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Studies on antioxidant activity and characterization of essential oil extracted from *Cinnamomum zeylanicum* Bark

Sontakke MD, Syed HM, Salve RV and Shinde EM

Abstract
The present investigation was undertaken to study extraction and characterization of essential oil of *Cinnamomum zeylanicum* bark. The extraction of essential oil from cinnamon bark was carried out by hydrodistillation method using clevenger type apparatus. Further, the obtained essential oil was characterized for chemical compounds by Gas chromatography-Mass spectroscopy (GC-MS) method. The results of GC-MS analysis identified 20 chemical compounds which included cinnamaldehyde, eugenol, α-pinene, eucalyptol, cinnamic acid, α-terpineole and other trace compounds were investigated. The cinnamon essential oil had antioxidant activity which showed 30.73% scavenged hydrogen peroxide and 15.23% scavenged nitric oxide.

Keywords: cinnamon bark, extraction of essential oil, hydrodistillation, GC-MS analysis

Introduction
Spices are natural food additives which contribute immensely to the taste of our foods. From ancient times they have been used to enliven our foods. Spices possess medicinal as well as nutritional based properties (Meghwal and Goswami, 2012) [11].
The name cinnamon refers to the tropical evergreen tree as well as the bark that is extracted from the plant Cinnamon spice is obtained by drying the central part of the bark and is marketed as quills or powder. The production of cinnamon is mostly limited to the wettest lowland areas of Southeast Asia. *Cinnamomum zeylanicum*, is reported to have originated in Sri Lanka and the Malabar coast of India. *C. cassia* is reported to have originated in South East China.
Cinnamomum is a large genus, many species of which yield a volatile oil on distillation. The composition of the oil, and therefore its value and the use to which it is put, depends very much on the species that is distilled as well as the part of the plant which is utilized. Cinnamon bark oil possesses the delicate aroma of the spice and a sweet and pungent taste. Its major constituent is cinnamaldehyde but other, minor components impart the characteristic odour and flavour. It is employed mainly in the flavouring industry where it is used in meat and fast food seasonings, sauces and pickles, baked goods, confectionery, cola type drinks, tobacco flavours and in dental and pharmaceutical preparations (Coppen, 1995) [3].
Cinnamon oil has strong lipolytic properties in dissolving fat and thus aids digestion. Once consumed, cinnamon helps break down fats in the digestive system, possibly by boosting the activity of digestive enzymes. Cinnamon also has a potential role in the treatment of diabetes. Cinnamon contains a chemical called methoxy hydroxy chalcone polymer which can reduce the blood glucose level (Thomas and Duethi, 2001) [15]. Essential oils are important components cinnamon bark, and these oils contain large quantities of terpenes and aromatic compounds. Specifically, cinnamaldehyde is the primary component of cinnamon oils (Li *et al.*, 2013) [10].
The cinnamon of commerce is the dried inner bark of the tree, *C. verum*. It is an essential item in curry powders and *masalas*. The bark oil, bark oleoresin and leaf oil are important value-added products from cinnamon. Bark oil is used in the food and pharmaceutical industries. Cinnamon leaf oil is cheaper than bark oil and is used in the flavour industry (Neela, 2008) [13].
Cinnamon is an aromatic and warm spice that is available in two forms - the cinnamon stick or ground cinnamon powder. The spice is actually obtained from the inner bark of the cinnamon tree (Maheshwari *et al.*, 2013) [11].

Material and Methods

Raw material

The raw material such as cinnamon bark were procured from the local market area of Parbhani District, Maharashtara.

Methods

Extraction of essential oil

The extraction of Essential oil was carried out by hydrodistillation method using Clevenger type apparatus. The raw cinnamon bark was coarsely ground using mortar and pestle were directly immersed in sufficient quantity of water into a extraction flask. The extraction flask was attached to the clevenger apparatus and extraction process was carried out for 4-5h. The condensed mixture containing oil was separated in a Florentine flask due to their immiscibility and density difference that oil was collected in a pre weighed vial and the weight of oil collected was calculated. The traces of water in the oil were removed using anhydrous sodium sulphate, pure oil was stored at 4°C in obscurity until the beginning of analysis (Al-Hashemi, 2014) [2].

