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Analysis of Volatile Oil of the Stem Bark of *Cinnamomum Zeylanicum* and its antimicrobial activity

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ABSTRACT

*Cinnamomumzeylanicum*Blume (Lauraceae)is a moderate sized tree native to Sri Lanka and southern India. Its stembark is used to treat stomach cramps, gastric irritation, dysentery, diarrhoea, nausea, neuralgia, rheumatism, toothache and paralysis of the tongue. The bark essential oil was characterized mainly by cinnamic aldehyde (91.5 %). There were thirteen monoterpenes (5.9 %) and ten of them were monoterpene hydrocarbons (5.1 %). The predominant monoterpene was *l*-limonene (3.1 %). There was three sesquiterpenes, *viz.* 3, 7-guaiadene, *cis*-calamenene and caryophyllene oxide, all of them were present in trace amounts. In addition to cinnamic aldehyde, the other aromatic components detected were benzaldehyde, *o*-cymene, eugenol , (E)–methyl cinnamate and benzyl benzoate, all of them occurred in lesser than 1 % yield. The oil was devoid of any aliphatic constituent. The volatile oil showed significant antimicrobial activity against pathogenic bacterial and fungal strains.

KEYWORDS: *Cinnamomumzeylanicum*, stem bark, Volatile oil, Analysis, Microbiological activity

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INTRODUCTION

*Cinnamomumzeylanicum*Blume, syn. *C. verum*Presl. (Lauraceae),commonlyknown asdalchini or Ceylon cinnamon, is a moderate sized tree up to 16 m in height, native to Sri Lanka and southern India; distributed in several tropical countries, including China, Madagascar, West Indies, Malaysia and Java[1]. Its bark is smooth, brown, thin, with a characteristic, strong pleasant odour and spicy burning taste. The bark is aromatic, astringent, expectorant and carminative; used to check nausea and vomiting and to treat stomach cramps, gastric irritation, dysentery, diarrhoea, neuralgia, rheumatism, toothache and paralysis of the tongue.Itis one of the oldest andmost valuable item in the spice trade usedforflavouring liquids, in cakes, curries, tomato ketchup and sauces. The bark essential oil is utilized in foodpreparations, confectionery, perfumery,beverages, pharmaceuticals and dental preparations. It is perfect in topical applications, and with its pleasant scent, a perfect additive to creams, lotions, and soaps[1,2].It stimulates the uterine muscles and is used in difficult deliveries due to inadequate contractions. Cinnamon promotes regular and easy menstruation and is taken as a warming herb for cold conditions, often in combination with ginger (*Zingiberofficinale*). It increases the circulation, especially to the fingers and toes and has been used to cure arthritis. It is also a traditional remedy for aching muscles and other symptoms of viral conditions such as colds and flue. Cinnamon tea is effective to stop bronchial asthma. Principally it is used as an aromatic to cover the unpleasant taste of other drugs.The cinnamon essential oil mainly containscinnamaldehyde, eugenol, linalool and benzyl benzoate[3-7]. The oil possessed antifungal, antibacterial,antiparasitic, antispasmodic, antiflatulent, appetitestimulant, antioxidant, antidiarrhoeal, refrigerant andanthlemintic properties[8-18].The presentwork describes analysis of the volatile and screening antimicrobial activity of the essentialoil of the stem bark of *C.zeylanicum.*

MATERIALS AND METHODS

Plant material

The dried stem bark of *C. zeylanicum* was purchased from the SangamVihar Market, New Delhi. The plant material was identified by Dr. S.R. Mir, Department ofPharmacognosy and Phytochemistry, JamiaHumdard, New Delhi. A voucher specimenis preserved in the herbarium of the Department.

Isolation of the volatile oil

The dried stem bark powder (1 kg) of *C. zeylanicum* washydrodistilled in an all glass Clevenger apparatus according to the method recommended in the British Pharmacopoeia, 1988**.** The colourless

volatile oil was dried over anhydrous sodium sulphate and stored at 4°C in the dark. The yield was 3.25 % based on the dried weight of the plant material.

