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Research Article

Comparative Evaluation of Cinnamon Plant for Their Antimicrobial Efficacy against Pathogenic Bacteria

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ABSTRACT

Cinnamon was grounded in mortar and pestle with addition of 10ml distilled water. Then it is subjected to vortex and centrifuged to collect the supernatant. This extract was collected in a test tube. In the qualitative phytochemical testing presence of various secondary metabolites were found viz. saponin, tannin, flavonoid, carbohydrate, protein, steroids, terpenoids and soluble starch. Saponin, terpenoids and carbohydrate were found in abundance while protein, steroid, soluble starch, tannin and flavonoid were found in moderate amount. Less or no amount of alkaloids, phytosterols and phenol were found. In the quantitative analysis titration of ascorbic acid and citric acid were performed and spectrophotometry of carbohydrates and phenols were performed. Ascorbic acid and carbohydrate were found in moderate amount while citric acid and phenols were found in fewer amounts. Then the antimicrobial activity of cinnamon was studied by the disk diffusion method. The antibacterial activity of cinnamon against *S. aureus* and *S. epidermis* was found to be nil. Whereas the antifungal activity of cinnamon against *A. niger* was found quite good while no activity in *A. candidus*. The inhibitory effect of cinnamon is quite poor against *P. corylophilum* and *Penicillium* species.

Keywords: Cinnamon, Phytochemical, Supernatant, Spectrophotometry.

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INTRODUCTION

Cinnamon is the aromatic, inner bark of certain bushy, tropical, evergreen shrubs or small trees of that is dried, ground, and used as a spice. The term is also used for the culinary name of the spice and for the plants yielding this bark.

Description

Notable *Cinnamomum* species include cinnamon (*Cinnamomum verum* or *C. zeylanicum*, also known as "true cinnamon" or Ceylon cinnamon), cassia (*C. aromaticum* or *C. cassia*), camphor laurel (*C. camphora*), Saigon cinnamon (*C. loureiroi*, also known as

Vietnamese cinnamon, Vietnamese cassia, or Saigon cassia). Cassia is an evergreen tree native to southern China and mainland Southeast Asia west to Myanmar. Like its close relative, *Cinnamomum zeylanicum*, also known as "true cinnamon" or "Ceylon cinnamon," it is used primarily for its aromatic bark, which is used as a spice, often under the culinary name of "cinnamon." Cassia's flavor however is less delicate than that of true cinnamon for this reason the less expensive cassia is *Cinnamomum* is a genus of evergreen trees and shrubs belonging to the Laurel family, Lauraceae. The species of *Cinnamomum* have aromatic oils in their leaves and bark. The genus contains over 300 species distributed in tropical and subtropical regions of North America,

Central America, South America, South America, Asia, Oceania, and Australasia. The buds are also used as a spice, especially in India and in Ancient Rome.

The name *cinnamon* is correctly used to refer to Ceylon cinnamon *C. verum* also known as "true cinnamon." True cinnamon is also sometimes confused with *Cinnamomum tamala* (Malabathrum). In particular, however, true cinnamon and cassia are confused in the marketplace. Ceylon cinnamon, using only the thin inner bark, has a finer, less dense and more crumbly texture, and is considered to be less strong than cassia. Cassia is generally a medium to light reddish brown, is hard and woody in texture, and is thicker (2-3 mm thick), as all of the layers of bark are used. Cinnamon sticks have many thin layers and can easily be made into powder using a coffee or spice grinder whereas cassia sticks are much harder usually are made up of one thick layer, and are capable of damaging a spice or coffee grinder if one attempts to grind them without first breaking them into very small pieces.

Medicinal uses

As a warm and dry substance in ancient times cinnamon was believed by doctors to cure snakebites, freckles, the common cold, and kidney troubles, among other ailments. In medicine, cinnamon has been used to treat diarrhea and other problems of the digestive system. It is high in antioxidant activity and the essential oil of cinnamon also has antimicrobial properties which aid in the preservation of certain foods. In the media cinnamon has been reported to have remarkable pharmacological effects in the treatment of type II diabetes.

