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Essential Oil Compounds and Antibacterial Activity of Leaves of *Cinnamomum chemungianum* Mohan et Henry (Lauraceae)

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Abstract

Essential oil of leaves of *Cinnamomum chemungianum* was obtained by hydro distillation and analyzed by GC-MS. The major components of the oil were Benzyl benzoate (66.36%), α -Terpine-4-ol (9.83%), Linalool (19.63%), and caryophyllene oxide (6.6%). Two compounds of the oils remained unidentified. The *in vitro* antibacterial activity was performed by agar disc diffusion method. It showed that maximum inhibition zone activity against *staphylococcus aureus*.

Keywords: Essential oil, hydro distillation, GC-MS, Antibacterial activity.

Introduction

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition (Buchbauer, 2000). This has recently attracted the attention of many scientists and encouraged them to screen plants to study the biological activities of their oils from chemical and pharmacological investigations to therapeutic aspects. Hopefully, this will lead to new information on plant applications and new perspective on the potential use of these natural products. Essential oils can be extracted from leaves, flowers, buds, twigs, rhizomes, heartwood, bark, resin, branches or whole plant, seeds and fruits (Sangwan, *et al.*, 2001).

The Lauraceae is a family of about 2000-2200 species of mostly tropical trees (C. Chaverri and J. F. Ciccio, 2005). The genus *Cinnamomum* comprises several hundred species, which occur in Asia and Australia. These are evergreen trees and shrubs and most of the species are aromatic (G.K. Jayaprakasha, *et al.*, 2002). Twelve *Cinnamomum* species are endemic to Peninsular India, of which nine are endemic to Southwestern Ghats, one of the mega centers of endemism in India (Baruah and Akhil, 2006).

Cinnamomum chemungianum Mohan et Henry (Lauraceae) was reported in 1991 from chemungi, Kerala, Southern India. 'Chemungi' is a botanically rich area in the south western ghats of India and the type locality of many taxa. The plant is an ever green shrub or small tree, 3-4 m tall with slender branches, leaves 3-7 by 2-4 cm, thinly coriaceous, ovate, caudate acuminate at apex, rounded at base with 0.6- 1 cm long petioles. The main purpose of this study is to know the essential oil chemical compositions and their antimicrobial potential of *C. chemungianum* leaves.

Materials and methods

Collection of plant materials

Cinnamomum chemungianum leaves were collected from Kalakad Mundanthurai Tiger Reserve forest, Southern Western Ghats, Tamil nadu, India.

Hydro-distillation of essential oil

The essential oil was extracted from the collected materials by hydro-distillation for 5 h using the Clevenger type apparatus (Guenther 1948). A clear, yellow coloured oily layer was obtained on top of the aqueous distillate which was separated from the later and dried with the anhydrous sodium sulphate. The extracted essential oil was kept in air tight sealed glass vials and covered with aluminum foil at 4°C until further use.

Gas chromatography analysis

The essential oil was analyzed using a Shimadzu QP 5000 gas chromatograph equipped with a FID detector and HP-5 MS capillary column (30m \times 0.25 mm, film thickness 0.25 μ m). Injector and detector temperatures were set at 220 and 290 °C, respectively. Oven temperature was kept at 50 °C for 3 min, then gradually raised to 160 °C at 3 °C /min, held for 10 min and finally raised to 240 °C at 3 °C /min. Helium was the carrier gas, at a flow rate of 1 ml/min. Diluted sample (1/100 in acetone, v/v) of 1.0 μ l was injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

The leaves of *C. chemungianum* yielded about 1.41% essential oils which were analyzed by GC/ MS using a Shimadzu – GC 17.A system with OV-I column (30m\0.25mm; 0.25 μ m film thickness). Mass spectra were taken at 70eV. Mass range was from m/z 35-350amu. The column temperatures were programmed from 70-250 °C at 4 °C /min. Helium was employed as carrier gas (1ml/min); injection of 1ml of a 1% solution of whole essential oil in chloroform split 1:50, scans range 35-350amu and scan time 1.0 sec. Identification of components in the oil was based on retention indices (RI) relative to *n*-alkanes and computer matching with the WILEY 275.L library, as well as by comparison of the fragmentation pattern of the mass spectra with data published in the literature (R. P. Adams, 2001).