GC analysis

Analytical GC was carried out by injection 01.µL of the leaf oil on a Varian 3300 gas chromatograph with FID detector fitted with silicone DB-I capillary column (30 m x 0.25 mm, film thickness 0.25 µm). GC operation condition was split mode, carrier gas was helium at a rate of 1.5 mL/min; temperature programme was from 80 to 225^0 C (4^0 C/min), injector temperature 280^o C and detector temperature 300^o C. Injection volume for all samples was 0.1 µl.

GC-MS analysis

GC-MS analysis was carried out by injection (0.1 µL) of the leaf oil on a QP-2000 instrument with a mass selective HP 597A detector fitted with Ulbon HR-1 capillary column (50 m x 0.25 mm, film thickness 0.25 µm)). GC-MS operation condition was split mode, carrier gas helium at a flow rate of 1.5 mL/min; temperature programme 70-225^0 C (10^0 C/min), injector temperature 250^o C and detector temperature 280^o C. The mass spectrometry conditioned were as follows: ionization voltage, 70 eV; emission current, 40 mA; mass range 0 – 400 Da, ion source temperature, 200° C.

Identification

The most constituents were identified by GC comparing their retention indices with those of authentic standard available in the laboratory or with the retention indices in close agreement with reference[19]. Further identification was achieved by analysis of GC/MS spectra. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer data base using the NBS 54 KL and Wiley L-built libraries and with those reported in the literature[19,20].

Antimicrobial Activity

Test organisms and inoculums

Escherichia coli (NCTC-6571), *Staphylococcus aureus* (NCTC-10418) and *Bacillus subtilis* were obtained from the Division of Biotechnology, Faculty of Pharmacy, JamiaHamdard, New Delhi. The fungus strains*Aspergillusflavus, Aspergillusniger*, *Aspergillusfumigatus*and *Candida albicans*were procured from the Institute of Genomics and Integrative Biology (CSIR), New Delhi.

Antimicrobial standard

Tetracycline solution with specific activity of 50 µg/ml was prepared in DMSO solution (antibacterial). Fluconazole with specific activity of 50 µg/ml was prepared in DMSO solution (antifungal).

Media

Dehydrated nutrient agar media was prepared in distilled deionized water. The media (g/100 ml) was composed of peptone (5.1 g), sodium chloride (5.0 g), beef extract (1.5 g), yeast extract (1.5 g) and agar (1.5 g).

Preparation of media

Dehydrated nutrient agar medium (28 g) was accurately weighed and suspended in 1000 ml of distilled water in a conical flask. It was heated on a water bath to dissolve the medium completely. The conical flask containing the nutrient agar medium was plugged with the helpof anon-absorbent cotton plug. Direct heating was avoided as it may lead to charring of the medium components and render it useless for the purpose.

Sterilization of Media

The conical flask containing the nutrient agar medium was plugged with the help of a non-absorbent cotton plug. The mouth of the conical flask and the cotton bung were properly covered with an aluminum foil. The medium was then sterilized by autoclaving at 15-lbs/in^2 pressure for 20 minutes.

Preparation of organisms

The test organisms were maintained on slants of medium and transferred to a fresh slant once a week. The slants were incubated at 37^{0}C for 24 hours. Using 3 ml of saline solution, the organisms were washed from the agar slant on to a large agar surface (medium) and incubated for 24 hours at 37+2^{0}C. The growth from the nutrient surface was washed using 50 ml of distilled water. A dilution factor was determined which gave 25 % light transmission at 530 nm. The amount of suspension to be added to each 100 ml agar or nutrient broth was determined by use of test plates or test broth. The test organisms were stored under refrigeration.

Temperature control

Thermostatic control is required in several stages of a microbial assay when culturing a micro-organism and preparing its inoculums and during inoculation in a plate assay.

