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Chemical Composition and Biological Activities of Essential Oils of *Cinnamomum Tamala, Cinnamomum Zeylenicum* and *Cinnamomum Camphora* Growing in Uttarakhand

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Abstract

Studies were undertaken to chemically examine the essential oils of different species of Cinnamomum growing in Kumaon region of Uttarakhand and screening of their antibacterial, and antioxidant activities. Eight samples of Cinnamomum tamala, two samples of Cinnamomum zeylenicum and one sample of Cinnamomum camphora were collected. Among all these oils, four chemotypes of C. tamala, two of C. zeylenicum and one of C. camphora are introduced by GC-MS analysis. Antibacterial activity of essential oils of Cinnamomum species were tested against three pathogenic bacteria viz. Pasturella multocida, Escherichia coli and Salmonella enterica enterica by disc diffusion method and compared with Gentamicine. Antioxidant activities of the essential oils were evaluated by three different methods viz. reducing power activity, DPPH radical scavenging activity and effect on the chelating activity of Fe (II) ions. Results showed that the essential oils of Cinnamomum species have effective antimicrobial and antioxidant activities. Therefore they could be used as food preservatives and as medicines.

Introduction

Spices are dried seeds, fruits, roots, barks, leaves and vegetative substances used for nutritive purposes in insignificant quantities as food additive for the purpose of flavor, color, or as preservatives. Many of these substances are also used for other purposes such as medicines, in religious rituals, as cosmetics, in perfumery or as vegetables [19].

Family Lauraceae or Laurel family comprises a group of flowering plants included in the order Laurales [28]. Himalayan region particularly Uttarakhand region represent 10 genera and 23 species of the plant belonging to family lauraceae [8]. The genus *Cinnamomum* contains over 300 species, distributed in tropical and subtropical regions of North America, Central America, South America, Asia, Oceania and Australasia. The genus *Cinnamomum* contains 250 species in Indo-Malaysia and south-east Asia and 16 species in India [23]. The species of *Cinnamomum* have aromatic oils and terpenoids in their leaves and bark.

Cinnamomum tamala Nees & Eberm also known as Indian *Cassia,* a native species of India [3] is widely distribution in Himalayan region (900–2000 mt) also known as "Tejpat", popular among the north Indians as flavoring ingredient in various dishes. *Cinnamomum tamala* is mainly used in pharmaceuticals preparations because of its hypoglycemic, stimulant and casuistic properties. It is also used in therapeutics, colic, diarrhea, enlargement of spleen and snake bite. Leaves are commonly used as spice and are reported to possess antioxygenic, antibacterial and antifungal properties. Essential oil of *Cinnamomum tamala* is important for spices and perfumes [4, 30]. *Cinnamomum camphora* is commonly known as camphor. It has medicinal importance as Anti-inflammatory, antiseptic, antiviral, bactericidal, counterirritant, diuretic, expectorant, stimulant, rubefacient, vermifuge. *Cinnamomum zeylanicum* Blume. (cinnamon) is known

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as ‚Dalchini' in India. Cinnamon bark is widely used as a spice. It is principally employed in cookery as a condiment and flavoring material [4, 30].

Materials and Methods

Isolation and Chemical Investigation

Plant materials were collected from different natural habitats of the Kumaon region of Uttarakhand during March to May 2009. Eight samples of *Cinnamomum tamala* from Munsyari, Pithoragarh, Lohaghat, Champawat, Pantnagar, Dogaon, Nepal and Tanakpur, two samples of *Cinnamomum zeylenicum* from south India and Pantnagar and One sample of *Cinnamomum camphora* from Pantnagar were collected. 1 Kg. each of the fresh aerial part of the plant were hydrodistilled in Clevenger's type apparatus for 8 hours. The oils were extracted in diethyl ether. Removal of solvent under vacuum and drying over anhydrous Na_2SO_4 yielded varies amount of essential oils. The oils were kept in refrigerator for further study. The chemical analysis of volatile oils was undertaken by Gas chromatography-mass spectrometry (GC-MS) technique. The GC-MS data were obtained on Thermo Quest, Trace 2000, GC coupled with Finnigan Mat Polaris Q MS. In GC-MS analysis column was RtX- 5 (Res tex corp.) fused silica capillary column (30 m×0.25 mm, 0.25 µm) film coating, helium used as carrier gas with 1 ml/ min flow rate, EI (70 eV) mode and 210°C injection temperature. Components of essential oils (Table 1) were identified by matching their Mass spectra and GC retention indices with those in NIST- MS Wiley Library, comparing with literature reports and published data [24].

