Comparative Analysis of In vitro Antimicrobial and Antioxidant Potential of Cinnamomum tamala Extract and their Essential Oils of Two Different Chemotypes

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ABSTRACT
Background: The essential oils of aromatic plants have wide range of biological applications. Natural food preservatives have been always a demanding for food industries in both developed and developing countries to prevent bacterial growth in food stuffs. Therefore, focused on Cinnamon leaves essential oils components against food pathogens have been investigated to confirm its potential use in food products.

Methods: The antimicrobial activity of two Cinnamon leaves oils and extracts (T-2 and T-19) were examined by disc diffusion assay and the minimum inhibitory concentration by two-fold serial dilution method against foodborn pathogenic microorganisms i.e. E. coli (MTCC 723), B. Cereus (MTCC 430), S. aureus (MTCC 3381), S. typhi (MTCC 734) and C. perfringens (MTCC 1349). The antioxidant activity of both essential oils and extract was determined by DPPH assay. The chemical profiling of Cinnamon essential oils were determined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

Result: The antimicrobial activity of both Cinnamon leaves oils and extract were evaluated by disc diffusion assay and it showed that in essential oils and extracts exhibited the highest zone of inhibition (ZOI) against S. aureus and E. coli. Minimum inhibitory concentration (MIC) of both oils and extracts ranged from 0.156 mg/ml to 5mg/ml and the antioxidant properties of oils and extract of cinnamaldehyde type Cinnamon possessed the highest antioxidant activity than linalool type. The chemical constituent of Cinnamon oil was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) revealed that T-2 contains cinnamaldehyde (75%) and T-19 contains Linalool (63.77%) were found as major constituents. Therefore the results shows that essential oil of cinnamaldehyde type Cinnamon could be a potential rich source of natural antioxidants and also more effective against food borne pathogens than linalool type and could be used as natural antibacterial agents in food preservation.

Key words: Cinnamaldehyde, Cinnamomum tamala, Essential oil, GCMS, Linalool.

INTRODUCTION
Recently, spices have attracted a lot of attention for their useful physiological functions and antimicrobial activity. Among the many of known spices, Cinnamon is one of the most popular and the oldest spice used in foods. It belongs to Lauraceae family and grows in South and South-East Asia (Sathish et al., 2009; Elaissi et al., 2011). The essential oil from Cinnamon is commonly used in the food industry because of its special aroma in addition to its medicinal properties. In the recent years, some studies have reported that Cinnamon oil had a broad range of antimicrobial activities against gram-positive and gram-negative bacteria (Tyagi and Malik 2011). These study results provided a possibility for the application of Cinnamon in the food preservation.

Cinnamon is an important spice as well as they are 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 (Huang et al., 2007). The phyto-constituents of Cinnamon include diverse components of which cinnamaldehyde and trans-cinnamaldehydeare the key ingredients. These components are present in the essential oil and are responsible for the fragrance and biological activities (Yeh et al., 2013). Studies conducted on some species of Cinnamon, viz. Cinnamomum osmophloeum (C. osmophloeum) indicated that the essential oil of this plant contains high level of Cinnamaldehyde while extracted from C. zeylanicum named (E)-cinnamaldehydehas an anti-tyrosinase activity (Marongiu et al., 2007). Cinnamon bark contains procyanidins which include both procyanidin A-type and B-type linkages the seprocyanidins extracted from Cinnamon and berries possess antioxidant activities (Peng et al., 2008; Anderson et al., 2004). Cinnamon also been used as...
antiproliferative, inflammation, gastrointestinal disorder, urinary infection (Kallel et al., 2019; Briley and Kleber 2011; Al-Jiffri et al., 2011).

Microbial pathogens in food may cause spoilage and contribute to foodborne disease incidence and the emergence of multidrug resistant and disinfectant resistant bacteria such as Staphylococcus aureus (S. aureus), Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa) has increased rapidly, causing the increase of morbidity and mortality (Miladi et al., 2016). Moreover, chemical preservatives cannot completely eliminate several pathogenic bacteria like Listeria monocytogenes in food products or delay the growth of spoilage microorganisms (Tajkarimi et al., 2010). Natural products, as substitutes of synthetic chemical preservatives, are increasingly being accepted because they are innately better tolerated in human body and have inherent superiorities for food industry (Silva and Domingues 2017). The antimicrobial activities of natural products are necessary to be studied for its application in food industries.

