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A Scientific Erudition

Phytochemical Screening and Antimicrobial Activity of "C*innamon zeylanicum*"

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ABSTRACT

Cinnamon (*Cinnamomum zeylanicum*) has been using since the ancient period due to its medicinal values. It has antimicrobial, antioxidants, anti-inflammatory properties. Cinnamaldehyde and eugenol are highly present in cinnamon bark. Methanol, ethanol, and water were used to produce cinnamon extracts. The anti-microbial activity was performed to determine the microbial activity of *Cinnamon zeylanicum* by observing the zone of inhibition and thus the anti-microbial activity of the cinnamon extract was determined. The antibacterial property was tested against *Esherichia coli* (gram-negative), *Enterococcus faecalis* (gram-positive) and *Salmonella typhi* (gram-positive) by agar diffusion method.

Keywords: *Cinnamomum zeylanicum,* Antibacterial, Agar diffusion

INTRODUCTION

Cinnamon (*Cinnamomum zeylanicum*), a member of the family *Lauraceae*, is a tropical evergreen tree, native to Sri Lanka and the Malabar coast of India. It is called differently in different languages such as dalchini in Hindi, cannelle in French, kaneel in German, canella in Spanish and yookgway in Chinese. The botanical name Cinnamomum is derived from the Hebraic and Arabic term amomon, meaning fragrant spice plant. Cinnamon has been used in food preparations and in traditional medicine by the Egyptians and the Chinese since ancient times approximately 250 species of cinnamon genus have been identified globallywhich are not only used as a flavoring agent but also in medicinal, antimicrobial and antioxidant applications**.** The cinnamon also acts as a natural food preservative. The medicinal applications of cinnamon are the treatment of diarrhea, flatulent dyspepsia, influenza, cough, bronchitis, angina, palpitations, controlling infections, reducing blood sugar levels in diabetics. Cinnamon exhibits anti-inflammatory, antimycotic, insecticidal and anticancer properties [1].

The chemical composition of cinnamon is broadly explored. Each part of the plant has different phytochemicals which has immense medical values. Table No. 1 has showed the presence of phytochemicals and the plant parts. The bark contains cinnamaldehyde, a vasodilator, and a hypoglycemic agent. Eugenol has analgesics, antipyretic properties. Gossypium act as a neuro-protective agent. Caryophyllene oxide, γ-muurolene, α-cadinol,

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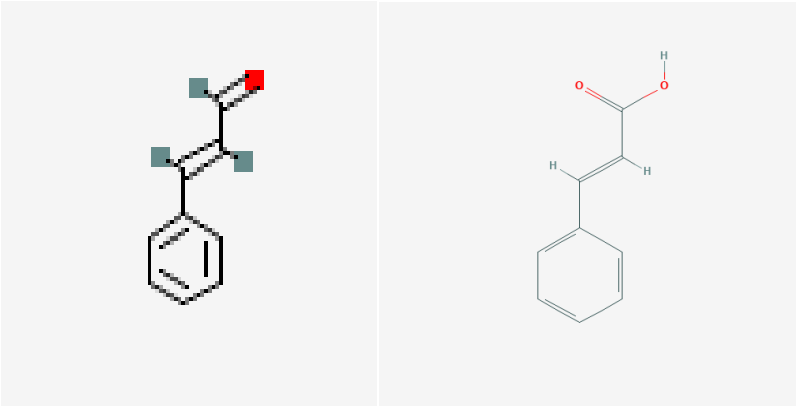
L-borneolare other chemicals present. A study has done by Lu J, Zhang Ketal have found a water-based extract from cinnamon was a potent inhibitor of VEGFR2 kinase activity, directly inhibiting the kinase activity of purified VEGFR2 as well as mitogen-activated protein kinase- and STAT3-mediated signaling pathway in endothelial cells [2]. The extracts of cinnamon have shown antioxidant activities. It can remove free radicals from the body. In accordance with the Department of Agriculture U.S (USDA), a teaspoon of powder cinnamon contains vitamins A, B, C and K, magnesium, iron, phosphorus, calcium, etc. The chemical structure of bio active compounds present in the cinnamon is given in Figure 1.