Antibacterial Activity

Antibacterial activity was screened against three gram negative bacteria viz., *Salmonella enterica enterica*, *Escherichia coli* and *Pasturella multocida*. These were collected from Department of Virology, Indian Veterinary Research Institute (Bareilly) and maintained in the laboratory by regular subculturing on to nutrient agar. Antibacterial screening of the essential oils against test bacteria was done by Disc diffusion method as reported by [14] with slight modification. 5µl, 10µl, 15µl, 20µl concentrations of essential oils were applied on each sterilized paper disc. The antibacterial screening was measured by zone of inhibition against bacteria. The plates were incubated at 37°C for overnight to observe the zone of inhibitions around the disc, which were compared with the standard antibiotics (Gentamicin).

Antioxidant Activity

Antioxidant potential of the essential oils were evaluated in terms of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability, effect on the chelating activity on Fe^{+2} and reducing power in comparison with the synthetic and natural antioxidants. Butylated hydroxyl toluene (BHT), catechin and gallic acid were taken as standards.

Reducing Power Activity

The reducing power of the essential oils was determined by the method reported earlier [14–16]. Different amount of essential oils (5µL, 10 µL, 15 µL and 20 µL) were mixed with 2.5mL of the phosphate buffer (200 mM, pH 6.6) and 2.5mL of 1% potassium ferricyanide, $K_3[Fe(CN)_6]$. The mixtures were incubated at 50°C. After incubation, 2.5mL of 10% trichloroacetic acid was added to the mixtures, followed by centrifugation at 650 rpm for 10min. The upper layer (5mL) was mixed with 5mL of distilled water and 1mL of 0.1% ferric chloride and absorbance of the resultant solution were measured at 700 nm using UV-Vis spectrophotometer (Visiscan-167, Indian).

DPPH Radical Scavenging Activity

The scavenging effect on DPPH radical was determined according to the methods developed earlier [14–16, 21]. Various amounts of essential oils (5µL, 10 µL, 15 µL and 20 µL) were mixed with 5mL of 0.004% methanolic solution of DPPH. Each mixture was placed for 30min. in the dark and the absorbance of the samples was read at 517 nm using UV-Vis spectrophotometer (Visiscan-167, Indian). The percentage (%) radical scavenging activity was determined according to the Equation [1].

Effect on the Chelating Activity of Fe^{2+}

The chelating activity of essential oils on ferrous ions (Fe^{2+}) was measured with slight modification of the method reported earlier [12]. Different amounts of essential oils (5μL, 10 μL, 15 μL and 20 μL) were first mixed in 1mL of methanol. Then mixtures were left for reaction with ferrous chloride (2mM, 0.1mL) and ferrozine (5mM, 0.2mL) for 10min at room temperature and, absorbance was measured at 562 nm in UV-Vis spectrophotometer (Visiscan-167, Indian). A lower absorbance indicates a higher chelating power. The chelating activity on Fe^{2+} of the oil was compared with that of EDTA (0.01mM) and Citric acid (0.025M) and percentage (%) chelating activity was calculated according to the Equation [2].

Results and Discussion

GC-MS Analysis of Essential Oils

The present investigation shows several chemotypes of *Cinnamomum tamala* which have mainly linalool, 1,8-cineol, cinnamyl acetate, *E*-cinnamaldehyde and eugenol were presented in major quantities**.** The essential oil of *Cinnamomum tamala* collected from Munsyari has linalool (52.5%) and E-cinnamaldehyde (26.4%) as major components another major component is 1,8-cineol (4.2%). 31 compounds have been identified in the oil which contributes to 98.4% of the oil. The oil is rich in monoterpenoids contributing of the total oil 94.12% of which 7.44% were hydrocarbons and 86.68% were oxygenated monoterpenoids. Only 3.68% sesquiterpenoids could be identified. Essential oil of *Cinnamomum tamala* collected from Nepal has similar pattern of essential oil composition. It has 53.2% linalool, 25.0% *E*-cinnamyl acetate and 16.1% 1,8-cineol**.** In oil 34 components were identified in the oil and the oil was rich in monoterpenoids (92.60%). The sesquiterpenes identified were epi-cubenol (0.6%) and caryophyllene oxide (0.6%). The above two species are linalool-*E*-cinnamaldehyde type. No camphor was detected in these samples. The essential oil of *Cinnamomum tamala* collected from Lohaghat showed the presence of linalool (29.8%), camphor (44.0%) and E-cinnamaldehyde (14.3%) along with other constituents**.** Its constituents contributing 96.6% of the oil were identified. Similar pattern of essential oil components was formed in the essential oil collected from Champawat collection. It has linalool (24.7%), camphor (25.5%) and *E*-cinnamaldehyde (30.4%). In all 25 constituents were identified in the oil which contribute to 96.3% of the oil. The above two collections are linalool-camphor-cinnamaldehyde type. The *Cinnamomum tamala* essential oils collected from Pithoragarh and Tanakpur showed similar pattern of the major constituents. Samples contains linalool (22.2%) and (38.0%), *E*-cinnamaldehyde (44.6%) and (25.0%) and cinnamyl acetate (15.1%) and (3.5%) respectively. Both the oils also contain borneol. The oil of *Cinnamomum tamala* collected from Dogaon also showed similar pattern in its linalool (27.2%), borneol (2.2%), cinnamaldehyde (42.5%) and E-cinnamyl acetate (1.8%). Essential oil of *Cinnamomum tamala* collected from MRDC Pannagar has eugenol (65.0%) as major constituent**.** It also contains *E*-cinnamaldehyde (3.8%) and *E*-cinnamyl acetate (2.6%) along with epi-cubenol (2.9%) and caryophyllene oxide (4.1%) hence it is eugenol type [1–2, 25–27].