Keeping in view of the importance of natural antimicrobial agents as a cheap source without any side effects, it is necessary to identify the screening of natural products from aromatic plants to develop new and efficient agents against microbial diseases and infections. The present study was designed to investigate the antimicrobial efficacy of food born pathogen against of Cinnamomum tamala oils and extract for their potential uses.

**MATERIALS AND METHODS**

**Plant materials**

Fresh Cinnamomum tamala leaves were collected from Cinnamon plant Cinnamaldehyde type (T-2) and Linalool type (T-19) grown in the premises of Centre for Aromatic Plants (CAP), Selaqui, Dehradun, Uttarakhand (Latitude N 30°21'44.70, Longitude E 77°50'58.14, Altitude 508 m) in September 2017. The plant material was authenticated in laboratory by referring herbarium records available in the Centre. The collected plant material were washed and dried for analysis.

**Test organisms**

The foodborne pathogenic microorganisms were procured from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh. E.coli (MTCC 723), B. Cereus (MTCC 430), S.aureus (MTCC 3381), S. typhi (MTCC 734) and C. perfringens (MTCC 1349) were selected for study. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

**Preparation of extract**

About 50 gm of sample material was pulverized and sequentially extracted with 250 ml of methanolic and aqueous solution in a Soxhlet apparatus for the period of 8 hr. The extracts were filtered thereafter and dried under reduced pressure using rotary evaporator.

**Isolation of essential oil**

Dry mass of 200 g of Cinnamom tamala leaf were subjected to hydrodistillation for 4 hr using Clevenger apparatus. The oil obtained were dried over anhydrous Na2SO4 and kept in a sealed glass vial at 4°C prior to analysis.

**Preparation of inoculum**

A single colony was transferred in sterile 50 ml of nutrient broth and incubated at 37°C for 24 h. The concentration of bacterial cells was optimized to 0.5 McFarland standards (that corresponds 150×10^8 CFU/mL) at 660nm for disc diffusion method.

**Antimicrobial bioassay**

Antimicrobial activity of both the oils and extracts was primarily screened by disc diffusion method. 100 µl of each bacterial inoculum was spread over Muller Hinton agar plate and sterile whatman filter paper disc 6 mm were soaked in 20 µl of different oils samples and 500mg/ml of extract dissolved in DMSO was placed on seeded petri plates. Standard antibiotic disc containing streptomycin (50µg/disc) was used as a reference control. The plates were incubated at 37°C for 24 h. (Mishra et al., 2010). After the incubation period the zone of inhibition (ZOI) was measured. All studies were performed in triplicate and mean values was calculated in Mean±SD.

**Determination of minimum inhibitory concentration (MIC)**

Two-fold serial dilution method was used to determine the MIC value against the selected food pathogens. A stock solution of oils and extracts were done by using 10% Tween 80 in a sterile Muller Hinton broth to obtain a concentration of 20, 10, 5, 2.5, 1.25, 6.25, 3.12, 1.56, 0.78 mg/ml. Each test tube of different concentrations were inoculated with microbial suspension, equivalent to 0.5 McFarland standards and incubated at 37°C for 24 h. The MIC value was determination as the lowest concentration that inhibits visible growth of pathogen in broth.

**Antioxidant activity**

The antioxidant activity of essential oils and extract was determined by DPPH assay, 2,2-diphenyl-1-picrylhydrazy (Brands-William, Cuvelier and Berset 1995). 50µl of 1mg/ml of the sample extracts and 50µl of essential oil was treated with 2ml of 0.06 mM methanolic solution of DPPH solution. Absorbance was recorded at 517nm was determined after 30 min of incubation. The percentage inhibition of the DPPH free radical was calculated in accordance to the given formula:

\[
\text{(% inhibition of DPPH)} = \left( \frac{A_0 - A_t}{A_0} \right) \times 100
\]

Where \(A_0\) is the absorbance of the control and \(A_t\) is the absorbance of the extract/standard.