Phytochemistry of *Cinnamon zeylanicum*

Table 1: Constituents of *Cinnamon zeylanicum*

Part of the plant	Compound
Leaves	Cinnamaldehyde: 1.00 to 5.00% Eugenol: 70.00 to 95.00%
Bark	Cinnamaldehyde: 65.00 to 80.00% Eugenol: 5.00 to 10.00%
Root bark	Camphor: 60.00%
Fruit	Trans-Cinnamyl acetate (42.00 to 54.00%) and caryophyllene (9.00 to 14.00%)
C. zeylanicum buds	Terpene hydrocarbons: 78.00% alpha-Bergamotene: 27.38% alpha-Copaene: 23.05% Oxygenated terpenoids: 9.00%
C. zeylanicum	(E)-Cinnamyl acetate: 41.98%
Flowers	trans-alpha Bergamotene: 7.97% Caryophyllene oxide: 7.20%

Chemical Structures



Cinnamaldehyde [3] and Cinnamic acid [4]

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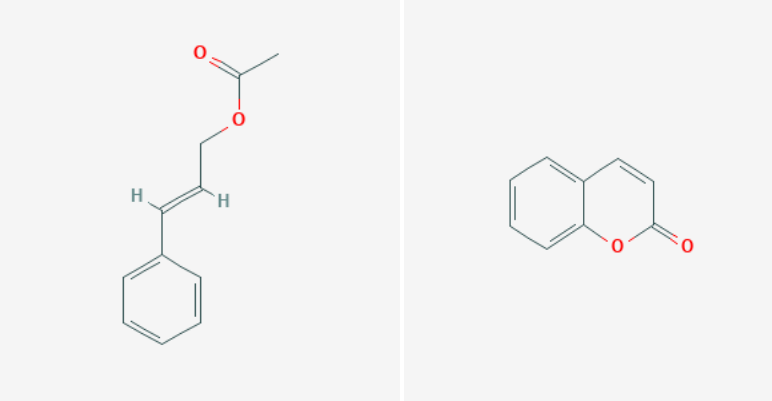

Cinnamylacetate [5] and Coumarin [6]

Figure 1: Bioactive components of cinnamon

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Cinnamomum zeylanicum healthy plant barks were collected from local areas of Bhadravathi near Shimoga, Karnataka, India. The *C. zeylanicum* washed 2-3 times thoroughly with running tap water and once with sterile distilled water, dried air on sterile blotting paper at room temperature. Using a pestle and mortar, barks were well powdered after complete drying. The powdered material was then weighed and kept tight in the air container and stored in a refrigerator. Chloroform, methane refluxed about 30 g to the powdered sample. Amber-colored bottles were used to store crude extracts. The powdering of cinnamon bark done by pestle and mortar shown in Figure 2.

Figure 2: Fresh Dried cinnamon powdered with pestle and mortar

Preparation of Cinnamon Extract

Fresh dry cinnamon bark was taken down and the extract was extracted with optimal conditions using a Soxhlet extractor. With 200ml of different solvents such as chloroform, methanol, ethanol, ethyl acetate and aqueous 50 g of fresh dry cinnamon was used. The operating temperature varied from 30 °C to

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40 °C. The sample obtained was distilled to recover solvent at 10 °C until all solvents were collected in the thimble and left in the distillation flask behind essential cinnamon oil. The essential oil obtained from cinnamon was collected and stored for further use at room temperature. The extraction and yields of all the five solvents were noted [7].

Qualitative Analysis

Qualitative analysis was done using fresh dry cinnamon bark extracted by using different solvents in Soxhlet extractor Table 2.

