Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd

Document heading

Antimicrobial activity and composition of the volatiles of *Cinnamomum tamala* Nees. and *Murraya koenigii* (L.) Spreng. from Uttarakhand (India)

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ARTICLE INFO

Article history:
Received 25 June 2012
Received in revised from 5 July 2012
Accepted 7 Octoberr 2012
Available online 28 October 2012

Keywords:
Cinnamomum tamala
Murraya koenigii
essential oil composition
cinnamaldehyde
α-pinene
antimicrobial activity.

ABSTRACT

Objective: To examine the composition of *Cinnamomum tamala* and *Murraya koenigii* essential oils and their antimicrobial activities against nine microbial strains. **Methods:** Essential oils were obtained by hydrodistillation from the leaves of two spice trees and were analyzed by GC and GC/MS. The oils were also tested for their antimicrobial activity using broth micro dilution method. **Results:** Cinnamaldehyde (37.85%) and cis-linalool oxide (29.99%) were the main components characterized in the oil of *C. tamala*, whereas α-pinene (39.93%), sabinene (13.31%) and trans-caryophyllene (9.02%) detected as the major constituents in *M. koenigii* oil. *C. tamala* oil exhibited significant antifungal activity and satisfactory antibacterial activity, while lesser antimicrobial activity was observed in *M. koenigii* oil. **Conclusions:** The present study suggested that *C. tamala* oil was more effective against bacterial and fungal strains as compared with *M. koenghii* oil.

1. Introduction

Spices are the building blocks of flavour in food application since ancient times. These are not only used due to flavouring agent but also as medicinal and food preservative. Many spices have been cultivated for their aromatic, fragrant, pungent, or any other desirable properties including the seed (coriander, caraway), berry (allspice, black pepper), bark (cinnamon), rhizome (ginger, turmeric), flower (saffron), bulb (garlic, onion), fruit (cardamom), flower bud (clove) and the leaf of *Cinnamomum tamala* (bay, tezpat) and *Murraya koenigii* (kari, curry)[1].

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Cinnamomum tamala Nees. (Lauraceae), a medium sized tree is distributed in tropical and subtropical Himalayas at the altitudes of 1000-2400m[2]. In India, the tree is cultivated commercially in certain parts of the country for leaf production and essential oils. In the leaf oil of *C. tamala*, four chemotypes namely cinnamaldehyde (Uttarakhand), eugenol (North East India), cinnamaldehyde-linalool (Himachal Pradesh) and linalool rich type (Assam) are reported in different parts of the country[3]. Tezpat leaves and oils are used in flavouring foods, beverages, in perfumery and pharmaceutical industries[4,5].

Murraya koenigii (L.) Spreng. (Rutaceae) is a strong smelling perennial shrub or small tree commonly found in forests as undergrowth, cultivated in India for its aromatic leaves and for ornament[6]. Fresh leaves, dried leaf powder and volatile oils are extensively used in India for flavouring many food

dishes and materials[7,8]. The oil is also used in soap and cosmetic industries[9]. In *M. koenigii* oil, four chemotypes – (1) β –pinene (70%), (2) α –pinene (65.7%), (3) β –caryophyllene (53.9%), (4) β –phellandrene (30.2%) + β –caryophyllene (24.2%) are reported from various parts of India[10]. The purpose of this investigation was to examine the oil composition and its antimicrobial activity against some important microbes.

2. Materials and Methods

2.1. Plant Material

The leaves of *C. tamala* and *M. koenigii* were collected from Dehradun in the month of April, 2011. Voucher specimens have been duly identified and deposited in the herbarium of Botanical Survey of India (BIS), northern circle, Dehradun (Acc. No.113522 and 113523 respectively).

2.2. Extraction of oils

The shade dried leaves of *C. tamala* and *M. koenigii* were subjected to separate hydro-distillation for 4 hours using a Clevenger apparatus. The oils obtained over dried over anhydrous Na_2SO_4 and stored at 4℃ for GC, GC/MS and microbiological analysis.

2.3. Gas chromatography and Gas chromatography-Mass spectroscopy analyses

About 0.1 μl of each pure oil sample was subjected to GC and GC/MS analyses. The GC was composed of an Agilent Technology 6890 N gas chromatograph data handling system equipped with a split-splitless injector and fitted with a FID using N_2 as the carrier gas. The column was HP-5 capillary column (30m x 0.32mm, 0.25 μm film thickness) and temperature program was used as follows: initial temperature of 60℃ (hold: 2 min) programmed at a rate of 3℃ /min to a final temperature of 220℃ (hold: 5 min). Temperatures of the injector and FID were maintained at 210℃ and 250℃, respectively.

