones, structurally constructed with fusion of a benzene ring and α-pyrone ring having molecular formula as $C_9H_6O_2$ (Fig.1). Coumarin is chemically known as 2H-1-Benzopyran-2-one and 1,2-Benzopyrone and it is a colorless crystal flakes or powder in appearance with a pleasant vanilla fragrant and a bitter aromatic burning or caramel taste. [1] [4] The molecular weight of coumarin is 146.145g/mol, and melting and boiling points are 71°C and 301.7°C respectively. Coumarin is completely soluble in organic solvents such as ethanol, either and chloroform, and insoluble in water.[2] Coumarin is sensitive to exposure of light and heat. Coumarins are incompatible with strong acids and bases and oxidizers. It is hydrolyzed by hot concentrated alkalis. It can be halogenated, nitrated and hydrogenated.[3]

Cinnamon, sweet clover, lavender oil and Tonka bean are the natural sources of coumarin compound and present in high concentration [5] [7]. Firstly, coumarin was extracted using Tonka beans in 1820 [5]. Coumarin and its derivatives form are major group of natural source and widely distributed in the natural kingdom [6]. Coumarin extraction with polar solvents (water, ethanol, methanol) ae shown to be the most efficient. Water extraction gives the highest total coumarin concentration whereas furanocoumarin is obtained by extracting using methanol and other organic solvents [8].

By reviewing different research study, it is demonstrated that coumarin and its derivatives represent one of the most active classes of heterocyclic compounds which possess a wide spectrum of biological activities such as antitumor, antibacterial, antifungal, anticoagulant, antioxidant, and anti-inflammatory [9][10][11][12]. Coumarin gives a remarkable formation of biochemical and pharmacological activity. These are natural benzopyrone derivatives found in plants as a free state and as glycosides, exhibit various pharmacological properties such as antitumor, anti-hypertension, analgesic and antiseptic, as well as toxicity (e.g. phototoxic and carcinogenic effects).[21] Structurally modified compounds come up with to speed up the process by providing insights to improve the potency, efficacy and selectivity of compounds.[13] Coumarins are categories by their benzopyran-2-one nucleus, have been confined from number of plants, belonging to the family Apiaceae, Rutaceae, and Fucaceae, and from some genera of Leguminosae [22]. Natural and synthetic coumarins are one of the demanding compounds due to its broad pharmacological activities. The synthesized coumarin derivatives showed purposeful activities against Gram positive and Gram-negative bacteria as well as on strains of Candida spp..[14]

The aims of this study are to prepare and identify the crude coumarin extracted from cassia cinnamon and to study its antimicrobial activity.

MATERIALS AND METHOD

PREPARATION OF CRUDE EXTRACT

The cinnamon sticks were washed, air dried and ground to obtain fine dry powder of cinnamon. The solvent. Then vigorously stirred for 10- 15 minutes followed by addition of small quantity of prepared 100 gram of cinnamon powder was mixed with 200 ml of 90% ethanol and anhydrous magnesium sulphate powder to remove any fine traces or water. Next, the solution is filtered using funnel and filter paper to achieve clear liquid filtrate (Fig.2). After that the collected filtrate content was placed on hot plate for evaporation to get the dry powdered as residue (Fig.3) [15]

IDENTIFICATION TESTS OF PREPARED EXTRACT

Ferric chloride test was performed in order to confirm the presence coumarin in crude extract of cassia cinnamon.To the concentrated alcoholic extract of drug few drops of alcoholic FeCl3 solution was added. Resulting of pale green colour, which turned orangish yellow on addition of conc. HNO3, indicates presence of coumarins.(fig. 4)The cinnamon extract passes the test and it confirms the presence of coumarin.

Fluorescence test-The alcoholic extract of drug was mixed with 1N NaOH solution (one ml each). Emission of blue-green fluorescence confirms the presence of coumarins.

ANTIMICROBIAL ACTIVITY OF CRUDE COUMARIN

The fungal and bacterial strains were obtained from MICROBIO LABORATORY vartak nagar thane, Maharashtra, India. Antimicrobial effect was studied using three microbial species. The two were bacterial species i.e. E Coli (Gram Negative) and Staphylococcus Aureus (Gram Positive).and other one was fungal species Candida Albicans.

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 and fungal strain media culture.