Cup and plate method

A Previously liquefied and sterilized medium was poured into plastic petri-plates of 100 mm size. Required plates were prepared and kept for solidifying. Six holes were made in each plate with a stainless steel borer having 6 mm i.d. Different dilutions of the volatile oil of*C.zeylanicum*were made having concentration of 3 µl/ml, 5 µl/ml, 7 µl/ml and 9 µl/ml of solution. Tetracycline and fluconazole solutions were used as standards. The plates were labeled as Co (control), S (standard), X (*B. subtilis*), Y

(*S. aureus*), Z (*E. coli*) with four or five different holes, labeled as 3, 5, 7 and 9 for different concentrations. All dilutions were made in dimethyl sulphoxide (DMSO) solvent, which were used in experiment. Co was used for Control and S was used for Standard. Micropipette was used to deliver the solutions into the holes. The plates were then left for standing for 1 h for proper diffusion of the drug solutions. They were incubated for about 24 h at 32 ± 2^{o}C. After 24 h the plates were examined and the diameter of zones of inhibition was accurately measured**.** Antifungal activity was determined against *Aspergillusflavus, Aspergillusniger, Aspergillusfumigatus*and *Candida albicans*similar to antibacterial activity.

RESULTS AND DISCUSSION

The components of the volatile oil of the leaves of *C.zeylanicum* , their retention indices and percentage are listed in Table -1. The constituents are arranged in the order of their elution on Ulbon HR-1 capillary column. Analysis of the oil by GC-MS led to identification of twenty one components comprising 100% of the total volatile oil. The oil was characterized mainly by cinnamic aldehyde (91.5 %). There were thirteen monoterpenes (5.9 %) and ten of them were monoterpene hydrocarbons (5.1 %). The predominant monoterpene was *l*-limonene (3.1 %). There was three sesquiterpenes, *viz.* 3, 7-guaiadene, *cis*-calamenene and caryophyllene oxide, all of them were present in trace amounts. In addition to cinnamic aldehyde, the other aromatic components detected were benzaldehyde, *o*-cymene, eugenol, (E)–methyl cinnamate and benzyl benzoate, all of them occurred in lesser than 1 % yield. The oil was devoid of any aliphatic constituent.

The oil of the *C.zeylanicum*stem bark was examined for antibacterial activity against *E. coli, S. aureus*and *B. subtilis*and antifungal activity against *Aspergillusniger, A. fumigatus, A. flavus*and *Candida albicum.* The oil showed significant antimicrobial and antifungal activity in comparison to standards, Tetracycline and Fluconazole (Fig.1 and Fig. 2) . The observations are recorded in the Tables 2 and 3.

Table 1: Percentage composition of volatile oil of the dried stem bark *C. zeylanicum.*

S.No.	Components	Retention Indices	Percentage
1	*α*-Pinene	925	0.2
2	Camphene	939	0.1
3	Sabinene	960	0.2
4	β-Pinene	964	0.2
5	Benzaldehyde	967	0.5
6	α-Phellandrene	991	0.1
7	*l*-Limonene	1017	3.1
8	*cis*-Ocimene	1019	0.1
9	*o*-Cymene	1023	0.8
10	Linalool	1077	0.3
11	Terpinenolene	1079	0.2
12	Γ-Terpenene	1083	0.1
13	Thujyl alcohol	1125	0.2
14	Terpinen-4-ol	1129	0.3
15	Cinnamicaldehyde	1210	91.5
16	Eugenol	1326	0.5
17	3,7-Guaiadene	1430	0.3
18	*cis*-Calamenene	1451	0.4
19	(E) –Methyl cinnamate	1507	0.5
20	Caryophyllene oxide	1520	0.2
21	Benzyl benzoate	1866	0.2

Table 2: Antibacterial activity of the stem bark of *C. zeylanicum*..

Sample conc. (μl/ml)	Zone of inhibition (mm) S. *aureus*	Zone of inhibition (mm) *E. coli*	Zone of inhibition (mm) *B. subtilis*
3 (A)	12	10	12
5 (B)	17	14	14
7 (C)	18	15	16
9 (D)	18	17	27
50 (Co)	---	---	---
50 (S)	11	16	27

Table 3: Antifungal activity of the stem bark of C. zeylanicum.