The effects which may even be produced by brewing a tea from cassia bark also may be beneficial for non-diabetics to prevent and control elevated glucose and blood lipid levels. Cassia's effects on enhancing insulin sensitivity appear to be mediated by polyphenols. It should not be used in place of anti-diabetic drug unless blood glucose levels are closely monitored and its use is combined with a strictly controlled diet and exercise program. Cinnamon has traditionally been used to treat toothache and fight bad breath. There is anecdotal evidence that consumption of cassia has an effect in lowering blood pressure, making it potentially useful to those suffering from hypertension. Cassia is used in traditional Chinese medicine, where it is considered one of the 50 fundamental herbs. European health agencies recently have warned against consuming high amounts of cassia generally known just as cinnamon in U.S. market due to a toxic component called coumarin. This is contained in much lower dosages in Ceylon cinnamon and in *Cinnamomum burmannii*. Coumarin is known to cause liver and kidney damage in high concentrations. Though the spice cassia has been used for

thousands of years there is concern that there is as yet no knowledge about the potential for toxic buildup of the fat-soluble components in cassia as anything fat-soluble could potentially be subject to toxic buildup.

REVIEW OF THE LITERATURE

CINNAMON

The bark of various cinnamon species is one of the most important and popular spices used worldwide not only for cooking but also in traditional and modern medicines. Overall approximately 250 species have been identified among the cinnamon genus with trees being scattered all over the world. Cinnamon is mainly used in the aroma and essence industries due to its fragrance which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products. The most important constituents of cinnamon are cinnamaldehyde and *trans*-cinnamaldehyde, which are present in the essential oil, thus contributing to the fragrance and to the various biological activities observed with cinnamon. A study on *Cinnamomum osmophloeum* indicated that the essential oil from cinnamon leaves contains a high level of Cinnamaldehyde. Consequently *C. osmophloeum* is also used as an alternative spice for *C. cassia*. One of the major constituents of essential oil extracted from *C. zeylanicum* named (E)-cinnamaldehyde has an antityrosinase activity while cinnamaldehyde is the principal compound responsible for this activity. Cinnamon bark contains procyanidins and catechins. The components of procyanidins include both procyanidin A-type and B-type linkages. These procyanidins extracted from cinnamon and berries also possess antioxidant activities.

MATERIALS AND METHODS

SAMPLE EXTRACTION

Extraction is the crucial first step in the analysis of Cinnamon plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. The basic operation included steps, such as pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system. Proper actions must be taken to assure that potential active constituents are not lost, distorted or destroyed during the preparation of the extract from Cinnamon.

Fresh cinnamon bark is ground into powder without any water. The powder is then dissolved in a specific amount of distilled water and mixed properly using vortex. The vortexed solution is then centrifuged and the supernatant is collected in a test tube.



Figure: 1 Crude sample extract (on left); sample extract after centrifugation (on right)

GROWTH AND MAINTENANCE OF MICROORGANISMS IN CINNAMON PLANT

The strains of bacteria *S. aureus* (MTCC 3160) , *S. epidermis* (MTCC 3382) , *E. coli* (and *B. subtilis* were provided by Rapture Biotech for this particular study. The

strain from the plate was inoculate in the nutrient broth and then the inoculum was left for 1-2 days at 30°C in the incubator. After the growth of bacteria in the broth, it is used to perform the disk diffusion method with the given cinnamon plant sample.



Figure 2: Strains of *S. aureus* and *S. epidermis* on MSA

PHYTOCHEMICAL TEST (Qualitative tests)

SAPONIN

2 ml sample was dissolved in 6ml distill water.

Shaked well. Froth formation took place.

Stability of the froth confirms the presence of saponin in the samples.

TANIN

1 ml sample was dissolved in 1 ml 5% FeCl3.

Appearance of dark blue or greenish black color confirms presence of tannin in the sample.

If no color changes then heating mantle is used for changing the color.

FLAVONOIDS

20 µl sample was drop wise added into 20 ml NaOH.

Again Conc. HCL was added drop wise, disappearance of yellow color confirms the presence of flavonoids in the sample.

CARBOHYDRATES

Fehling's reagent was prepared by mixing Fehling A and Fehling B solution.

For Fehling A 0.35g CuSO₄ was dissolved into 5 ml distill water followed by addition of 2-3 drops of Conc. H₂SO₄.

