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Antibacterial and Phytochemical Analysis of Cinnamon Bark (Cinnamomum Zeylanicum) Extract against Clinical Pathogens

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ABSTRACT:
Cinnamon (Cinnamomum zeylanicum) has been using daily documented to be antimicrobial and have medicinal value as well. It has antimicrobial, antioxidants, anti-inflammatory properties. Cinnamaldehyde and eugenol are highly present in cinnamon bark. Different concentrations of extracts were prepared by using methanol and water. The extracts were analysed for phytochemicals which give cinnamon its antimicrobial activity.Chemical analysis showed that Cinnamomum zeylanicum bark powder extract contained different active compounds (Phenols, alkaloids, tannins, glycosides, saponins, resins, steroids, terpenoids, carbohydrates and flavones). Antibiotic sensitivity test were done by disc diffusion method. The Antibacterial activity was tested against Staphylococcus aureus, Streptococcus mutans, Pseudomonas aeruginosa. The extracts were very effective against Staphylococcus aureus than the antibiotic used.

Key words: Cinnamon (Cinnamomum zeylanicum) Solvent extraction, Phytochemical analysis, Antibacterial activity.

I. INTRODUCTION:

The use of plant based drugs for treating various ailments is known to humans since thousands of years. Herbs and spices have been used since ancient times, not only as antioxidants and flavoring agents,but also for their antimicrobial activity against pathogenic microorganisms. Cinnamomum zeylanicum , a member of the family Lauraceae , has a long history both as a spice and as a medicine. Cinnamon possesses potent antibacterial, antifungal, antitermitic,larvicidal, nematicidal, and insecticidal properties. The chemical composition of cinnamon is broadly explored. Plants produce large amounts of compounds known as phytochemical and each part of the plant has different phytochemicals which has immense medical values. Research investigation by Vangalapati et al., 2012 reported that Cinnamon bark is rich in cinnamaldehyde which has been proven to be active against many pathogenic gram positive and gram negative bacteria. A recent study reported the activity of the aqueous extract of cinnamonand other plants against oral microflora. One of the potential compounds in controlling plaque formation is in cinnamon bark. Puspita et al. (2013) showed that cinnamon extract has an effect on the growth of Streptococcus mutans which is a carriogenic bacteria. The aim of the present study was to determine the phytochemical composition of the cinnamon (Cinnamonum zeylanicum) and to assess the antibacterial activities of cinnamon against various human pathogens.

II. MATERIAL AND METHODS:

Collection of sample:

The spice sample Cinnamomum Zeylanicum was purchased from the Supermarket in Nehru Nagar, Coimbatore (Fig. 1). The cinnamon bark was grounded in a grinding machine (Moulinex) in order to obtain a fine dry powder.

Collection of Clinical Pathogens:

The Clinical Pathogens such as Staphylococcus aureus, Streptococcus mutans, Pseudomonas aeruginosa cultures were collected from the Bio line Laboratory, R S Puram, Coimbatore . The cultures were preserved under 4°C.

Preparation of spice extract:

Aqueous extract:

20 gram of spice powder was dissolved in 200 ml of Sterile Distilled water and mixed . It was kept in room temperature for 7 days with regular shaking . Then it was filtered using Whatman filter paper. The extract was used for antibacterial analysis.

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Solvent extract:

20 gram of spice powder was dissolved in 200 ml of Ethanol in a conical flask, mixed and tightly plugged. It was kept in room temperature for 7 days with regular 33 shaking. Then it was filtered using Whatman filter paper, filtrate was evaporated in a petri dish at room temperature for 2-3 days until it evaporates. Then the dried sample was resuspended with a sterile Deionised (DI) water and stored in vials at 4˚c.

Preparation of broth cultures:

All the three clinical pathogens (Staphylococcus aureus, Streptococcus mutans, Pseudomonas aeruginosa) was cultured in Nutrient broth and grown overnight at 37˚c for 24 hours, it was used in antibacterial analysis.

