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Proximate composition and antimicrobial analysis of cinnamon collected from various regions of Asia; A comparative assay

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ABSTRACT

Globally, an increased use of pesticides has resulted in the occurrence of drug resistant microbes. This has urged the investigators and researchers to use and develop the antimicrobial agents derived from some natural resources. Among various naturally occurring medicinal plants, Cinnamaldehyde, owing to its acid generation, acid tolerance and virulence gene expression has gained a considerable attention. Keeping in view its beneficial aspects, the present study has been conducted to investigate the contents and compare the nutritional and antimicrobial activities of Cinnamon collected from various regions of Asia. In this study, antimicrobial activity of cinnamon was estimated using well diffusion method against various bacterial and fungal strains. As shown by the findings of the present study, Cinnamon oil is found a potent antibacterial agent against all microbes used. Additionally, it was found that cinnamon samples collected from Sri Lanka has the highest oil content (3.1%), followed by China (2.8 %) and India (1.9 %), where all tests were standardised using fixed oil levels. In terms of antimicrobial properties, obtained results demonstrate that Indian cassia (*Cinnamomum tamala*) exhibited the highest antimicrobial activity as compared to the samples collected from other regions. The findings can be beneficial in establishing a synergistic relation to the extent of oil content and its combating properties to the microorganisms in various species of cinnamon.

Keywords: Cinnamon, extract, fatty acids, minerals, antimicrobial activity

INTRODUCTION

The past decade has seen a tremendous increase in the use of naturally occurring herbs as preserving compounds against degradation induced by food borne pathogens and food spoilage bacteria. These herbs have been used to secure the edibles from biotic and abiotic stresses (Nabavi *et al*., 2015). Nowadays, for health care almost 65% of the world population relies on the use of conventional medicine (Nabavi *et al*., 2015; Nabavi *et al*., 2014). Accordingly, much consideration has been paid to the disclosure and improvement of new antimicrobial operators that may present a good defensive demonstration against various microorganisms and Cinnamaldehyde has proven to be an intriguing applicant in this regard (Högberg *et al*., 2010; Nabavi *et al*., 2015).

Cinnamon (*Cinnamomum zeylanicum),* an evergreen shrub of Lauraceae family is a world widely known species. It is found native to Sri Lanka and India (Adarsh *et al*., 2020) and has been marked as an important ingredient in various food products mainly chocolate, beverages, spicy candies, alcohols savory dishes, pickles, soups, and Persian sweets (Ravindran *et al*., 2003). Mostly all parts of cinnamon tree (bark, leaves, flowers and oils), Cinnamon sticks and powdered Cinnamon, its oil (i.e. leaf oil & bark oil), capsules and tablets are of vital importance.

Since, 2000 BC people have been using this spice in Ancient Egypt for medicinal purposes. The umbrella term of “natural antimicrobial system” i.e. cinnamon usually refers to the dried bark of *C. zeylanicum* and *C. aromaticum* (Jakhetia *et al*., 2010). Cinnamon has also been reported to be concerned with the food safety along with beneficial health effects as an anti-oxidant, anti-diabetic, anti-inflammatory, anti-microbial, anti-cancer agent al (Nasir *et al*., 2015). In several studies, Cinnamon is

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found to be pharmacologically effective against numerous ailments; diarrhea, in the reduction of blood sugar levels in diabetics, urinary infections cough, bronchitis, inflammation, palpitations, controlling infections and gastrointestinal disorders (Kim *et al*., 2006, Brierley & Kelber, 2011). Another potential restorative utilization of cinnamon would be concerning with its antimicrobial properties, particularly antibacterial action. Cinnamon oil contains phenolic compounds such as cinnamaldehyde (65 to 75% of the oil) and eugenol (about 10% of oil), which have been recognized as being responsible for its antimicrobial property (Vijayan & Thampuran, 2004). The activity of *C. zeylanicum* oils against dermatophytes and other pathogenic microorganisms was studied by Nasir *et al*, where minimum inhibition concentration (MIC) was observed in the range 0.31 µL/mL to 0.16 µL/mL. In one of the similar study conducted by Nimje, Cinnamon was found to give maximum average activity index at 60^{o}C against E. Coli (Nimje *et al*., 2013). Zone of inhibition (ZOI) is found to be most important part in studying the antimicrobial or antibacterial effect. A larger ZOI confirms the sensitivity of antimicrobial agent against antibiotic used. Likewise, cinnamon extracts were found to be more sensitive with large inhibitory zone as compared to smaller Inhibitory zone reported in literature (Moreira *et al*., 2005; Philip *et al.*, 2012). In one of the study, an agar diffusion test of various cinnamon oils extracted to validate antimicrobial activity, it was found that Cinnamon extracts reflected different inhibition zone against gram positive and gram negative bacteria (*Escherichia coli*, *Enterococcus faecalis* and *Salmonella typhir).* Chloroform extract of cinnamon was found to be more effective as compared to the standard antimicrobial drug (Hameed *et al.*, 2016).