For Fehling B 1.75g NaK tartarate was dissolved in 5 ml distill water, 1.25g NaOH was added in the solution and mixed well to dissolve it.

Then Fehling A and Fehling B was mixed well in the ratio of 1:1(FA+FB=10ml).

Now 1ml Fehling's reagent was dissolved in 2ml sample and heated for over 20 mins.

Appearance of red ppt. confirms the presence of carbohydrates in the sample.

PROTIEN

500µl of 1% CuSO₄ was prepared and 500µl of 5% NaOH was prepared.

Mixed together.

Sample was added in the solution, occurrence of purple color confirms protein in the sample.

ALKALOIDS

500µl extract was centrifuged and 500µl Wagner's reagent was mixed into it.

Shaked well and left for sometime.

Reddish brown color appears and confirms presence of alkaloids.

STEROIDS

500µl extract was dissolved in 1ml chloroform and 1ml H₂SO₄ was added side by side in the test tube.

Upper layer appears red and H₂SO₄ shows yellow with green fluorescence.

After that acetic acid was added to confirm the test.

PHENOLS

500µl extract was dissolved in distill water. 2 drops of aq.

FeCl₃ was added.

Appearance of blue color or green color indicates presence of phenols.

PHYTOSTEROLS

500µl sample was dissolved in 500µl chloroform and 500µl H₂SO₄ was added side by side.

For confirmation dil. acetic acid was added in the solution.

Bluish green color of the solution confirms presence of phytosterols in the solution.

TERPENOIDS

500µl sample was dissolved in 250µl chloroform.

625µl Conc. H₂SO₄ was added to the solution.

Reddish brown ppt. of the solution confirms presence of terpenoids.

STARCH SOLUTION

500µl sample was dissolved in 500µl 5% KOH.

Solution was boiled and cooled down.

400µl H₂SO₄ was added to the solution.

Yellowish color confirms presence of starch solution in the sample.

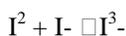
QUANTATIVE ANALYSIS OF PHYTOCHEMICALS**ASCORBIC ACID TEST****Aim**

This test is performed using titration method. It is to see the amount of ascorbic acid present in the mentions plant samples used.

Principle

Ascorbic acid is an important antioxidant and a major source of vitamin C that is essential for human nutrition. to determine the amount of ascorbic acid in the given plant samples, redox titration is used.

Iodine oxidises vitamin C to form dyhydroascorbic acid. as long as vitamin C is present in the solution, the iodine is converted to iodide ion very quickly. However, when all the vitamin C is oxidised, the iodine present will react with starch to form a blue-black complex. The blue-black colour is the end point of titration.

Reactions

Iodine is complexed with iodide to form triiodide.



Triiodide oxidises vitamin C to form dehydroascorbic acid.

Chemicals used

Iodine is used as titrate - 5 ml iodine dissolved in 45 ml distilled water.

0.5% 500 µl starch solution is prepared of volume 10 ml.

□ 5% ascorbic acid.

Procedure

- Take a burette and fix it properly in the burette stand. Wash the burette properly with distilled water.
- The iodine solution prepared is poured into the burette upto a specific mark and note its reading.(IBR)
- Prepare the solutions to be titrated in a culture bottle as mentioned below (-ve control, +ve control , sample.).
- Place the bottle exactly below the burette so that when the knob is opened, the iodine solution in the burette falls drop wise into the bottle.
- Continuously shake the bottle gently with each drop of iodine falling into it.
- Just when the colour of the solution in the culture bottle turns blue, tighten the knob immediately.
- Note the reading at which the iodine level has reached in the burette. (FBR)

-ve control : 19.5 ml distilled water + 500 µl starch.

+ve control : 19.4 ml distilled water + 500 µl starch + 100 µl ascorbic acid.

sample : 19.4 ml distilled water + 500 µl sample + 100 µl ascorbic acid.