Media preparation:

Mueller Hinton agar (MHA) Composition (grams/ L)

HM infusion B form- 300.00
Acicase -17.50
Starch -1.50
Agar -17.00
Final pH -7.3±0.1

19 grams of MHA (Muller Hinton Agar) was dissolved in 500 ml of distilled water and then autoclaved for 15 minutes at 121˚c. Once the medium was about 45˚-50˚c, it was poured into sterile petri dishes. Then it was allowed to set completely.

Antibacterial activity:

Disc diffusion method:

Select a pure culture plate of one of the organisms to be tested.

Aseptically emulsify a colony from the plate in the sterile saline solution.

Mix it thoroughly to ensure that no solid material from the colony is visible in the saline solution.

Repeat until the turbidity of the saline solution visually match that of the standard turbidity. Take a sterile swab and dip it into the broth culture of Pseudomonas aeruginosa, streptococcus mutans, staphylococcus aureus .

Gently squeeze the swab against the inside of the tube in order to remove excess fluid in the swab.

Take a sterile Mueller-Hinton agar (MHA) plate Use the swab with the test organism to streak an MHA plate for a lawn of growth.

After the streaking is complete, allow the plate to dry for 5 minutes.

Overnight soaked disk can be placed on the surface of the agar using sterilized forceps. Gently press the discs onto the surface of the agar using flame sterilized forceps or inoculation loop.

Carefully invert the inoculated plates and incubate for 24 hours at 37° C.

After incubation, use a metric ruler to measure the diameter of the zone of inhibition for each antibiotic used.

The zone of inhibition was observed and tabulated.

Phytochemical analysis:

Preparation of Cinnamon bark extract:

The collected spice sample was dried and grinded into a fine powder, which can be used for extraction.

Aqueous extract:

The 10 grams of cinnamon bark powder was mixed with 100ml of distilled water and kept for 3 days . Then the extract was filtered by whatman filter paper.

Solvent extract:

The 10 grams of powdered spice sample was mixed with 100 ml of Ethanol and kept 3 days and filtered by whatman filter paper.

Qualitative phytochemical analysis of the cinnamon bark extracts as follows,

Test for alkaloids :

2 ml of spice extracts was mixed with few ml of dilute Hydrochloric (Hcl) Acid and filtered. The filtrate was added with few drops of Hager's reagent (Aqueous solution of Picric acid). A yellow precipitate indicates the presence of Alkaloids.

Test for glycosides :

2 ml of spice extract was added with glacial acetic acid, ferric chloride (FeCl3) and H2So4 of each 1 ml. A green blue colour indicates the presence of Glycosides.

Test for steroids :

2 ml of spice extract was mixed with 5 ml of chloroform, 2 ml of acetic anhydride and 1 ml of concentrated H2So4 and the colour changes was observed. Reddish brown colour indicates the presence of Steroids.

Test for flavonoids :

To a 2ml of spice extracts, few drops of NaOH solution was added, a yellow colour solution was formed. Then add few ml of diluted Hydrocholoric (Hcl) Acid which turns yellow

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colour solution into a colorless solution, which indicates the presence of Flavonoids.

Test for tannis :

A small amount of spice extract was mixed with 2ml of ferric chloride (FeCl3) and the colour change was recorded. The formation of green grey / dark blue colour indicates the presence of Tannins.

Test for saponins :

The spice extract and the distilled water was mixed as same volume and the mixture was shaken vigorously. The formation of a layer of foam indicates the presence of Saponins.

Test for resins :

10 ml of distilled water was added to 5ml of spice extract. A precipitate indicating the presence of Resins.

Test for phenols :

To a 2 ml of spice extract, 1 ml of ferric chloride (FeCl3) solution was added . Deep blue black colour indicates the presence of Phenols.

Test for terpenoids :

Extract was taken in chloroform with few drops of concentrate sulphuric acid, shaken well and allowed to stand for some time. Formation of yellow colour layer indicated the presence of terpenoids .