Analysis of two sample of *Cinnamomum zeylenicum* cultivated at MRDC Pantnagar and CIMAP (introduced from South India) showed quit difference in the chemical makeup. The essential oil of *Cinnamomum zeylenicum* of Pantnagar contains linalool (7.4%), *E*-cinnamaldehyde (10.9%) and *E*-cinnamyl acetate 58.5% and 92.3% of the oil contributed by 25 compounds, identified by GC-MS. The essential oil of *Cinnamomum zeylenicum,* south Indian collection showed dominance of eugenol (74.1%). Unlike other oils, this was also rich in sesquiterpenoids. β-caryophyllene (1.2%), γ-elemene (1.9%), aromadendrene (0.1%), γ-gurjunene (2.3%), spathulenol (0.5%) and caryophyllene oxide (9.6%) were among the identified sesquiterpenoids [11, 18, 20, 31]. GC analysis of essential oil of *Cinnamomum camphora* cultivated at MRDC revealed the presence of a camphor single major constituent (82.4%). However other chemotypes of *C. camphora* are also reported in literature [5, 7, 9–10, 17, 22, 29, 32].

Antibacterial Investigation

All the oils showed activity against *Pasturella multocida, Escherichia coli* and *Salmonella enterica enterica* in comparison to gentamicine which was used

as standard. Antibacterial activity of essential oils of the essential oil of *Cinnamomum tamala* collected from Pithoragarh, Lohaghat and Champawat showed zone of inhibition against *Pasturella multocida* but minimum activity against *Salmonella enterica enteric. Cinnamomum tamala* collected from Dogaon, Tanakpur and Munsyari were found to show maximum zone of inhibition against *Salmonella enterica enterica, Escherichia coli* and *Pasturella multocida. Cinnamomum camphora* oil collected from Pantnagar was effective against *Pasturella multocida* but not against *Salmonella enterica enterica* and *Escherichia coli.* Concluding all these results, it can be said that the volatile oils of *Cinnamomum* species posses excellent antibacterial properties even at very low concentration against tested bacterial strains due to their major components. The results demonstrate that the essential oils of *Cinnamomum* species posses antibacterial activity against *Pasturella multocida*, *Escherichia coli* and *Salmonella enterica enterica* which are pathogenic bacteria which are responsible for diseases in animals and human beings.

Antioxidant Investigation

Antioxidant activities of the essential oils were evaluated by three different methods viz. reducing power activity, DPPH radical scavenging activity and effect on the chelating activity of Fe (II) ions. In presence of chelating agents, the complex formation between ferrous and ferrozine is disturbed, resulting in decrease the color of the complex. Measurement of color reduction therefore allows estimating the metal chelating activity of the coexisting chelator. Among all the essential oils tested essential oil of *C.zeylenicum* of south India (A_{700}=1.685 to 2.396 (±0.000 to 0.002)), *C. tamala* from Pantnagar (A_{700}= 3.812 to 4.000 (±0.020 to 0.000)) and from Pithoragarh (A_{700}=1.565 to 1.895 (±0.003 to 0.005)) possess maximum reducing power, followed by essential oil of *Cinnamomum tamala* from Tanakpur (A_{700}=1.243 to 1.693 (±0.001 to 0.000)). The reducing powers of essential oils were significantly lower compared to the BHT, gallic acid and catechin (Fig 1).