PREPARATION OF CULTURE MEDIA

1)***FOR FUNGUS***

Preparation of Sabouraud Agar

We Suspended 65 gm of the medium in one liter of purified water. Heat with continuous agitation and boil for 1 minute to dissolve completely the medium. Autoclave at 121°C for 15 minutes. Cool till 45ºC to 50°C

and then pour it into Petri dishes or tubes for slants. .For the processing of specimens, streak the specimen onto the medium with a sterile inoculating loop in order to obtain isolated colonies. Incubate the plates at 25-30 C in an inverted position (agar side up) with increased humidity.[17]The agar used for fungal strain (candida albicans) was sabouraud dextrose.

Composition of Sabouraud Dextrose Agar It consist of 10gm Mycological peptone (enzymatic digest of casein and animal tissues), Dextrose 40gm, Agar 15gm[17].

2)*FOR BACTERIA*

Preparation of Soyabean Casein Digest[18]

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 bacteria was soyabean casein.

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 leaned on the determination of an inhibition zone proportional to the bacterial susceptibility to the antimicrobial present in the given disk. As the concentration of the antimicrobial becomes diluted that it can no longer inhibit the growth of the test microbial, the zone of inhibition is bounded. The diameter(maximum length in petri plate) of the zone of inhibition around the antimicrobial disk is concerned to minimum inhibitory concentration (MIC) for that particular antimicrobial agent. The zone of inhibition is related inversely with the MIC of the testing agent. Generally, if the zone of inhibition is greater, lower will the antimicrobial concentration required to inhibit the growth of the organisms. Although, these things depend on the concentration of antibiotic in the disk and its diffusibility.

PH ANALYSIS FOR COUMARIN:

First, we had prepared the pH paper with the use of Watts's man filter paper by soaking it in the extracted solution of coumarin extract in the petri plate and allowed for drying. Dried Whatman filter papers cut in to 5 strips. Different buffer and chemical solutions of different pH were prepared. The color change of strips was checked with prepared various pH ranges of solutions.

The pH analysis was performed & it was found to be non-reactive with different acid & bases (Strong & Weak), So it cannot be used for titrimetric analysis although it has pigment.

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)[23]. Reddish brown liquid extract (shown in fig. 2) of cinnamon powder was collected using ethanolic solution. 5.12 gm crude ethanolic extract coumarin was successfully prepared (shown in fig.3), 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(showed in fig.4). The pH analysis was performed & it was found to be non-reactive with different acid & bases (Strong & Weak), So it cannot be used for titrimetric analysis although it has pigment.(showed in fig.8).

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 antimicrobial pattern against gram positive, gram negative bacteria and fungi. 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(shown in fig.6).Other gram positive bacteria staphylococcus aureus was also found susceptible to ethanolic extract and inhibition zone was noted as a 12.61mm (shown in fig.7). C.albicans fungi was also found susceptible to ethanolic extract and inhibitory zone was recorded Ambiguously in a wide range between 5-19 mm. However, there was no zone of inhibition found for aqueous extract form.