Two separate clinical strains of bacteria were employed in one of the investigations to validate Cinnamaldehyde's antibacterial efficacy. Results revealed that Cinnamon extracts serve as an effective antimicrobial agent against both strains used and results were further validated for clinical usage (Rasheed & Thajuddin, 2011). (Boyko *et al*., 2017) reported that cinnamaldehyde and eugenol rupture the bacterial cell by inhibition of an essential enzyme required to reproduce. Therefore, it was concluded that high anti-microbial activity of essential oils is due to presence of phytochemicals found in cinnamaldehyde. In several studies, Cinnamon aqueous extract were found to be active against bacteria and fungi causing urinary tract infections and against pathogens like *Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enterica serotype typhi, Shigella flexneri,* and *Enterobacter* (Thiyagarajan & John, 2020).

Keeping the literature review in account and pharmacological benefits of cinnamon, the present study is aimed to analyze the comparative assay of nutritional and antimicrobial activities of cinnamon collected from different regions of Asia viz. Sri Lankan cinnamon (*Cinnamoum zeylanicum*), Indian cassia (*Cinnamomum tamala*) and Chinese Cassia (*Cinnamomum cassia*). It is hoped that findings of this study will lead in developing the new insight of regional effects of medicinal and nutritional values of Cinnamon plant.

MATERIALS AND METHODS

Sample Preparation

About 250 gm of Cinnamon samples collected from Sri Lanka, China and India (labeled as “S”, “C” and “I” respectively) were pulverized to fine powder and kept at room temperature in desiccators within 24 hrs prior to sample preparation.

Determination of Moisture Content

Weighed 3gm of sample, dried in an oven at 100-102 ^{o}C for 16 hrs to 18 hrs and then placed into the desiccating unit. Sample was cooled at room temperature for 30 minutes and weighed accurately and process was repeated for accurate readings. Eq (i) was used to calculate moisture content (Srilekha *et al*., 2019).

$$\% \text{ age moisture} = \frac{\text{loss in weight}}{\text{Weight of Sample}} \text{ x } 100 \qquad \text{(i)}$$

Determination of Ash

About 1gm of sample was weighed, burnt at 550 ^{o}C and incinerated. The remaining residue of inorganic matter was demonstrated and ash weight was calculated using Eq. (ii).

$$\%\text{Ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \text{ x } 100 \qquad \text{(ii)}$$

Determination of Fat

Weighed 10gm of cinnamon sample, dried in oven at 100^{o}C for 3 hrs, afterwards fat was extracted with hexane. By the process of evaporation, the solvent was totally removed from the extract and the residue was weighed. Following Eq. (iii), the percentage of crude fat was calculated (Srilekha *et al*., 2019).

$$\% \text{ Fat} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \text{ x } 100 \qquad \text{(iii)}$$

Determination of Protein

Weighed 0.5g cinnamon sample combined it with an equimolar digestion tablet and 20 mL concentrated H_2SO_4 in a digestion flask, and heated it to make a clear and transparent ammonium sulphate solution. Under the condenser tube, a flask containing around 10 mL of 2% boric acid solution was inserted, and the burner was positioned underneath the $KMnO_4$ solution. 5 mL digested sample and 15 mL NaOH solution were added to convert ammonium borate to ammonia. The ammonia was released once the combination started. The ammonium borate complex is made up of ammonia and boric acid. Titration against HCl was used to establish the end point after distillation. The nitrogen and crude protein content in the sample was determined by the following Eq. (iv) and (v) (Srilekha *et al*., 2019).