Table 2: Qualitative Analysis of Cinnamon Extracts

Phytochemical tests	Test name	Reference
Alkaloids	Wagner's test	8
Carbohydrates	Molish test	8
Steroids	Salkowski test	9
Terpenoids	Chloroform test	8
Flavonoids	Alkaline reagent test	9
Reducing sugar	Benedict's test	9
Amino acids	Ninhydrin test	9
Glycosides	Keller-kilian test	8
Phenols	Fecl3 test	8
Saponins	Foam test	8

Anti-microbial Activity

E. coli, Salmonella Typi and Enterococcus *faecalis* which were Standard culture were collected from JSS Hospital, Mysore and DFRL Mysore. Three pure standard cultures were collected from JSS Medical College. The standard was a sub cultured in subsequent nutrient broth and definite media where they favored i.e., Mannitol salt agar (MSA) for *S. aureus* and MacConkey for *E. Coli* and Petri dishes were prepared on a slant [10].

Inoculums Preparation

Bacterial culture from the test organisms on both nutrients were maintained. Briefly, with an inoculating loop, three to four colonies were picked and suspended in 5 ml of broth and incubated for 24 hours at 37 °C. The broth culture's turbidity was then balanced to match 0.5 MacFarland's standards. This provides organisms ranging from 1 to 10 to 5 to 10 CFU/mol, which is pathogenic to the test [10].

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Agar Diffusion Method

The molten agar is mixed at a molten state of 45-50 °C with different concentrations of the test samples and mixed aseptically with different amounts of cinnamon extracts at a concentration of 30µl, 40µl, and 50µl. Then it could solidify the prepared media. To provide an adequate growth of organisms, a separate agar plate without sample or drugs were also prepared (simultaneously with control). Two standard drugs were also tested against these microorganisms as a positive control. It was Gentamicin as well as the solvent i.e. distilled water, methanol, and chloroform the negative control used in the cork borer. Direct visual comparison of the growth of the test cultures determined the antibacterial effect. All tests were conducted, and the results were reported as the average replication [11].

Antibacterial Activity Test

The Agar diffusion method used to test the antibacterial activity of the cinnamon crude extract against standard isolates [12].

Cork Borer Method

In this method, using a mixer (vortex) 20 ml of sterile nutrient agar was poured into sterile Petri dishes. The seeded agar was punched out at equally spaced positions with a sterile bore (back hole) to make five holes after congealing. Five of the holes in the test sample solution were filled with 30µl, 40µl and 50µl while the fifth one was filled with standard antibiotics (Gentamicin) per hole. The plates were then left for 2 hours at room temperature (to promote diffusion over microbial growth) and incubated for 24 hours in an incubator at 37 °C. The antibacterial activity was evaluated using ruler to measure the diameter of the inhibition zone (the media was prepared in both methods according to the manufacturer's instructions) [13].

RESULTS

The freshly extracted cinnamon using the extractor of Soxhlet, yield (ml) was tabled as follows:

Solvent Extraction from Fresh Dry Cinnamon

Table 3: Yield of Fresh Dry Cinnamon Extract Indifferent Solvents

Solvent used	Fresh cinnamon extract yield in ml
Ethyl acetate	12
Chloroform	10

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Methanol	22
Ethanol	18
Aqueous	20

Phytochemical analysis report

Table 4: Phytochemical Analysis of Bioactive Compounds in the Cinnamon Extract

S. No	Bioactive compounds	Non-polar solvents	Polar solvents			Aqueous
		Chloroform	Methanol	Ethanol	Ethyl acetate	Hot water
1	Alkaloids	+	+	+	+	+
2	Carbohydrates	+	+	+	+	+
3	Phlobotannins	+	+	-	+	+
4	Steroids	+	+	+	+	+
5	Terpenoids	+	+	+	+	+
6	Flavonoids	-	-	-	-	-
7	Antraquinanes	+	+	-	-	+
8	Reducing sugar	+	+	+	+	+
9	Amino acids	-	-	-	-	-
10	Glycosides	+	-	+	-	-
11	Proteins	-	-	-	-	-
12	Phenolic and tannins	-	-	+	+	-
13	Saponins	+	+	-	+	+
14	Volatile oil	+	+	-	-	+

