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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *CINNAMOMUM ZEYLANICUM* (COMMERCIAL SPECIES)

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Abstract –Cinnamon is a member of family Lauraceae, found in tropical area and it has been used as a spice in India. In the present study, *Cinnamomum zeylanicum* was used for the detection of phytochemical components and antimicrobial activity. The dried powder of cinnamon was extracted using Methanol. The extract was tested for the presence of alkaloids, flavonoids, terpenoids, tannins, saponins, phenols and carbohydrates. The phytochemical screening of cinnamon was rich in alkaloids, flavonoids, and terpenoids. The effect of cinnamon extract against *Escherichia coli* and *Salmonella typhi* were studied. The strongest antimicrobial activity were observed at 100 mg/ml of methanolic extract of cinnamon was 20.67±0.58 mm zone of inhibition against *E.coli* and 24.57±0.58 mm zone of inhibition against *Salmonella typhi.* The antibiotic Gentamycin was kept as a control.

INTRODUCTION

Cinnamon zeylanicum is originated from Sri Lanka and southern parts of India. It is also known as *Ceylon cinnamon* (the source of its Latin name, zeylanicum) or 'true cinnamon' (Paranagama *et al.,* 2010; Ranasinghe *et al.,* 2013). Cinnamon (*Cinnamomum zeylanicum*) belongs to family Lauraceae. Cinnamon is widely used in many savory dishes, pickles, and soups (Ranasinghe *et al.,* 2012; Liu *et al.,* 2017). Three main constituents of cinnamon are Cinnamaldehyde, Cinnamyl acetate and Cinnamyl alcohol (Khasnavis and Pahan, 2012; Liu *et al.,* 2017). In cosmetics and food products, cinnamon is used due to its antimicrobial activities (Nabavi *et al.,* 2015; Liu *et al.,* 2017). It is also used as health-promoting agents to treat diseases like inammation, gastrointestinal disorders, and urinary infections (Al-Jiffri *et al.,* 2011; Brierley and Kelber, 2011; Liu *et al.*, 2017).

The genus *Cinnamomum* (family Lauraceae) consists of more than 300 evergreen aromatic trees and shrubs (Nabavi *et al.,* 2015; Ranasinghe *et al.,* 2013). *Cinnamon zeylanicum* Blume (a synonym of *Cinnamon verum* J. Presl, known as Sri Lanka cinnamon), *Cinnamon loureiroi*Nees (known as Vietnamese cinnamon), *Cinnamon burmanni* (Nees and Nees) Blume (known as Indonesian cinnamon) and *Cinnamon aromaticum* Nees (a synonym of *Cinnamon cassia* (L.). J. Presl, known as Chinese cinnamon) are the four species which have great worldwide economic importance for their multiple culinary uses (Nabavi *et al.,* 2015; Ravindran *et al.,* 2004). *Cinnamomum zeylanicum* and *Cinnamomum aromaticum* are the two main varieties (Ranasinghe *et al.,* 2013).

Since ancient times, spices have been used as food ((Lai *et al.,* 2004). Now, spices are used as medicine as well as food preservatives (Nabavi *et al.,* 2015 and Zheng *et al.*, 2016). Many spices like clove, oregano, thyme, cinnamon, and cumin have been proved to possess antimicrobial activities against pathogenic fungi and bacteria. These spices are used to protect food and treat infectious diseases (Arora and Kaur, 1999; De *et al.,* 1999). Secondary metabolites are the antimicrobial agents in spices. Therefore, spices could be best candidates to discover and develop new antimicrobial agents against foodborne and human pathogens (Liu *et al.,* 2017).

Around the world, many cultures used bark and leaves of *Cinnamomum* spp as a common spice (Ranasinghe *et al.,* 2013). Also, it's distilled essential oil and synthetic analogs are used as flavouring agent in the food and beverage industries. Some

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scientific studies have shown essential oils of *Cinnamomum cassia* presl. *C. osmophloeum* kaneh. and *C. zeylanicum* Blume have antimicrobial activity against many micro-organisms (Sambasivan *et al.,* 2011).