$$\% \text{ Nitrogen} = \frac{\text{Titer x Total Vol.made x Normality of acid x 14 x 100}}{\text{Volume Taken x weight of Sample x 1000}} \quad \text{(iv)}$$

$$\% \text{ Protein} = \% \text{ Nitrogen x Factor } (6.25) \quad \text{(v)}$$

Determination of Fiber Content

Weighed 2gm of fat-free sample, put to round bottom flask and mixed with 1.25% NaOH 100 mL to residual with hot distilled water and alcohol. Filtered using Whattman's No.40 filter paper, dried at room temperature. Pre-weighed sample filter paper was put in pre-weighed crucible. Placed in the oven100^{o}C, weighed it after burning carbonaceous material. Then calculated the amount of fiber by following Eq. (vi) and (vii),

$$\text{Crude fiber= weight of dry sample–weight of ash} \quad \text{(vi)}$$

$$\% \text{ Crude fiber} = \frac{\text{Crude fiber}}{\text{weight of original sample}} \text{ x } 100 \quad \text{(vii)}$$

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was performed using “Well diffusion method” (Okeke *et al*., 2001) on Mueller Hinton agar (Oxoid UK) in accordance with clinical laboratories standards Institutes (CLSI). For antimicrobial activity test, two strains of *Staphylococcus aureus* along with two bacteria i.e. *Escherichia coli* & *Bacillus subtilis* and three strains of fungi were selected. Typical insulated bacterial colonies were picked and dissolved in normal saline or sterile water to establish antimicrobial susceptibility inoculum. The concentration was adjusted to 0.5 McFarland index. Spread plastic was used to inoculate bacterial saline or water in MH agar plates applied to plates evenly split into four plates, 8 mm drilling wells, followed by an antibiotic disc (Vancomycin for strains and a sodium propionate in case of a different food-borne pathogen). Stock solution was made using equal quantity (200 µL) of cinnamon oil and DMSO. In the MRSA zone wells, 100 µL stock solution was administered for all cinnamon oil samples. Vancomycin (Oxoid UK) was also used as controls. Repeated the same procedure for all strains of pathogens used. Another negative control used was 100 µL DMSO. Plates were incubated at 35^{o}C for 18 hrs (Patel *et al*., 2015). Incubation plates were evaluated and expressed in millimeters for inhibition regions.

Determination of Antifungal Activity of Cinnamon Oil

Fungal isolates were sub-cultured for PDA at 28^{o}C for 3-4 days to assess the antifungal activity of cinnamon oil. Agar plates were used to drill pools in the agar using the sterile cork borer (8 mm in diameter). In each peripheral well a volume of 100 µL was inserted while a fungal propionate sodium disc was seeded into the middle. Negative controls were also set up as above. The assessments were undertaken on daily basis, starting at the beginning of the trial 24 hours later and ending with two thirds of the platform area of the control treatment covered by fungus (Fiori *et al*., 2000). For antibacterial activity in the test material, inhibitor zones were judged positive (Abony *et al*., 2018). The micro-organisms used in this research are mentioned in Table I.

Table I: List of Microorganism used for antimicrobial of Cinnamon oil

Sr. No.	Organism Name	ATCC No.	References
1.	*Me`thicillin Susceptible Staphylococcus aureus* (MSSA)	25923	(Okeke *et al*., 2001)
2.	*Methicillin Resistance Staphylococcus aureus* (MRSA)	33591	(Okeke *et al*., 2001)
3.	*Escherichia coli*	25922	(Al-Numair *et al*., 2007)
4.	*Bacillus subtilis*	6633	(Al-Numair *et al*., 2007)
5.	*Aspergillus niger*	16404	(Muller *et al*., 1996)
6.	*Penicillium digitatum*	201167	(Al-Numair *et al*.)
7.	*Aspergillus fumigatus*	8001	(Al-Numair *et al*.)

The inhibitory zone diameter results were reported: x 9 mm inactive; 9-12 mm slightly active; 13-18 mm

active (Alves-Silva *et al*., 2013).

RESULTS AND DISCUSSION

Proximate Analysis

In different cinnamon varieties the different percentages of nutritional values were observed is shown in Fig. 1. The obtained results for moisture contents revealed that maximum moisture content is present in Chinese sample (12.12%), followed by Sri Lankan sample (11.56%) than in Indian (11.16%). The moisture contents in all varieties were higher than the value (5.1%) as reported in recent research (Gul & Safdar, 2009).

When we compare the results for ash contents we conclude that the maximum occurrence of ash contents is in Sri Lankan sample (4.56%) followed by Chinese sample (4.33%) than Indian sample (2.71%). The ash contents in Indian variety (2.71%) were closer to the value (2.4%) as reported by (Gul & Safdar, 2009). But Sri Lankan sample and Chinese sample showed large values than reported value.