**Gas chromatography and gas chromatography-mass spectrometry analyses**

GC were carried out by an Agilrent Technology 6890 N gas
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The gas chromatography-mass spectrometry (GC-MS) of the oils were performed with a Perkin Elmer Clarus 500 gas chromatograph equipped with a split/splitless injector (split ratio 1:50) data handling system. The column was Rtx-5 capillary columns (30 m × 0.32 mm, 0.25 µm film thickness). Helium was the carrier gas at a flow rate 1.0 mL/min. The GC was interfaced with (Perkin Elmer Clarus 500) mass detector operating in the EI+ mode. Temperature program used was the same as described above for GC analyses. The temperatures of the injector, transfer line and ion source were maintained at 210, 210 and 200°C, respectively. Mass spectra was taken over m/z 40-500 amu that revealed the total ion current, using an ionizing voltage of 70 eV.

Identification of compounds
The identification of constituents was performed on the basis of retention index, determined with reference to the homologous series of n-alkanes, C_8-C_24 with co-injection of standards (Sigma Aldrich, USA) under same analytical conditions and by matching their recorded mass spectra with installed MS library (NIST and Wiley) and available literature (Adams, 2007 and Avies, 1990). Quantification of each compound was performed on the basis of their GC peak area, using the normalization procedure without using correction factors.

RESULTS AND DISCUSSION
In the present study, the antimicrobial activity of essential oils and extracts of Cinnamon leaf were evaluated against five food borne pathogens by disc diffusion method. The essential oils and extracts of Cinnamon showed varied inhibitory antimicrobial activities against all tested pathogen which are represented in the form of zone of inhibition (ZOI) in Table 1. The essential oils of Cinnamon T-2 and T-19 were more effective against S. aureus with inhibition zone 24.50±0.41 and 22.50±0.41 mm and minimum against C. perfringens with ZOI 21.16 ± 0.23 and 19.06 ± 0.32, respectively.

Table 2: MIC value of oils and extract of Cinnamaldehyde (T-2) and Linalool (T-19) against food borne pathogen.

| Microorganisms       | T-2(mg/ml)      | T-19(mg/ml)   |
|---------------------|-----------------|--------------|
|                     | Oil MeE AqE     | Oil MeE AqE  |
| E. coli (MTCC 723)  | 0.312 1.25 1.25 | 0.312 2.5    |
| B. cereus (MTCC 430)| 0.625 2.5 2.5  | 0.625 2.5    |
| S. aureus (MTCC 3381)| 0.156 2.5 2.5  | 0.312 2.5    |
| S. typhi (MTCC 734) | 0.156 1.25 1.25 | 0.312 2.5    |
| C. perfringens (MTCC 1349) | 0.625 2.5 | 0.625 2.5 |
extract had maximum ZOI against *E. coli* with 11.26±0.55 and 11.66±0.47 mm while aqueous extract against *S. aureus* with 10.50±0.40 and 10.80±0.74 mm in Cinnamom T-2 and T-19 extract, respectively. The antibacterial and activity of the tested essential oils was compared against the standard antibiotics (Streptomycin sulphate). Similarly, antimicrobial activity of Cinnamon leaf oils against food spoilage microorganisms *i.e.* *B. cereus*, *S. aureus* and *E. coli*, Clostridium sp. has been shown already in earlier studies, (Dobre et al., 2011; Silva et al., 2016; Nimje et al., 2013). In Cinnamon extract Sharma et al., (2009), reported the antibacterial activity in aqueous extract of *C. cassia* against 33 pathogens and the methanolic extract of Cinnamon resulting more efficient in its antimicrobial properties as compared to aqueous extract against *S. aureus* and *E. coli*. (Mohamed et al., 2015).