This study was carried out to screen phytochemicals and to evaluate the antimicrobial activity of methanolic extract of *Cinnamomum zeylanicum* against *Escherichia coli* and *Salmonella typhi*.

MATERIALS AND METHODS

Collection of cinnamon

Cinnamomum zeylanicum bark was brought from APMC market, Vashi, Navi-Mumbai, Maharashtra from authorized dealers. Cinnamon barks cleaned and washed in sterile distilled water and air dried at room temperature. The dried bark pieces were powdered using blender and stored in clean dry air tight containers.

Extract preparation

Plant extract prepared in 1:10 concentration using Methanol. It was kept in rotatory shaker at 150 rpm for 72 hours. After 72 hours, it was filtered through Whatman No. 1 filter paper. This extract was stored in cold conditions (Harborne, 1973).

Bacterial cultures

Escherichia coli and *Salmonella typhi* were collected from the Department of Microbiology, Ramnarain Ruia Autonomous College, Mumbai, and Maharashtra. All microbes are sub-cultured on nutrient agar.

Phytochemical screening

Qualitative phytochemical screening for alkaloids, flavonoids, saponins, carbohydrates, proteins, tannins, phenols, terpenoid and organic acid were performed (Harborne, 1973; Harsha *et al.,* 2013).

Antimicrobial activity

Antibacterial activity of *Cinnamomum zeylanicum* was carried out by the agar well diffusion method (Aneja *et al.,* 2009). In this method, pure isolate of each microbe was subcultured on the nutrient agar for each microorganism at 37 ^{o}C for 24 hrs. Density of each microbial suspension was adjusted equal to that of 10^{6}cfu/ml (standardized by 0.5McFarland standard) and used as the inoculum for performing agar well diffusion assay. The inoculum (100 µl) of each test organism was spread onto the nutrient agar plates so as to achieve a confluent growth. The agar plates were allowed to dry and wells of 8mm were made with a sterile borer. Different concentration of test extract (100 µl) was propelled directly into the wells (in triplicates). The plates were allowed to stand for 10 minutes for diffusion of the extract to take place and incubated at 37 ^{o}C for 24hrs (Aneja *et al.,* 2009).

Statistical Analysis

The antibacterial activity was determined by the measuring diameter of zone of inhibition in mm. It was carrying out in triplicates. The inhibition zones were calculated as mean ± SD (n=3). The Microsoft Excel was used for statistical analysis.

Broth dilution assay

Minimum inhibitory concentration (MIC) was determined by the Broth dilution method (Balouiri *et al.,* 2016).

Preparation of dilutions:-The two-fold dilutions were prepared of the *Cinnamomum zeylanicum* methanolic extract (100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.390625, 0.1953125 mg/mL) in a liquid growth medium and dispensed in 96-well microtitration plate (micro dilution). Each well is inoculated with a microbial inoculum. The inoculum was prepared in the same medium. Microbial suspension was adjusted to 0.5 McFarland scale. After well mixing, plate was kept in incubator at 37 ^{0}C for 24 hrs. After incubation of 24 hours add 20 µl of 5mg/ml TTC (Tetrazolium) in each well. Incubate for 30-45 minutes (till pink colour develops). 2, 3, 5-Triphenyl tetrazolium chloride (TTC) was used as an indicator of bacterial growth. End point is colourless to pink colour. The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism. In microdilution wells, MIC was detected using unaided eye. A positive control containing broad spectrum antibiotic Gentamycin (5mg/ml) was included in microplate.

RESULTS

Phytochemical screening

Qualitative phytochemical screening was done for *Cinnamomum zeylanicum* methanolic extract. It shows the presence of alkaloids, flavonoids, carbohydrates, tannins, phenols, terpenoids, saponins gum and organic acid in extract. The results of phytochemical

analysis is given in Figure 1 and Table 1.