Sample conc. (µl/ml)	Zone of inhibition (mm) *A. niger*	Zone of inhibition (mm) *A. fumigatus*	Zone of inhibition (mm) *A. flavus*	Zone of inhibition (mm) *C. albicum*
3 (A)	22	25	27	23
5 (B)	27	27	28	25
7 (C)	28	33	35	26
9 (D)	33	38	37	36
50 (Co)	---	----	---	---
50(S)	22	12	14	30

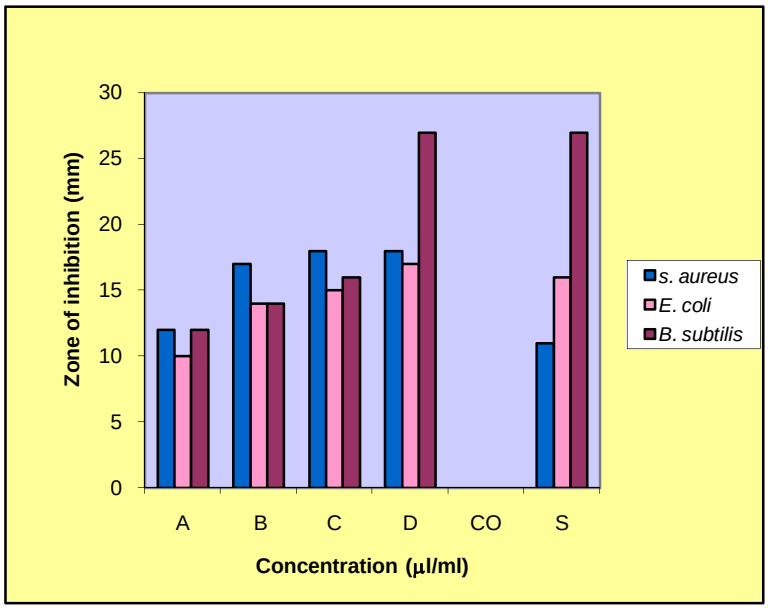

Fig. 1: Antibacterial activity of the stem bark of *C. zeylanicum*

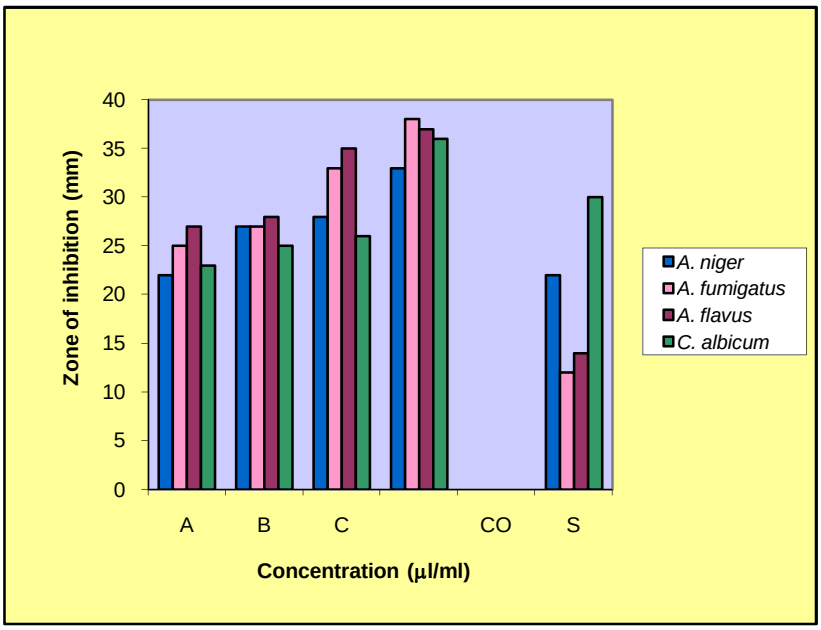

Fig. 2: Antifungal activity of the stem bark of *C. zeylanicum*

CONCLUSION

The volatile oil of *C. zelanicum* was consisted mainly of mainly by cinnamic aldehyde (91.5 %). There were thirteen monoterpenes (5.9 %) and three sesquiterpenes. It showed significant antimicrobial activity.

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