Analytical methods

Characterization of cinnamon essential oil

The characterization extracted essential oils was determined using Gas chromatography method (Adinew, 2014) [1]. GC-MS analysis of the essential oil sample was carried out on a PerkinElmer Auto System XL GC interfaced with Turbomass Quadrupole Mass Spectrometer fitted with an Equity-5 fused silica capillary column (60 m × 0.32 mm i.d., film thickness 0.25 µm). The oven temperature was programmed from 60-210 ^{0}C at 3^{0}C/min using helium as the carrier gas at 1.0 ml/min. the injector temperature was 210^{0}C, injection volume 0.1 µl prepared in n-hexane (dilution 10%), split ratio 1:40. MS were taken at 70 eV with mass scan range of 40-450 amu and scan rate 1 sec with interscan delay 0.5 sec.

Identification of components

The component were identified on the basis of a Retention Index (RI), co-injuction with standards or known essential oil constituents. The relative amount of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

Antioxidant activity of cinnamon essential oil

H_2O_2 radical scavenging activity

The H_2O_2 scavenging activity was determined according to a method described by (Kumar *et al.*, 2007) [9]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of ginger extract were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min. The percentage scavenging of hydrogen peroxide by extracts and standard compounds (gallic acid, quercetin, and ascorbic acid) was calculated using the following formula:

Scavenged activity of H_2O_2 (%) = $[(A0 - A1)/A0] \times 100$

Where, A0 represents the absorbance of the control and A1 represents the absorbance in the presence of the extracts and standards. EC50 was the effective concentration at which 50% of hydrogen peroxide was scavenged.

Nitric oxide radical scavenging activity

Nitric oxide scavenging activity was determined given by (Haripriya *et al.*, 2013) [7]. 3 ml of reaction mixture (CA) containing sodium nitroprusside in PBS and sample was incubated at 25°C for 150 minutes. Controls were kept without test compound in an identical manner. After incubation, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance values of control and those of test compounds.

Antioxidant activity of cinnamon bark essential oil

Antioxidants scavenge free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or increasing the antioxidant defence mechanisms. Natural antioxidants that are present in spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress (Haripriya *et al.*, 2013) [7].

Table 1: Antioxidant activity of cinnamon bark essential oil

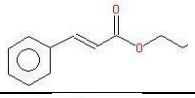
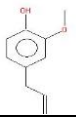
Sr. No.	Radical scavenging methods	Antioxidant activity (%)
1.	Scavenged hydrogen peroxide (H_2O_2)	30.73
2.	Scavenged nitric oxide (NO)	15.23

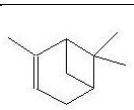
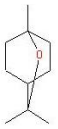
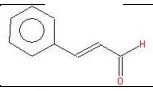
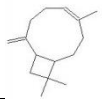
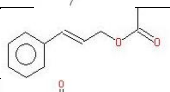
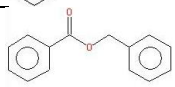
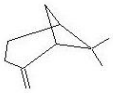
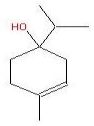
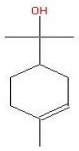
Antioxidant activity of cinnamon bark essential oil was determined by scavenging hydrogen peroxide and nitric oxide. The cinnamon essential oil showed maximum free radical scavenging activity against hydrogen peroxide which scavenged 30.73%. Comparatively, antioxidant activity with nitric oxide showed 15.23% radical scavenging activity. The cinnamon essential oil showed maximum hydrogen peroxide scavenging activity. The compound cinnamaldehyde found to possess significant reducing power (Haripriya *et al.*, 2013) [7].