RESULT AND DISCUSSION

Table: 1 Qualitative phytochemical test results

TESTS	CINNAMON
Saponin	+++
Tanin	++
Flavonoid	++
Carbohydrate	+++
Protein	++
Alkaloids	-
Steroids	++
Phenols	-
Phytosterols	-
Terpenoids	+++
Soluble starch	+

*+++ = highly present, ++ = moderately present, + = slightly present, - = absent

Cinnamon is rich in saponin, carbohydrate and terpenoids. While tanin, flavonoid, protein, steroid and soluble starch are present in moderate/less amount.\

Quantitative Phytochemical Tests Results

Ascorbic Acid Concentration Estimation By Titration

Table 2: Ascorbic acid estimation by titration

	IBR	FBR	RESULT
-VE	13.5	13.7	0.2
+VE	13.7	14.2	3.5
CINNAMON	14.9	17.3	2.4

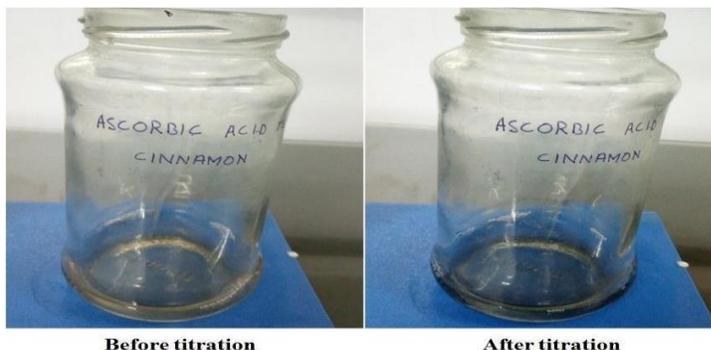


Figure: 3 Ascorbic acid titration of cinnamon

Citric Acid Concentration Estimation By Titration

Table 5: Citric acid estimation by titration

	IBR	FBR	RESULT
-VE CONTROL	6.6	6.7	0.1
+VE CONTROL	6.7	7.3	4.5
CINNAMON	7.6	7.8	0.2



Figure 4 Citric acid titration of cinnamon

Carbohydrate Concentration Estimation By Spectrophotometr Of Cinnamon Plant Sample

ABSORBANCE

Table 6: Carbohydrate concentration estimation by spectrophotometre

Tubes	OD	Concentration (mg/ml)
Blank	0.000	0
T1	0.029	0.3
T2	0.243	1.6
T3	0.509	3.3
T4	0.632	4.9
T5	0.809	6.6
Cinnamon	0.989	7.7

PHENOLIC CONCENTRATION ESTIMATION BY SPECTROPHOTOMETRE

ABSORBANCE

Table 7: Phenolic concentration estimation by spectrophotometre

TUBES	OD	CONCENTRATION (mg/ml)
Blank	0.000	0
T1	0.105	0.2
T2	0.314	2.0
T3	0.441	4.0
T4	0.949	10.0
Cinnamon	0.253	1.8

CONCLUSION

India is the country where plants have great medicinal value. From ancient time plants are used as source for curing diseases. Most of the medicines prepared are recipes

which require proper documentation and research. Herbal medicines have been very useful for curing most of the diseases. Even the consumption of these herbs in little amount is useful in keeping away diseases. Now-a-days these herbal medicines have gathered lot of attention of

general public, researchers as well as government because of no side effects and cost factor. Some of the medicinal plants are used by us daily in different food materials in form of spices to enhance the taste of food, for seizing, for aroma etc.

Cinnamon was grounded in mortar and pestle with addition of 10ml distilled water. Then it is subjected to vortex and centrifuged to collect the supernatant. This extract was collected in a test tube. In the qualitative phytochemical testing presence of various secondary metabolites were found viz. saponin, tannin, flavonoid, carbohydrate, protein, steroids, terpenoids and soluble starch. Saponin, terpenoids and carbohydrate were found in abundance while protein, steroid, soluble starch, tannin and flavonoid were found in moderate amount. Less or no amount of alkaloids, phytosterols and phenol were found. In the quantitative analysis titration of ascorbic acid and citric acid were performed and spectrophotometry of carbohydrates and phenols were performed. Ascorbic acid and carbohydrate was found in moderate amount while citric acid and phenols were found in fewer amounts. Then the antimicrobial activity of cinnamon was studied by the disk diffusion method. The antibacterial activity of cinnamon against *S. aureus* and *S. epidermis* was found to be nil. Whereas the antifungal activity of cinnamon against *A. niger* was found quite good while no activity in *A. candidus*. The inhibitory effect of cinnamon is quite poor against *p. corylophilum* and *Penicillium* species.

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