The MIC values of the oils and extracts of Cinnamon ranged from 0.312 to 1.25 mg/ml and 1.25 to 5 mg/ml, respectively for both the Cinnamon spices (T-2 and T-19). The MIC of the essential oil T-2 showed the highest activity for *S. aureus* and *S. typhi* in oil 0.156 mg/ml and the lowest for *B. cereus* 0.625 mg/ml. However, the T-19 oil showed the highest inhibition against *E. coli*, *S. aureus* and *S. typhi* at the concentration 0.312mg/ml respectively and the lowest for *B. cereus* and *C. perfringens* 0.625mg/ml. While the MIC value of both Cinnamon extract was found in the range of 2.5-5.0 mg/ml against all the test organisms presented in Table 2. From the microbial sensitivity point of view, *S. aureus* and *S. typhi* was the most sensitive bacterium whereas *B. Cereus* and *C. perfringens* proved to be the most resistant among the tested food borne pathogen in both essential oils.

Antioxidant activity through DPPH assay revealed that the highest oxidation suppressing or radical scavenging activity was shown by essential oil of both chemotypes T-2 and T-19 of Cinnamon than the extract with per cent inhibition 75.66±0.33% and 63.33±0.33% at 50µl, respectively. In extracts, higher antioxidant activity was possessed by the methanol with 65.50±5.07% and 53.93±0.67% inhibition as compared to aqueous extract with inhibition 42.80±0.33% and 35.53±0.61%. The results demonstrate that the cinnamaldehyde chemotype (T-2) has more antioxidant activity than the linalool chemotype (T-19). Furthermore, similar pattern of antioxidant activity were also obtained for the extracts of methanol and aqueous in both chemotype presented in Table 3. Antioxidant activity of Cinnamon leaf oils was reported by Padalia (2015) and Kapoor (2009). Similarly Sudan et al., (2013), reported that methanolic extract has shown higher activity than the aqueous extract of *Cinnamomum cassia* and *Cinnamomum tamala* leaf, Pandey et al., (2012), also showed that the aqueous extract of *C. tamala* had less activity as compared to alcoholic extract.

The chemical constituents of essential oils of Cinnamon leaf T-2 and T-19 is responsible for their wide range of antimicrobial activity. The major constituents identified by GC-MS analysis in essential oils, included Cinnamaldehyde was the major component of T-2 oil comprising 75% of the total oil composition and linalool in T-19 oil was identified 63.77% as dominant components coupled with other constituents *i.e.* Benzaldehyde, β-pinene, 1,8, cineole,
Cinnamyl acetate, Caryophyllene oxide and Trans-caryophyllene was also detected Table 4. These essential oils containing the complex mixture of the different classes of compounds such as phenols, aldehyde, ketones, alcohols or hydrocarbons present in essential oils having multiple antimicrobial properties. It has been early reported that essential oils containing aldehyde or phenols, as major components showed the highest antibacterial activity followed by terpene alcohols and ketones or esters while terpene hydrocarbons have no activity (Dormans et al., 2000; Griffin et al., 1999; Sachetti et al., 2005; Ait-Ouazzou et al., 2011). The present results are in accordance with previous report that essential oils containing aldehyde as major component, exhibited the highest antibacterial activity followed by those containing terpene alcohols. Mith et al., (2014), reported that trans-cinnamaldehyde shows stronger antibacterial activity in comparison with eugenol and linalool. As earlier report antimicrobial activity of Cinnamon bark oil is due presence of cinnamaldehyde had been reported as the main constituents Moarefian et al., 2013; Tiziana et al., 1998 and Oliveira et al., 2012. Lopes et al., (2014), also reported the antimicrobial activity of cinnamaldehyde and used to developed antimicrobial film to pack bread and pastry made without using any preservatives with widely acceptance and it is commonly used in food and beverages as a flavouring ingredient Jo et al., (2015).

CONCLUSION
In the present study, we have showed that Cinnamomum essential oil and extract of T-2 and T-19 justify the significant antimicrobial activities against food born pathogen. The high antimicrobial activity of Cinnamon oil is due to the presence of the high amount of cinnamaldehyde as compared to linalool in essential oils. Finally these result made cinnamaldehyde rich essential oils could be widely used as a food additive and flavoring agent and to extend the shelf life of the food products, cinnamaldehyde rich Cinnamon oils may be suggested to uses as natural alternative to synthetic preservatives in foodstuffs.

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