Chemical constituents of cinnamon bark essential oil

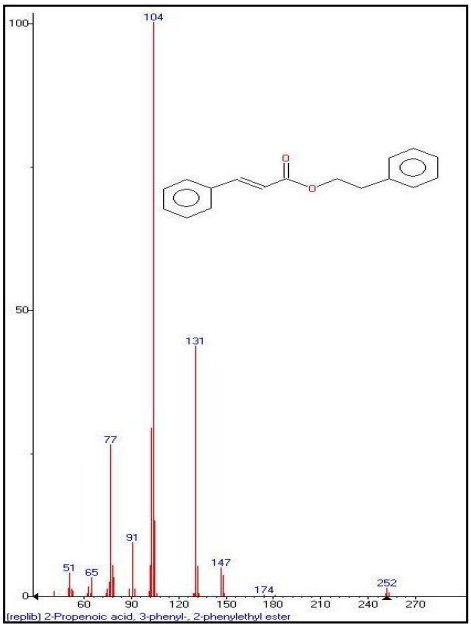
GC-MS analysis of cinnamon bark essential oil indicated the presence of 20 compounds comprised of cinnamicaldehyde, eugenol, α-pinene, eucalyptol, cinnamic acid, α-terpineole were investigated as the main chemical constituents.

Table 2: Chemical constituents of cinnamon bark essential oil

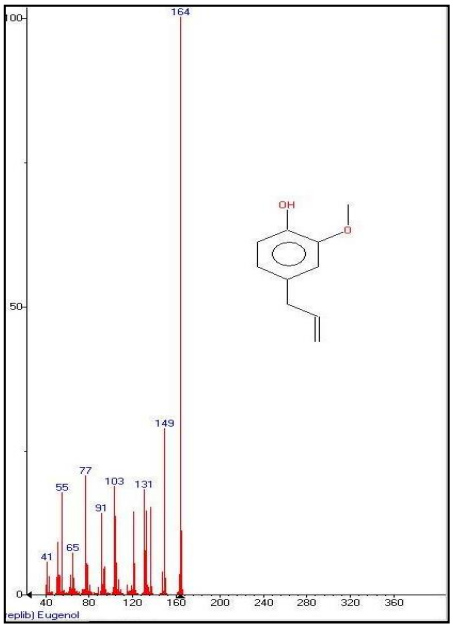
Retention time (min)	Compound	Structure	Molecular formula	Molecular weight
30.6	2-Propenoic acid, 3-phenyl, 2-phenylethyl ester		$C_{17}H_{16}O_2$	252
37.9	Eugenol		$C_{10}H_{12}O_2$	164

4.69	1S-α-Pinene		$C_{10}H_{16}$	136
6.73	Eucalyptol		$C_{10}H_{18}O$	154
13.4	Cinnamaldehyde-[E]		C_9H_8O	132
15.87	Caryophyllene		$C_{15}H_{24}$	204
16.53	2-Propen-1ol, 3-phenyl-acetate		$C_{11}H_{12}O_2$	176
22.53	Benzyl Benzoate		$C_{14}H_{12}O_2$	212
5.56	Bicyclo[3,1,1] heptanes, 6,6,-dimethyl-2methylene		$C_{10}H_{16}$	136
10.21	3-Cyclohexen-1-ol, 4-methyl-1-[1-methylethyl]		$C_{10}H_{18}O$	154
10.72	3-Cyclohexen-1-methanol, α, α,4-trimethyl		$C_{10}H_{18}O$	154

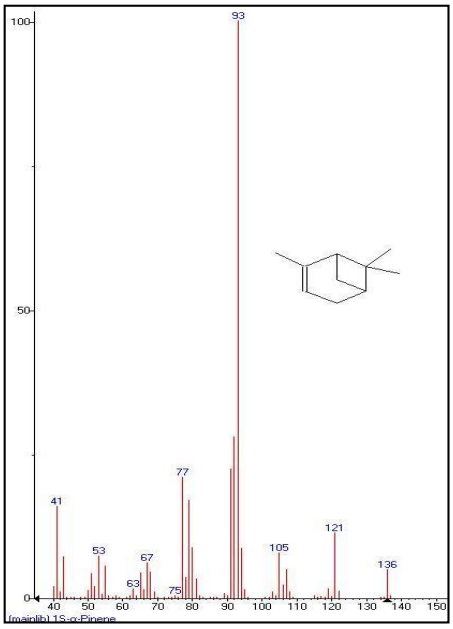
Cinnamon essential oil mainly contains cinnamaldehyde (65 to 95 per cent), cinnamyl acetate, cinnamic acid, benzaldehyde, and small amount of coumarin and trace amount of eugenol (Raghavan, 2007) [14]. The main constituent of cinnamon oil is cinnamaldehyde which provides the distinctive odour and flavour of cinnamon.