For the determination of radical scavenging activity, DPPH radical method is a very fast method to evaluate the antiradical power of an antioxidant activity by measurement of the decrease in absorbance of the DPPH radical at 517 nm. In the radical form this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule. The DPPH radical scavenging activity was tested for the essential oil of *C.zeylenicum* of Pantnagar (47.11±0.000%) and *C. tamala* of Tanakpur (39.64±0.006%) exhibited maximum DPPH radical scavenging activity among all collections which is followed by sample of Champawat (78.08±0.468%) and Lohaghat (75.14±0.573%) (Fig 2).

All the essential oils showed dose dependent chelating activity on Fe (II) ions. Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxide to reactive free radicals like Fenton type reaction. Among all the essential oil, the oil of *Cinnamomum tamala* cultivated at Pantnagar (62.48±0.126%) showed maximum chelating activity, followed by oil of *Cinnamomum tamala* from Munshyari (57.29±0.165%) and oil of *Cinnamomum zeylenicum* collected from South India (54.33±0.044%) (Fig 3). Therefore it can be concluded that the essential oils are an effective chelating agent and could effort protection against oxidizing agent. With the increase in the amount of essential oils, increase in chelating activity was observed for all the oils. Therefore we assume that the observed variation in antioxidant potential is related to differential chemical composition and concentration level. Therefore we can use the essential oils as natural antioxidant in place of synthetic antioxidant.

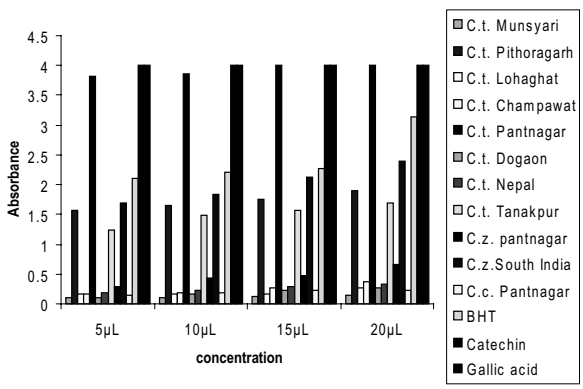

Fig. 1: Reducing power activity of essential oil of *Cinnamomum* species

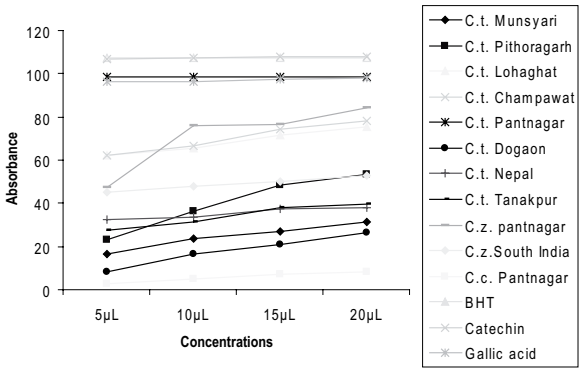

Fig. 2: Radical scavenging activity of essential oils of *Cinnamomum* species on DPPH

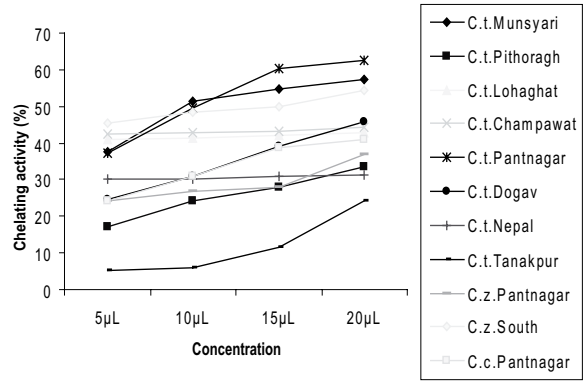

Fig. 3: Effect of the essential oils of *Cinnamomum* species on the chelating activity of Fe (II) ions

Equations

Equation [1]

$$\text{DPPH Radical Scavanging Activity (\%)} = [1- (A_t/A_o)] \times 100$$

(Where A_t is the absorbance of the sample at 517 nm and A_o is the absorbance of the control at 517 nm).

Equation [2]

$$\text{Chelating activity (\%)} = [1- (A_t/A_o)] \times 100$$

(Where A_t is the absorbance of the sample at 562 nm and A_o is the absorbance of the control at 562 nm).

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