The GC/MS analyses were carried out on a Perkin Elmer Clarus 500 (Shelton, CT 06484, USA) gas chromatograph equipped with a split-splitless injector (split ratio 50:1) data handling system. The column was an Rtx®-5 capillary columns (60 m x 0.32mm, 0.25 μm film thickness). Helium (He) 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. The mass spectra were generally recorded over 40-500 amu that revealed the total ion current (TIC) chromatograms. Temperature program was used as 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℃, respectively.

Identification of the individual components was made by matching their recorded mass spectra with the library (NIST/ Pfleger /Wiley) provided by the instrument software, and by comparing their calculated retention indices with literature value[11]. Relative area percentages of the individual components were obtained from GC-FID analyses.

2.4. Antimicrobial activity

The susceptibility of essential oils of *C. tamala* and *M. koenigii* were tested on nine reference strains, which included five bacterial strains (*Staphylococcus aureus* ATCC 29213, Methicillin Resistant *S. aureus* {MRSA} 15187 Clinical Isolate, Vancomycin Resistant *E. faecalis* (VRE) Clinical Isolate, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and four fungal strains (*Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Aspergillus fumigatus* MTCC 1811 and *Aspergillus niger* ATCC 16404). These strains were procured from the American Type Culture Collection (ATCC, Manassas, VA, USA) and Microbial Type Culture Collection (MTCC, Chandigarh, India).

The antibacterial and antifungal activities of the essential oils of *C. tamala* and *M. koenigii* were performed by broth micro dilution methods as per the guidelines of Clinical and Laboratory Standard Institute (formerly, the National Committee for Clinical Laboratory Standards)[12] by using Mueller-Hinton broth (MHB; Becton-Dickinson, Cockeysville, MD, USA) supplemented with calcium (25 mg/L) and magnesium (12.5 mg/L) for bacterial strains while as RPMI 1640 medium buffered to a pH of 7.0 with 0.165M MOPS (both from Sigma) was used for fungal strains. Two fold serial dilutions of the oils were prepared in the respective test medium in amounts of 100 μl per well in 96- well U-bottom microtiter plates (Tarson, Kolkata, India). Stock inoculum suspensions of the bacteria, *Candida* species and *Aspergillus* species were prepared in sterile normal saline (0.85 %) containing 0.05 % polysorbate 20 (NST) from overnight (7-days for *Aspergillus* species) cultures grown on Trypticase soy agar (TSA; Becton-Dickinson) and potato dextrose agar (Difco Laboratories, Detroit, Mich.) respectively at 35℃. Inocula were verified for each assay by plating onto agar plates for colony enumeration. These suspensions were further diluted in the respective test medium and a 100 μl volume of this diluted inoculum was

added to each well of the plate, resulting in a final inoculum concentration of 5 x 10^5 CFU/ml for bacteria, 0.5 × 10^4 to 2.5 × 10^3 CFU/ml for *Candida* species while 0.4 × 10^4 to 5 × 10^4 CFU/ml for *Aspergillus* species[12,13,14]. The final concentration of essential oils ranged from 0.003 μl/ml to 5 μl/ml. Ciprofloxacin and Amphotericin B were served as the standard drug control for bacterial and fungal cultures respectively. The microtiter plates were incubated at 35℃ for 24 h for bacterial cultures and 48 h for fungal cultures. The plates were read visually and the MIC was defined as the lowest concentration of test sample that prevented visible growth with respect to the growth control.

3. Results

The composition of the oils of *C. tamala* and *M. koenigii* are given in Table 1. As is shown, the oil of *C. tamala* was characterized by large amount of oxygenated monoterpenes (92.10%) with cinnamaldehyde (37.85%) and cis-linalool oxide (29.99%) being the major constituents found. Earlier, cinnamaldehyde, linalool and cinnamyl acetate rich types were reported from Uttarakhand region[5]. The present study reports the occurrence of cis-linalool oxide in abundance from Dehradun region. The other notable constituents in *C. tamala* oil were found as linalool (7.71%), cinnamyl acetate (5.37%) and 1,8-cineole (3.23%). The oil of *M. koenigii* was found to be rich in monoterpenes (70.17%) with α-pinene (39.93%), sabinene (13.31%), β-pinene (7.62%) and limonene (4.42%) being the major constituents. The percentage of identified sesquiterpenes was relatively small (16.86%) with trans-caryophyllene (9.02%) being the major one. 2-Furyl methyl ketone (4.80%) was also found in appreciable amount in this oil. The chemical profile of *M. koenigii* wildly grown in Dehradun region was investigated earlier[15]. Our result was found qualitatively similar, but differs quantitatively due to the presence of α-pinene (51.7%) in higher amount than our study. Overall, the *M. koenigii* oil from Dehradun region is α-pinene rich chemotype.