Table No. 4 shows the phytochemical analysis of bioactive compounds. Wagner's test for the alkaloids showed the presence of reddish brown precipitate. Molish test for carbohydrates showed the presence of violet ring at the junction. Alkaline test for flavanoids was negative. Benedict's test for the test of

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reducing sugar showed the presence of green or yellow or red colour. The ninhydrin tests for amino acids was negative. An upper layer green colour solution was obtained in test for volatile oils. Salkowski's test for steroid showed red colour in the lower layer of the extracts. Borntrager's test for glycosides showed the presence of red colour solution in both chloroform and ethanol extracts. . Blue colour solution observed in the tests for phenols and tannins by Ferric chloride test. Tests for terpinoids , Salkowski's test showed the presence of yellow colour at the bottom. Negative results was obtained in Biuret test for proteins. In Froth test for saponins observed foam formation except in ethanol extracts.

Anti-microbial Activity

Table 5: Zone of Inhibition of Cinnamon Extract against *Escherichia coli* (gram-negative)

Dilution of cinnamon extract(µl/ml) Zone of Inhibition (mm)			
Cinnamon extract	**Concentration of extract**		
	30	40	50
Methanol	7 ± 2	7 ± 4	7± 6
Chloroform	10± 3	10± 5	10 ± 7
Aqueous	-	-	6 ± 2
Control	-	-	-

Table 6: Zone of Inhibition of Cinnamon Extract against *Enterococcus faecalis* (gram-positive)

Dilution of cinnamon extract(µl/ml) Zone of Inhibition (mm)			
Cinnamon extract	**Concentration of extract**		
	30	40	50
Methanol	7 ± 4	7± 5	7± 6
Chloroform	11 ± 5	11 ± 8	11 ± 10
Aqueous	6 ± 3	6 ± 4	6 ± 7
Control	-	-	-

Table 7: Zone of Inhibition of Cinnamon Extract against *Salmonella typhi* (gram-negative)

Dilution of cinnamon extract(µl/ml) Zone of Inhibition (mm)			
Cinnamon extract	**Concentration of extract**		
	30	40	50
Methanol	14 ± 9	14 ± 10	14± 13
Chloroform	15±8	15±10	15± 11

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Aqueous	8 ± 3	8 ± 4	8 ± 6
Control	-	-	-