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

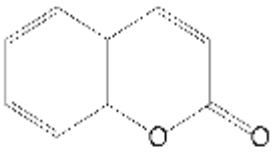

Figure 1 Basic coumarin structure

Figure2.LIQUID FILTERATE OF COUMARIN

Figure 3 DRY EXTRACT OF CRUDE COUMARIN

Figure 4 fecl3 test results showing presence of coumarin

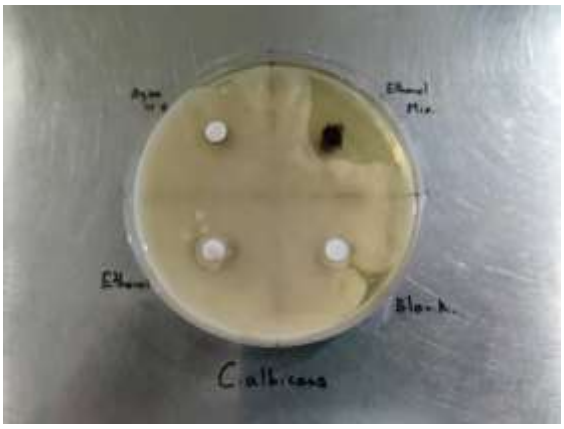

Figure 5 antifungal activity of crude coumarin against Candida Albicans

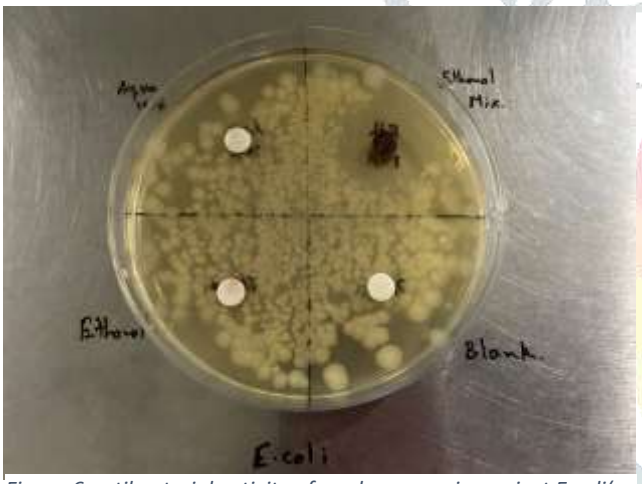

Figure 6 antibacterial activity of crude coumarin against E.coli(gram negative)

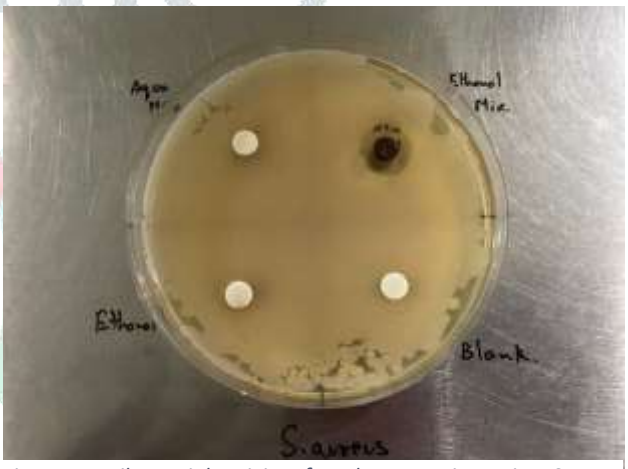

Figure 7 antibacterial activity of crude coumarin against S. aureus(gram positive)

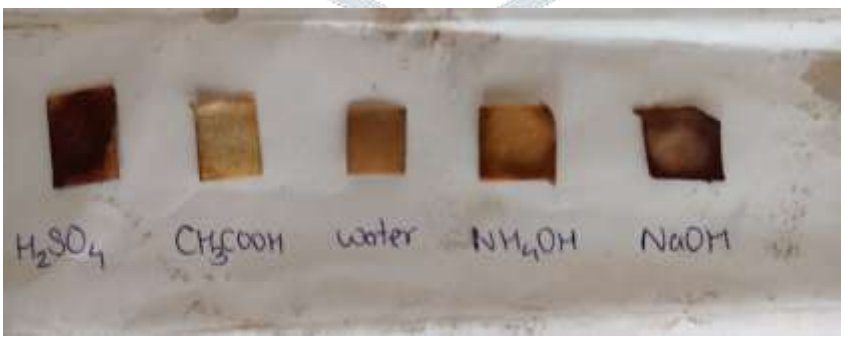

Figure 8. ph. analysis of coumarin extracted soaked paper

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 antimicrobial 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 and fungal infections. Coumarin and its derivatives are known to be medicinally important by having different pharmacological activities including antimicrobial activity. Coumarin serve as valuable source of lead compounds for the drug design and development of effective antimicrobial therapy. The pH analysis was also performed & it was found to be non-reactive with different acid & bases (Strong & Weak), So it cannot be used for titrimetric analysis although it has pigment.

ADVANCEMENT & FUTURE ASPECTS

The concern in the synthesis of coumarin derivatives has becoming an importance over the last decades, reflecting the importance of such compounds in both medical and chemical research. Future motive for this in field of research includes the discovery, synthesis, and development of compounds which display increased potency, as well as fueling structure-activity relationship studies aimed at understanding the modes of action of the most biologically active members of these classes of products.

The presence of an electronegative atom is powerful for hydrogen bond formation and for solubility, to little extent and aromatic ring is also responsible for having hydrophobicity. These events are the cause of better interaction of the molecule with a receptor site. The substitution of coumarins promotes them more significant for effective bioactivity. Number of coumarins have been synthesized and also are present in natural based too. With unlike structures due to the multi types of substitutions in their basic nuclei, they are important in showing effective and many classes of biological activity.

Based on the substitution pattern, coumarins show *anticancer,* 67 *anti-HIV*, *anticoagulant*, *antimicrobial*, *antioxidant*, *hepatoprotective*, *antithrombotic*, *antituberculosis*, *antiviral*, *anticarcinigenic*, *and anti-inflammatory* activities.