The MIC of the essential oil of *C. tamala* and *M. koenigii* against nine microorganisms were tested and the results are tabulated in table 2. The strongest activity (MIC = 0.3 − 0.6 μl/ml) was shown in *C. tamala* oil against all the four fungal pathogens, whereas oil was found comparatively less effective against bacterial pathogens (MIC = 2.5 μl/ml). On the other hand, *M. koenigii* oil exhibited weak antimicrobial activity against all the microorganisms (>MIC = 5 μl/ml).

Table 1

Percentage composition of leaf essential oils of *C. tamala* and *M. koenigii*

Components	RI	% in oils	
		C. tamala	*M. koenigii*
2-furyl methyl ketone	893	–	4.80
α-pinene	933	0.95	39.93
α-thujene	926	–	0.35
camphene	949	0.69	0.65
benzaldehyde	963	3.15	–
sabinene	972	–	13.31
β-pinene	980	0.55	7.62
β-myrcene	994	–	1.02
α-terpinene	1021	–	0.46
p-cymene	1029	1.06	0.48
limonene	1031	0.26	4.42
1,8-cineole	1035	3.23	–
cis-ocimene	1038	–	0.64
γ-terpinene	1062	–	0.96
acetophenone	1065	0.19	–
cis-sabinene hydrate	1070	–	0.21
cis-linalool oxide	1078	29.99	–
α-terpinolene	1085	–	0.33
linalool	1103	7.71	0.92
trans-sabinene hydrate	1109	–	0.39
3-phenyl propanal	1165	1.68	–
borneol	1166	0.55	–
terpinen-4-ol	1180	0.27	–
α-terpineol	1184	0.63	–
geraniol	1268	–	0.44
cinnamaldehyde	1282	37.85	–
bornyl acetate	1286	1.48	0.75
α-copaene	1379	–	0.24
β-bourbonene	1386	–	0.30
β-elemene	1393	–	0.36
cis-jasmone	1394	–	1.10
trans-caryophyllene	1420	–	9.02
β-cedrene	1421	–	0.10
β-gurjunene	1426	–	0.11
cinnamyl acetate	1443	5.37	–
spathulenol	1453	0.21	–
β-farnesene	1454	–	0.17
α-humulene	1456	–	1.38
germacrene D	1485	–	1.96
bicyclogermacrene	1492	–	0.51
α-farnesene	1508	–	2.26
β-bisabolene	1510	–	0.20
δ-cadinene	1524	–	0.35
cis-nerolidol	1534	–	0.14
caryophyllene oxide	1581	0.30	0.51
τ-cadinol	1611	–	0.12
δ-cadinol	1636	–	0.28
monoterpene hydrocarbons		3.51	70.17
oxygenated monoterpenoids		92.10	8.61
sesquiterpenes hydrocarbons		–	16.86
oxygenated sesquiterpenoids		0.51	1.05
Total identified compounds		96.12	96.79

Table 2
The Minimum Inhibitory Concentration (MIC) values of essential oils of *C. tamala* and *M. koenigii* against selected microorganisms

Micro-organisms Tested	MIC (μ l/ml)	
	C. tamala	*M. koenigii*
Bacterial strains		
Staphylococcus aureus ATCC 29213	2.5	5
Methicillin Resistant *S. aureus* (MRSA) 15187, Clinical Isolate	2.5	>5
Vancomycin Resistant *E. faecalis* (VRE), Clinical Isolate	2.5	>5
Escherichia coli ATCC 25922	2.5	>5
Pseudomonas aeruginosa ATCC 27853	2.5	>5
Fungal strains		
Candida albicans ATCC 90028	0.6	>5
Candida parapsilosis ATCC 22019	0.6	>5
Aspergillus fumigatus MTCC 1811	0.3	>5
Aspergillus niger ATCC 16404	0.6	>5

4. Discussion

The present study suggested that the volatile oil of *C. tamala* possess effective antifungal and antibacterial activity due to their major compounds as compared with *M. koenghii* oil. It can be said that the concentration of cinnamaldehyde and linalool oxide, the major compounds in *C. tamala* oil was responsible for inhibition of tested pathogens, especially against fungal strains viz. *Candida albicans*, *Candida parapsilosis*, *Aspergillus fumigatus* and *Aspergillus niger*. Although in some earlier studies, the aqueous and alcoholic extracts of *M. koenghii* effectively inhibited bacterial and fungal growth[16,17,18]. Further, the antimicrobial activity of essential oils is required to be analyzed repeatedly from different locations of the country so as to exploit these for logical scientific research.

Conflict of interest statement

We declare that there is no conflict of interest.

Acknowledgements

Authors are thankful to Dr. I.A. Khan, Clinical Microbiology Division, Indian Institute of Integrative Medicine, Jammu (J&K) for providing laboratory facilities regarding microbial testing. Two of the authors (MM, RS) are thankful to Department of Science & Technology (DST), Govt. of India for providing research fellowship (Grant no. DST/TDT/WTI/2k7/31 dated 12.07.10).

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