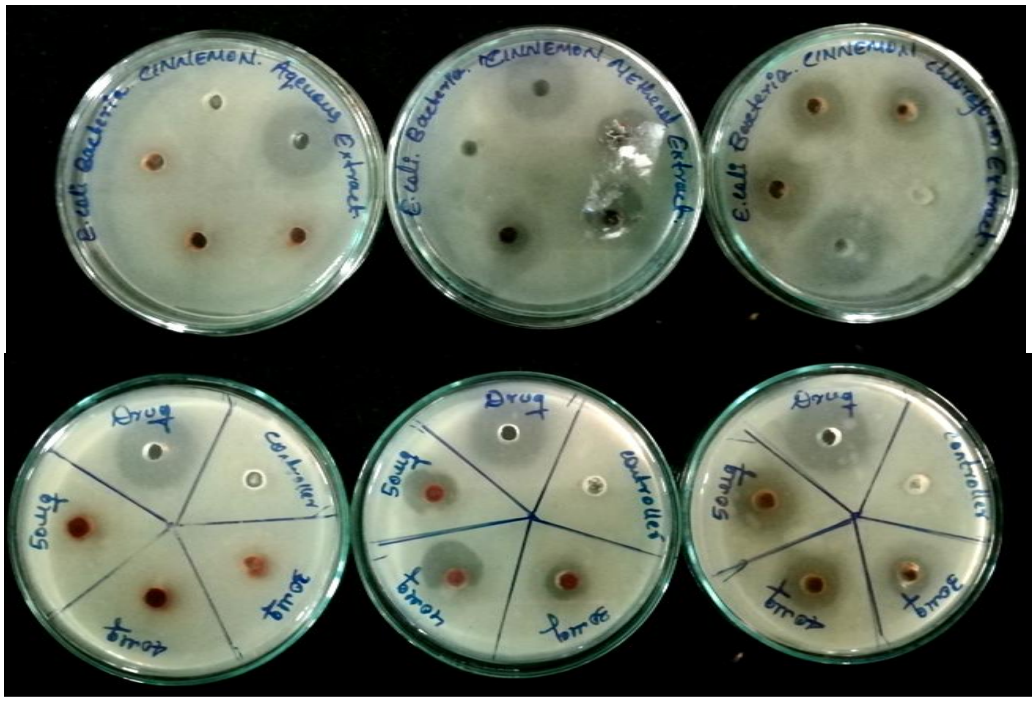

Figure 3: Zone of Inhibition of Cinnamon Extract against *Escherichia coli*

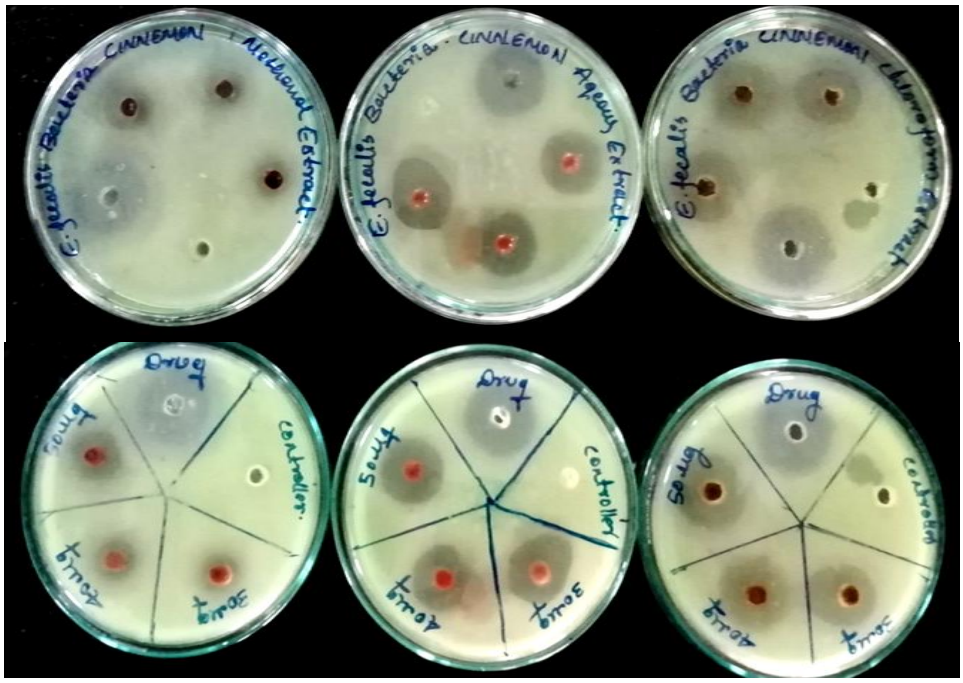

Figure 4: Zone of Inhibition of Cinnamon Extract against *Enterococcus faecalis*

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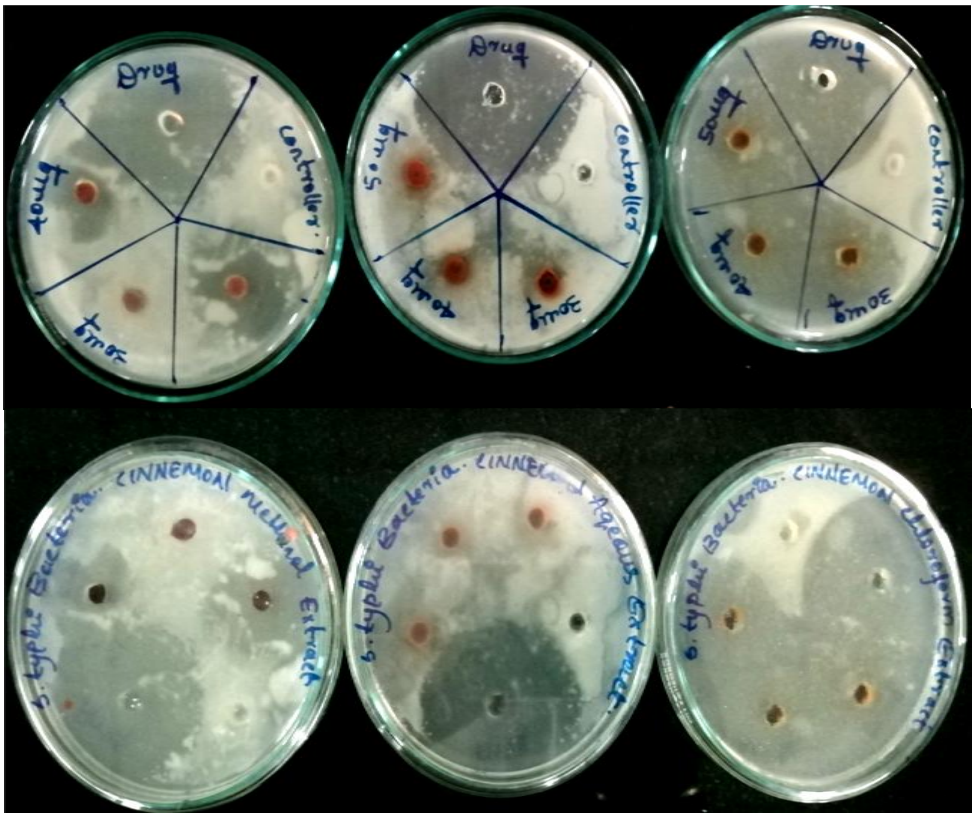

Figure 5: Zone of Inhibition of Cinnamon Extract against *Salmonella typhi*

The Yield of Extracts from Successive Extraction of *Cinnamomum zeylanicum* Bark

The yield of successive extracts (ml) is shown in Table 4. The amount of the chloroform extract obtained from the extraction was 10% w/w yield, Methanol extract was 22 % w/w yield, ethyl acetate extract 12% w/w yield, Ethanol extract 18% w/w yield and aqueous extract was 20% w/w yield.

Phytochemical Analysis of Successive Extracts of *Cinnamomum zeylanicum* Bark

The presence or absence of different phytoconstituents viz. carbohydrate, glycoside, protein, tannins, saponins, flavonoids and terpenoids were detected by the phytochemical screening methods with different chemical reagents. Phytochemical components are responsible for both pharmacological and toxic activities in plants. These metabolites are said to be useful to a plant itself but can be toxic to animals, including man. The presence of these chemical constituents in this plant is an indication that the plant if properly screened could yield drugs of pharmaceutical significance. This is better supported by the fact that members of the family of this plant have been known to be involved in ethnomedicine in the management