Antimicrobial activity

Detection of antimicrobial activity of methanolic extract of *Cinnamomum zeylanicum* carried out in triplicate against *Escherichia coli* and *Salmonella typhi.* For this test, 20, 40, 60, 80 and 100 mg/ml concentrations of methanolic extract were used. Gentamycin (5 mg/ml) was kept as a positive control and sterile distilled water was negative control. Observed zone of inhibition was shown in Table 2 (Zone of inhibition in mm with ± standard deviation). The diameter of well was 8mm. Methanolic extract had shown antibacterial activity against Gram-negative bacteria.

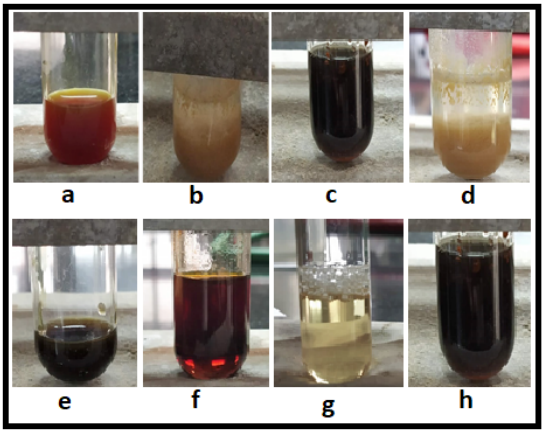

Fig. 1. Phytochemical analysis using different methods. a. Alkaloids (Wager test) b. Flavonoids (Lead acetate test) c. carbohydrate (Fehling's test) d. Tannins (Lead acetate test) e. Phenol (Ferric chloride test) f. Terpenoids (Salkowaski reaction) g. Saponins (froth test) h. gum

Table 2. The Zone of inhibition in mm (± SD) of methanolic extract of *Cinnamomum zeylanicum*.

Concentration of plant extract (mg/ml)	*Escherichia coli*	*Salmonella typhi*
0 (Methanol)	10.67±0.58	10.67±0.58
20	11.67±0.58	12.67±0.58
40	13.67±0.58	13.67±0.58
60	15.67±0.58	16.67±0.58
80	16.67±0.58	20.67±0.58
100	20.67±0.58	24.67±0.58
Gentamicin(5 mg/ml)	28.67±0.58	32.33±0.58

Table 1. Qualitative analysis of phytochemicals using methanolic extract of *Cinnamomum zeylanicum* (+ indicates presence and – indicates absence)

Phytoconstituents	Tests performed	Methanol extract of Cinnamon
Alkaloids	Wager's test	+
	Hager's test	+
	Dragendorf's test	+
Flavonoids	Lead acetate	+
	Ferric acid	+
Carbohydrate	Fehling's test	+
	Benedict's test	+
Tannins	Ferric chloride test	+
	Lead acetate test	+
	Dilute $KMnO_4$	+
Phenols	Ferric chloride test	+
	Lead acetate test	+
	Dilute $KMnO_4$	+
Terpenoids	Salkowaski reaction	+ red
Glycosides	Legal's test	-
	Keller Killani	-
Saponins	Froth test	+
Proteins	Biuret test	-
	Millions test	-
Starch	Tannic acid test	-
Gums	Fehling's test	+
	Benedict's test	+
Amino acids	Million's test	-
Organic acid	Oxalic acid	+
Inorganic acid	Sulphate test	-
	Coumarins	-