For effective antifungal activity-

- Nitro group at C3 position is active,
- Acetyl group at C4 position is significant
- Both nitro and acetyl groups are active at C6 position.
- Aliphatic chain, phenyl group, acetyl & nitro group are prominent at C7 position.

For effective antibacterial activity-

- Methyl group at C8 position shows effective antibacterial activity.
- Nitro group at C5 position.
- At C3 position on the coumarin ring, the presence of ethylene moiety with carboxylic or tertiary amine group, alkoxyamine, carbonyl, and benzyl group with hydroxyl or halide group substitution is effective.

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.[20]

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.

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REFERENCES

1. Lewis, R.J., Sr (Ed.). Hawley's Condensed Chemical Dictionary. 13th ed. New York, NY: John Wil ey & Sons, Inc. 1997., p. 307
2. https://pubchem.ncbi.nlm.nih.gov/compound/Coumarin
3. https://cameochemicals.noaa.gov/chemical/20052
4. https://www.degruyter.com/database/IUPAC/entry/iupac.compound.323/html?lang=en
5. https://www.researchgate.net/publication/282672605_Coumarins__An_Important_Class_of_Phytoc hemicals
6. Keating GJ, O'Kennedy R. The chemistry and occurrence of coumarins. O'KennedyRTRD, editor. England: John Wiley & Sons West Sussex; 1997
7. Miranda M, Cuellar A. Farmacognosia y productos naturales. La Habana: Félix Vare-la; 2001

8. https://www.researchgate.net/publication/230195990_Extraction_of_coumarins_from_plant_materia l_Leguminosae
9. bacteriostatic & antitumor activity. (P.K. Jain & Himanshu Jashi"; "Coumarin:- Chemical & Pharmacological Profile" Journal of applied Pharmaceutical Science 02(06); 2021:236-240)
10. Anti-coagulant, antioxidant, antimicrobial. (anti-viral, anti-fungal, and anti parasitic, anticancer, antidiabetic and analgesic Coumarins - An important class of phytochemicals http://dx.doi.org/10.5772/59982.
11. Cristina Montagner, Simone M. de Souza; Antifungal activity of coumarins: http://www.research 0/5467121 gate.net/publication/5467121
12. Anti hemorrhagic and anti-inflammatory effect (-Richard o kennedy & Enaprosser;" The pharmarcology, metabolism, Analysis & Application of Coumarin and Coumarin- Related Compound -https://www.researchgate.net/publication /225028868)
13. Evaluation of Antifungal Activity and Mode of Action of New Coumarin Derivative, 7-Hydroxy-6-nitro-2H-1-benzopyran-2-one, against Aspergillus spp ;Felipe Queiroga Sarmento Guerra 1, Rodrigo Santos Aquino de Araújo;https://pubmed.ncbi.nlm.nih.gov/26175794/
14. 4-Methyl-7-hydroxycoumarin antifungal and antioxidant activity enhancement by substitution with thiosemicarbazide and thiazolidinone moieties Bojan Šarkanj a , Maja Molnar www.elsevier.com/locate/foodchem139(2013)488-495
15. Celeghini, R. M. S.; Vilegas, J. H. Y.; Lancas, F. M. Extraction and Quantitative HPLC Analysis of Coumarin in Hydroalcoholic Extracts of Mikania glomerata Spreng: ("guaco") Leaves. J.
16. http://www.pharmacy180.com/article/chemical-tests-of-glycosides-166/
17. http://slideshare.net/Vamsilntellectual/preparation-of-fungal-culture-media
18. https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/345/476/s3931dat. pdf
19. Felipe QueirogaSarmento Guerra, Rodrigo Santos Aquino de Araújo; Evaluation of Antifungal Activity and Mode of Action of New Coumarin Derivative, 7-Hydroxy-6-nitro-2H-1benzopyran2one,against Aspergillus spp;Hindaw;volume 2015 |ArticleID 925096 | https://doi.org/10.1155/2015/925096
20. Coumarins; A.Garrard; journal of pharmaceutical and biomedical analysis,2013; https://www.sciencedirect.com/topics/chemistry/coumarins
21. https://www.sciencedirect.com/science/article/pii/S0731708512004931
22. https://www.researchgate.net/publication/265216184_Furanocoumarins_Biomolecules_of_Therapeutic_Interest
23. https://pubs.acs.org/doi/10.1021/jf4005862