of various ailments. The result showed the presence of alkaloids, phenolics, flavonoids, saponins, tannin, protein, carbohydrates, glycoside and traces amount of oil & sugars in the successive extract of *Cinnamomum zeylanicum* bark and the result of the phytochemical test is presented in Table

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5. All these phytochemicals possess good antioxidant activities and have been reported to exhibit multiple biological effects including anti-microbial and anti-inflammatory.

Anti-microbial Activity

The results of the agar diffusion test (Cork borer method) revealed that the various extracts of cinnamon showed different degrees of growth inhibition, depending upon the bacterial strains (Table5-7). The chloroform extract of cinnamon showed notable antibacterial activity against gram-positive bacteria and gram-negative bacteria. It is well known that most spices are more active against gram-positive bacteria than gram-negative bacteria. This study showed that chloroform and methanol extracts of cinnamon were more effective against gram-positive bacteria in vitro.

The sequence of antibacterial activity against *E. coli* is as follows with their zone of inhibitions:

Chloroform extract (10) > Methanol extract (7) > Aqueous extract (6)

The sequence of antibacterial activity against *Enterococcus faecalis is* as follows with their zone of inhibitions

Chloroformextract (11)> Methanol extract (7)> Aqueous extract (6)

The sequence of antibacterial activity against *Salmonella typi is* as follows with their zone of inhibitions.