Maximum crude fat contents were found in Sri Lankan variety (3.18%) followed by Chinese sample (2.83%) than Indian sample (1.9%). This data was the average of the two determinations of each sample. The crude fat contents in all varieties were less than the reported value i.e.(4%) (Gul & Safdar, 2009).

In consideration to the protein contents it was found that maximum of protein contents were determined in Chinese sample (4.80%) followed by Sri Lankan sample (4.15%) than Indian sample (3.68%). The crude protein contents in Indian variety were closer to the value (3.5%) as mentioned in the recent research (Gul & Safdar, 2009). But Sri Lankan sample and Chinese sample showed slightly large values than reported value. This variation in results might be due to the difference in specie used and environmental conditions.

The results obtained for crude contents reveals that maximal crude fiber contents were determined in Indian sample (22.56%) followed by Chinese sample (20.77%) than Sri Lankan sample (20.59%). The crude fiber contents in all varieties were within the range as reported in recent research (Al-Numair *et al*., 2007). A graphical representation of proximate analysis of the selected cinnamon species is given in Fig 1.

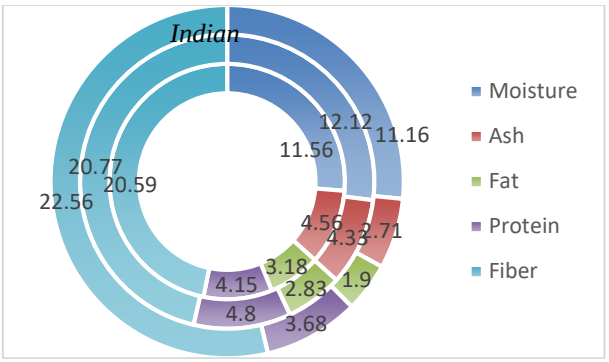

Fig. 1: Graphical representation of proximate analysis of collected samples of cinnamon

Antimicrobial Analysis

Fig 2 (a-e) indicates some of the representative images of testing antimicrobial activity of cinnamon oil extracted from the selected species. Oil extraction of three chosen varieties of cinnamon was shown to vary from highest (3.18%) in Sri Lankan (S), through intermediate (2.83%) in Chinese, to least (1.9%) in Indian (I).

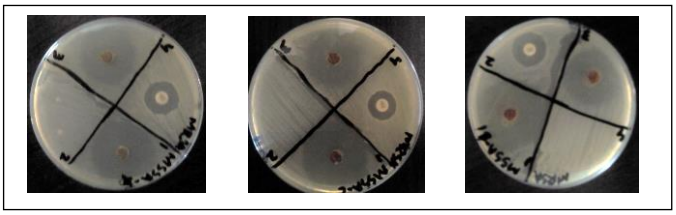

Fig 2 (a): Inhibition zone diameter of Cinnamon oil against *Staphylococcus aureus* (MRSA & MSSA)

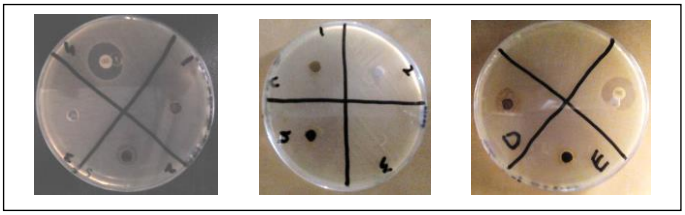

Fig 2 (b): Inhibition zone diameter of Cinnamon oil against *Bacillus subtilis*

From the images given in Fig 2 (a-e), it is clear that the sample of India exhibited rather wide area and findings were efficient, which revealed that the effect of antimicrobial activity in Indian cassia (*Cinnamonium tamala*) compared with that of both Sri Lanka cinnamon and Cinnamon (*Cinnamomum cassia*).