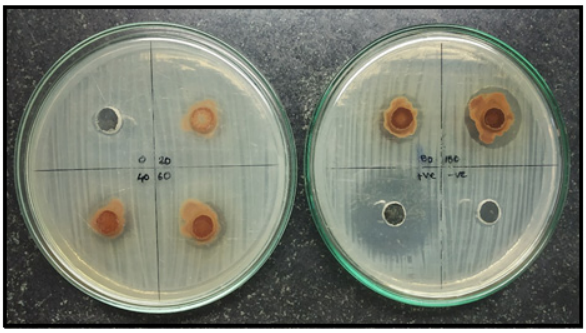

Fig. 2. Antibacterial activity of extracts (0-100 mg/ml) on *Escherichia coli*

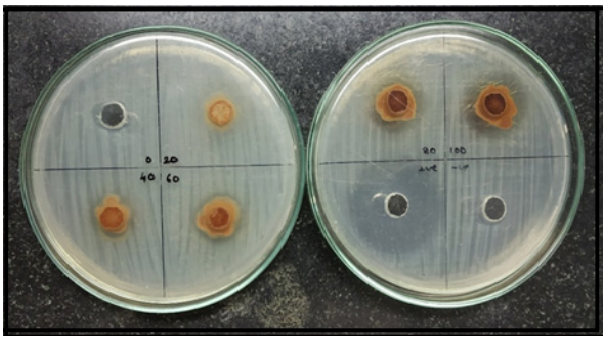

Fig. 3. Antibacterial activity of extracts (0-100 mg/ml) on *Salmonella typhi*

Broth microdilution method

The MIC of *C. zeylanicum* methanolic extract was determined by broth dilution method (Table 3). *Escherichia coli* was found to be the most sensitive pathogen to *C. zeylanicum* methanolic extract with MIC value of 1.563 mg/ml, while *Salmonella typhi* was showing MIC 12.5 mg/ml. The standard drug Gentamycin was active against all reference bacteria (5mg/ml).

Table 3. Determination of minimal inhibitory concentration

Bacteria	MIC (mg/ml)
Escherichia coli	1.563
Salmonella typhi	12.5

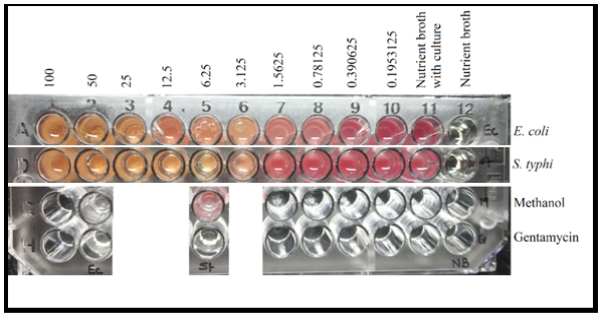

Fig. 4. Determination of minimum inhibitory concentration (MIC) for *Escherichia coli* (Ec), *Salmonella typhi* (St) and Nutrient broth (NB) as negative control. Gentamycin (G) as positive control. Methanol (M) was kept as solvent control and Nutrient broth with culture as microbial control.

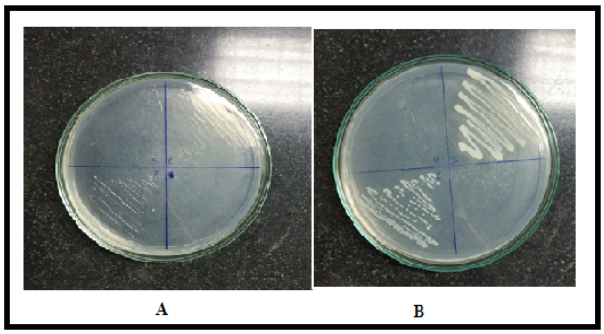

Fig. 5A. Determination of MIC for *E. coli* was 1.563mg/ml, growth was not observed in 5th to 7th well and. * was a Gentamycin control.

Fig. 5B. Determination of MIC for *S. typhi* was 12.5mg/ml, growth was not observed in 4th well but growth of microorganism was observed in 5th and 6th well. Fourth quadrant was kept as Gentamycin control. No growth was observed in it.