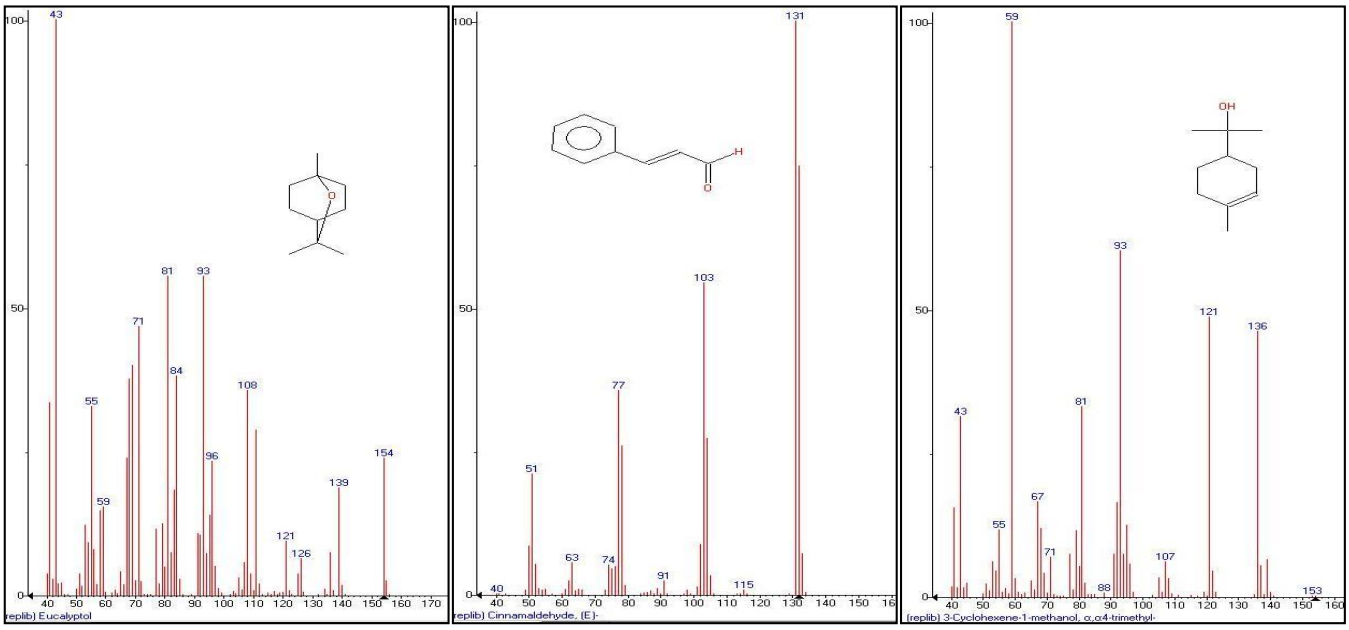
a) Cinnamic acid



b) Eugenol



c) α- Pinene



d) Eucalyptol e) Cinnamaldehyde f) α- Terpineole

Fig 1: Major GC-MS peaks of chemical constituents of cinnamon bark essential oil

Cinnamon oil exhibits a pleasing warm spicy aftertaste, characteristic spicy aroma, and preservative properties that made it attractive as a food flavouring and fragrance. The sweet taste of cinnamon is due to the presence of cinnamaldehyde (Husnu, 2010) [8].

Conclusion

The major chemical constituents detected in cinnamon essential oil consists cinnamicaldehyde, eugenol, α-pinene, eucalyptol, cinnamic acid, α-terpineole and some other were identified. Most of these compounds in essential oil are responsible for characteristic flavour or aroma, hence, widely used as flavourings in food and beverages. Spices essential oils shown to possesses antimicrobial as well as antioxidant properties due to its chemical constituents. Cinnamon essential oil shown to have strong antioxidant activity or free radical scavenging activity towards hydrogen peroxide and nitric oxide.

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