Carbohydrates

Fehling test :

Volume of Fehling A and Fehling B were mixed together and 2ml of it was added to crude extracts and gently boiled, a brick red precipitate at bottom of test tube indicated presence of reducing sugar.

III. RESULTS AND DISCUSSION

Antibacterial effect of cinnamon bark extract against clinical pathogens:

From the results obtained, the Cinnamon bark extracts inhibits the majority of clinical pathogens used in this study.The cinnamon bark extract showed more effective antibacterial activity for aqueous extract. The methanol and aqueous extracts of cinnamon bark showed inhibition againstPseudomonas aeruginosa, Streptococcus mutans, Staphylococcus aureus.

Phytochemical analysis of cinnamon bark extract:

Majorly, all the phytochemicals like Alkaloids, Glycosides, Steroids, Flavonoids, Tannins, Saponins, Resins, Phenols, Terpenoids and Carbohydrateswere present in cinnamon extracts. Aqueous extract of Cinnamon bark showed the presence of Phenol in addition.

IV. LIST OF TABLES

Table 1. showed Antibacterial activity of cinnamon bark methanol extract

S.No	Org anisms	Zone of inhibition (in mm)		
		Sample	Methanol (blank)	Control (Antibiotic disc)
1.	Staphylococcus aureus	23	20	18 (Methicillin)
2.	Streptococcus mutans	21	16	17(Ciprofloxacin)
3.	Pseudomonas aeruginosa	17	16	12 (Ciprofloxacin)

Table 2. showed Antibacterial activity of cinnamon bark aqueous extract

S.No	Organisms	Zone of inhibition (in mm)		
		Sample	Antibiotic disc	Control (Antibiotic disc)
1.	Staphylococcus aureus	23	21	19 (Methicillin)
2.	Streptococcus mutans	20	15	14(Ciprofloxacin)
3.	Pseudomonas aeruginosa	17	-	11(Ciprofloxacin)

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Table 3. showed phytochemical analysis of cinnamon bark extract

S. No.	Phytochemical	Cinnamon bark extract	
		Methanol extract	Aqueous extract
1.	Alkaloids	+	+
2.	Glycosides	-	-
3.	Steroids	+	+
4.	Flavonoids	-	-
5.	Tannins	+	+
6.	Saponins	+	+
7.	Resins	+	+
8.	Phenols	-	+
9.	Terpenoids	-	-
10.	Fehling test	+	+

Note: (+) – present, (-) - absent

V. SUMMARY CONCLUSION

In the present study the samples were collected fromSupermarket in Nehru Nagar, in Coimbatore. The collected sample were dried and powdered. And clinical samples collected from a Bio line laboratory in Coimbatore such as (Streptococcus mutans, Pseudomonas aeruginosa andStaphylococcus aureus).The powdered sample weremixed with solvents such as methanol and aqueous. The mixed solvent filtered by Whatman filter paper.They were kept in petri dish at room temperature for evaporation.

The Sample were powdered.The powdered samples were characterized using Antibacterial activity, Phytochemical analysis.

Antibacterial activity was done by Disc diffusion method. Soaked the discs in already prepared concentrations of extracts and left overnight. After the 24 hours of incubation the zone of inhibition were measured and recorded

The cinnamon bark extract showed more effective antibacterial activity for aqueous extract. The methanol and aqueous extracts of cinnamon bark showed inhibition against Pseudomonas aeruginosa, Streptococcus mutans, Staphylococcus aureus.

Majorly, all the phytochemicals like Alkaloids, Glycosides, Steroids, Flavonoids, Tannins, Saponins, Resins, Phenols, Terpenoids and Carbohydrateswere present in cinnamon extracts. Aqueous extract of Cinnamon bark showed the presence of Phenol in addition.

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CONFLICTS OF INTEREST:

The author has no conflicts of interest to publish this Research article in this journal.

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