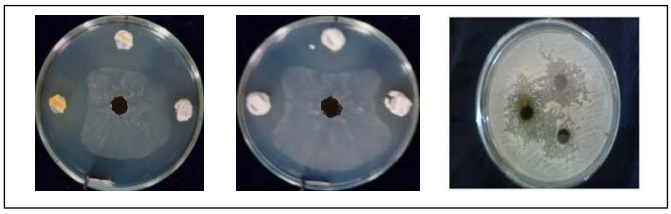

Fig 2(c): Inhibition zone diameter of Cinnamon oil against *Aspergillus niger*

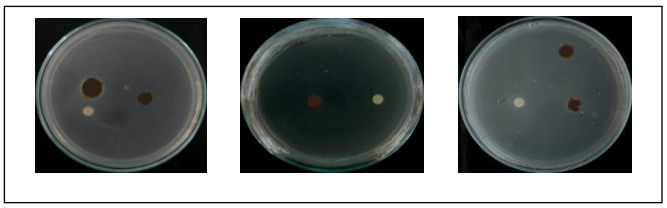

Fig 2 (d): Inhibition zone diameter of Cinnamon oil against *A. fumigates*

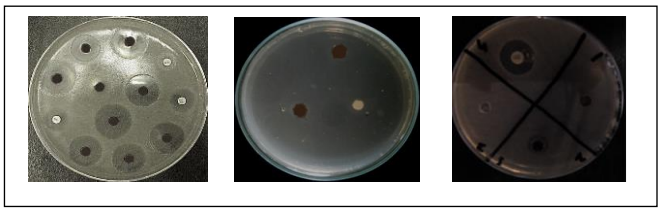

Fig 2 (e): Inhibition zone diameter of Cinnamon oil against *Penicillium sp*.

A broad zone of inhalation against two *Staphylococcus aureus* strains was shown in the antibacterial activity results of three distinct samples of cinnamon (as shown in Table II). When dissolved with DMSO and although Sri Lankan sample and Chinese samples exhibited IZD 34.5 mm and 29.5 mm zones for inhibition, the Indian mined oil zone (44.5 mm) with *Staphylocoque aureus* (MRSA) had methicillin resistant. In the event of negative control, no zone of inhibition was achieved. The Vancomycin IZD was 17.8 mm whereas the antibacterial trend for MSSA was comparable.

In terms of diameter of inhibitory zones trend observed from maximum to minimum efficiency profile has been presented in Table III.

CONCLUSION

This research focuses mostly on the antimicrobial activity and consequences of cinnamon samples collected from different regions of Asia. The results of this comparison investigation show that cinnamon oil is a powerful antibacterial agent for all microorganisms.

Table II: Zone of Inhibition (IZD mm) Of Cinnamon Oil against Test Microbes

Test microbial species	SriLankan Sample	Chinese Sample	Indian Sample	Positive control	Negative control
Methicillin Susceptible Staphylococcus aureus (MSSA)	35.5	38.5	35.5	17	0
Methicillin Resistance Staphylococcus aureus (MRSA)	34.5	29.5	44.5	17.8	0
Escherichia coli	16	17	18	12	0
Bacillus subtilis	15.3	14.8	16.3	14	0
Aspergillus niger	28.7	29	28.9	15	0
Penicillium digitatum	35.2	33.9	34.6	32	0
Aspergillus fumigates	37.6	39	38	18	0

Table II: Zone of Inhibition (IZD mm) Of Cinnamon Oil against Test Microbes

Test microbial species	Sri Lankan Sample	Chinese Sample	Indian Sample	Positive control	Negative control
Methicillin Susceptible Staphylococcus aureus (MSSA)	+++	+++	+++	++	-
Methicillin Resistance Staphylococcus aureus (MRSA)	+++	+++	+++	++	-
Escherichia coli	++	++	++	+	-
Bacillus subtilis	++	++	++	++	-
Aspergillus niger	+++	+++	+++	++	-
Penicillium digitatum	+++	+++	+++	+++	-
Aspergillus fumigates	+++	+++	+++	++	-

It was also found that the maximum oil content in Sri Lanka (3.18 %) is available followed by Chinese (2.825 %) than Indian (1.9 %) according to the unit method when all tests were standardized using fixed oil levels, therefore, this research shows that Indian cassia (*Cinamonium tamala*) showed maximum antimicrobial activity compared to botanical activity (*Cinnamomum cassia*). Thus, owing to varied oil

content in cinnamon bark, the entire effect of diverse cinnamon samples may vary. Moreover, Srilankan and Chinese samples exhibited higher amounts of ash, protein and fat compared to Indian samples but exhibited less fiber content than Indian samples. The critical requirement of the hour is to screen a rising number of natural products or plant parts in the hope of discovering new antibacterial medicines that are effective against resistant bacterium illnesses. Extensive research is thus required to elucidate the mechanism of action of other compounds found in cinnamon and to maximize their therapeutic potential in the treatment of various illnesses.

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