Antibacterial activity

Bacterial strains

Four different bacterial strains used in this study, which were supplied by Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India. Some bacterial species were chosen namely *Staphylococcus aureus* (MTCC 1430), *Pseudomonas aeruginosa* (MTCC 2642), *Klebsiella pneumoniae* (MTCC 2405), and *salmonella typhi* (MTCC 733).

Preparation of Inoculums

The bacterial strains preserved in the nutrient agar at 4⁰C were revived in nutrient broth (liquid medium) and incubated at 37 \pm 1⁰C for overnight and the suspensions were checked to provide approximately 10⁶cfu ml⁻¹.

Antimicrobial activity assay

The essential oil was tested for their antimicrobial activity using the disc diffusion technique on solid media. Sterile 5 mm diameter filter paper discs were impregnated with 20µl of oil extract and placed on nutrient agar seeded with the microorganisms (10^6 cfu ml⁻¹). The plates were incubated for 24 hrs at 37°C for bacteria. Control discs were soaked with the same extraction solvents and treated as the sample discs. The experiments were carried out as duplicate three times and corrected for the control discs. Additionally, Ampicillin was tested as positive standards at a concentration of 20µg/disc (Janssen *et al.*, 1987).

Results and discussion

Essential oils of aromatic plant species are used in industry in the production of perfumes and toiletries. Many of them are also used in traditional medicine for various purposes and have been screened for their potential uses as alternative remedies for treatment of many infectious diseases, as food preservatives, and have shown insecticidal and antiparasitic properties (Burt. S, 2004).

Components of essential oil

The leaves of *C. chemungianum* on hydrodistillation yielded 1.41 % (v/w) essential oil which was yellow in color. The results of the quantitative analysis are presented in table 1. The active constituents were identified by comparisons of their retention time (Rt) and retention indices. A total of nine constituents were identified. Namely, Benzyl benzoate (66.36%), a-Terpine-4-ol (9.83%), Linalool (19.63%), and caryophyllene oxide (6.6%) were found to be the major constituents in the oils of the leaves. Remaining two compounds of the oils were unidentified.

Antibacterial activity

Table 2 summarizes the microbial growth inhibition by essential oil of leaves of *C. chemungianum*, which showed good antibacterial activities against all the tested organisms. The antibacterial activities of essential oil of *Cinnamomum chemungianum* leaves were assayed *in-vitro* by agar diffusion method against four bacterial strains.

These data also revealed that the essential oils of leaves of *C. chemungianum* exhibits strong antibacterial activity. This is used as a popular ingredient in soaps, perfumes, foods and drinks (Souwalak Phongpaichi *et al.*, 2006). The essential oils of *C. chemungianum* leaf showed that maximum zone inhibition active against *Staphylococcus aureus*. And it also showed moderate activity against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and less activity studied only against *salmonella typhi*.

According to these results, it is possible to conclude that *C. chemungianum* leaves essential oil had a strong and a broad spectrum of antibacterial activity and which may provide to develop novel antibiotics.

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Table-1: Essential oil compositions of *Cinnamomum chemungianum* leaves.

S. No	Compositions	Percentage	Identification methods
1	Phenyl ethyl benzoate	0.58	RT,GC-MS
2	a –bisobolol	0.62	RT,GC-MS
3	Caryophyllene oxide	6.6	RT,GC-MS
4	Unknown	0.96	RT,GC-MS
5	Unknown	3.6	RT,GC-MS
6	b-cymen-8-ol	4.9	RT,GC-MS
7	Linalool	19.63	RT,GC-MS
8	Terpinen-4-ol	9.83	RT,GC-MS
9	Benzyl benzoate	66.36	RT,GC-MS

RT- Retention time; GC- Gas Chromatography; MS- Mass spectrophotometer

Table-2: Antibacterial activities of essential oil of *Cinnamomum chemungianum* leaves

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S. No	Tested bacteria	Zone of Inhibition (mm)				Ampicillin 20µg/disc
		Control	25µl	50µl	100 µl	
1	<i>Salmonella typhi</i>	0	8	10	13	16
2	<i>Klebsiella pneumoniae</i>	0	12	15	16	28
3	<i>Pseudomonas aeruginosa</i>	0	9	13	15	28
4	<i>Staphylococcus aureus</i>	0	16	18	21	24