DISCUSSION

Food borne disease is any illness that results from the consumption of contaminated food, contaminated with pathogenic bacteria, viruses, or parasites. There are 31 main foodborne pathogens causing diseases, the significant ones such as *Salmonella nontyphoidal, Campylobacter, Listeria,* and Shiga toxin producing *Escherichia coli* (Adleyand Ryan, 2016). *Salmonella typhi*and Shiga toxin-producing *E. coli* (STEC), can causes infections in humans and animals due to ingestion of contaminated food and water. Cinnamon is used in cosmetic, food industry and as health promoting agents. Many medicinal plants are the rich resource of drugs. The presence of secondary metabolites gives healing properties to medicinal plants. The purpose of present studies was to find effect of *Cinnamomum zeylanicum* methanolic extract on *Escherichia coli and Salmonella typhi.*

The present investigation deals with qualitative detection of phytochemicals, presence of alkaloids, flavonoids, carbohydrates, tannins, phenols, terpenoids, saponins gum and organic acid was observed in an extract. Similar results were reported by Harsha *et al.* (2013); Ahuja *et al.* (2015); Mazimba

et al. (2015) and Paliwal *et al.* (2018).

The qualitative screening by Shiney Ramya, 2012; Pandey *et al.*, 2014 and Goud Gajula *et al.*, 2016 studies showed presence of alkaloids, cardiac glycosides, tannins, terpenoids, saponins but absence of flavonoids in methanolic extract. The present study was found different results from previous studies, the presence of flavonoids in methanolic extract. Flavonoids are hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. 2', 4'- or 2', 6'-dihydroxylation of the B ring and 5, 7-dihydroxylation of the A ring in the flavanone structure are important for significant anti-MRSA (Methicillin-resistant *Staphylococcus aureus*) activity (Tsuchiya *et al.*, 1999).

In the present studies methanolic extract of *Cinnamomum zeylanicum*was tested for bactericidal activity against Gram negative bacteria by using well diffusion method. As given in Table 1 maximum zone of inhibition was formed by methanolic extract of cinnamon against *S. typhi* (24.67±0.58) as compared to *E.coli* (20.67±0.58) at concentration of 100 mg/ml, which was superior results than the observation made by Varalakshmi *et al.*, 2013 study of *E. coli*, methanolic extract concentration of 20 mg/50µl gave 7 mm zone of inhibition for antimicrobial assay. Antibacterial activity of 100 µg/ml of ethanolic extract was 10 mm zone of inhibition against *E coli*. (Kwak *et al.*, 2017). Antimicrobial activity of diethyl ether extract of cinnamon (30 µl/disc) was 13mm against *E.coli* (Agaoglu *et al.*, 2007). Therefore, it suggests that the strains were used in the present study seem to be sensitive to methanolic extract of *Cinnamon zeylanicum*.

In the present investigation, MIC of methanolic extract of *Cinnamomum zeylanicum* against *E.coli* was 1.563 mg/ml the result were superior to study of Varalakshmi *et al.*, 2013, MIC methanolic extract of more than 5mg/ml for *E.coli.* According to study of Vazirian *et al.*, 2015, MIC of essential oil detected for *E. coli* and *S. typhimurium* as 0.5 µL/ml each. For cinnamon essential oil, 0.5 mg/ml MIC of *E. coli* was observed in Firmino *et al.*, 2018. *E. coli* and *S. typhimurium* were more sensitive to essential oil of cinnamon as compared to methanolic extract of *C. zeylanicum* of present study which showed MIC at 1.563mg/ml for *E.coli* and 12.5 mg/ml for *S. typhi*.

CONCLUSION

The preliminary phytochemical screening of methanolic extract of *Cinnamomum zeylanicum* showed the presence of alkaloids, flavonoids, carbohydrates, tannins, phenols, terpenoids, saponins gum and organic acid in extract. The results of phytochemical analysis were given in Table 1. The *Cinnamomum zeylanicum* have medicinal properties. The antimicrobial activity shown by the cinnamon extracts may be due to the presence of Cinnamaldehyde.

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