Chloroformextract (15)> Methanol extract (14)> Aqueous extract (8)

DISCUSSION

Spices have played an important part in the lifestyle of people in certain areas of the world since ancient times. They have played many roles in the history of dyeing agents, aromas, preservatives, food additives and medicines. The molecular basis for these actions was the active phytochemicals derived from these spices. Cinnamon is a commonly used cooking spice with potentially medicinal effects. Cinnamon is medicine for respiratory and digestive conditions in native ayurvedic medicine. In this study, cinnamon bark has been analyzed using standard methods against pathogenic bacteria for the phytochemical and antibacterial evaluation. The phytochemical screening included qualitative chemistry and primary and secondary metabolite determination tests. It has a variety of active plant chemicals that attributes the medicinal properties of this plant. There is a global concern about the emergence of resistant bacterial and fungal strains due to the overuse of antibiotics.

The anti-microbial activity of Cinnamomum has been tested. Methanol, chloroform, and water were used to produce cinnamon extracts. Table No. 5,6,7 shows the antimicrobial properties of the extracts. In order to find out the cinnamon-based secondary metabolites that govern its antimicrobial properties, the extracts were phytochemically analyzed. Phytochemicals are an essential component of our diet, that is, secondary metabolites of plants. Figure 3, 4 and 5 shows the zone of inhibition of extracts

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against *Escherichia coli*, *Enterococcus faecalis* and *Salmonella typhi* respectively. With antioxidant properties imparted by flavonoids and tannins, saponins contain anti-cancerous and lower levels of cholesterol. As anti-malarial compounds and as analgesics, alkaloids are used. The results of the phytochemical analysis found the chloroform and methanol extract of cinnamon bark containing all six tested plant chemical products. Different biochemical tests characterized the isolates and were susceptible to cinnamon extracts. Cinnamon chloroform extracts, cinnamon methanol extract, cinnamon aqueous extracts with a minimum inhibitory concentration of 50mg / ml were the order of bactericidal activity. The anti-microbial activity was performed to determine the microbial activity of cinnamon by observing the zone of inhibition and thus the anti-microbial activity of the cinnamon extract was determined.

For medicinal purposes, one-fifth of all plants found in India are used. Due to its distinct strong smell of different compounds, the bark of cinnamon is widely used as a spice. Research and experimentation have been carried out on the spice's phytochemistry and antimicrobial properties. The presence of certain phytochemicals in the extracts has been certified for antibacterial activity. Studies suggested that Cinnamomum antibacterial activity might be different because of its major component cinnamaldehyde, and its properties. Cinnamaldehyde is a natural antioxidant and an extract of cinnamon bark taken orally may help to check for stomach ulcers in animal studies. Comparing the antimicrobial behavior of all the five extracts tested against the bacterial strains, it was finally concluded that chloroform cinnamon extract started to emerge as the potent agent exhibiting much higher antibacterial activity than the standard antimicrobial drug Gentamicin. The need for the hour is to screen more and more natural products or plant parts that may open the possibilities of finding new clinically effective antibacterial compounds against the resistant bacteria pathogens. In order to discover the mechanism of action of other compounds in cinnamon and exploit their therapeutic potential to combat various diseases, extensive research is therefore required. Cinnamon, therefore, plays an important role as a multi-purpose medicinal spice in the modern medicine system. This antibacterial study of plant extracts reveals that folk medicine can be effective in counteracting pathogenic microorganisms as modern medicine. It is concluded from this study that different bacterial strains have antimicrobial activity in the cinnamon extract, because of its bioactive components present in the cinnamon which is determined by phytochemical analysis. Where here in cinnamon the cinnamaldehyde and eugenol plays the main bioactive compound, which acts against different microbial strains. And bioactive compound extracted in chloroform and methanol is more than aqueous, ethyl acetate and ethanol.

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