SHORT COMMUNICATION

Gas chromatography-mass spectrometry analysis of volatile oil obtained from *Aegle marmelos* leaves collected from foothills of Shivalik range

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Abstract

Aim: *Aegle marmelos* (L.) Correa belongs to the Rutaceae family and is abundantly available all over India, and almost every part of this plant is used in traditional medicine. **Materials and Methods:** In the present work, we isolated volatile oil using the standard method of hydrodistillation from the leaves of *A. marmelos*. Further, the analysis of the oil was performed by capillary gas chromatography and gas chromatography-mass spectrometry. **Results and Discussion**: The percentage yield of oil was 0.91 (v/m). Further, the analysis of the oil resulted in the identification of 13compounds comprising more than 85 % of the total oil. The leaf oil was constituted mainly of monoterpene compounds, and the major compounds included a relatively stable terpene-limonene (67.83 %) and caryophyllene (8.76 %).

Key words: *Aegle marmelos*, gas chromatography-mass spectrometry, volatile oil

INTRODUCTION

Traditional or indigenous drugs used by different ethnic groups of the world for treatment of various diseases have special significance of having been tested on wide population over a long time scale. Conventional herbal therapies coupled with dietary measures are prescribed by Ayurveda and other complementary systems of medicines in India as remedies to many ailments in human and animals. A sizeable inhabitant in almost all developed countries uses at least one form of unconventional therapy including herbal medicines.[1,2]

Aegle marmelos Correa (syn. *Feroniapellucida* Roth, *Cratarea marmelos* L., vern. Bael, Vilwam Kuvalam, Bengal Quince, Golden Apple, Stone Apple, and Wood Apple) belongs to the family Rutaceae is a handsome deciduous aromatic tree growing up to 8.5m height which is widely distributed throughout the Indian peninsula along with Sri Lanka, Burma, and Thailand.[3,4] In addition to being regarded as good dietary supplement,[5,6] it has been valued in the treatment of various diseases in complementary systems of medicine.[7] Various parts of the plant have been reported to have a number of bioactive compounds and secondary metabolites belonging to various classes of natural products[8-10] mainly marmenol,[11] marmin,[12] marmelosin, marmelide, psoralen, alloimperatorin, rutaretin, scopoletin.[13-15] aegelin,[16,17] marmelin,[15] fagarine, anhydromarmelin,[18-19] b-carotene, limonene, α-phellandrene, betulinic acid, marmesin,[20] imperatorin[21] marmelosin, luvangentin,[22,23] and auroptene.[24] Many reviews on the plant with special emphasis on pharmacological activities and phytoconstituents have been published in literature.[25-29]

Various studies reported the isolation of volatile compounds from the leaves of *A. marmelos* available in different parts of the world.[30-33] Furthermore, the literature data available from previous publications reported the variation in the amount of volatile oil yield from 0.31% to 1.5% in leaves

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of *A. marmelos*.[31-33] In addition, qualitative analysis of the volatile components of the oil revealed the presence of limonene, α-phellandrene, (E)-β-ocimene, α-pinene, (E)-caryophyllene, β-elemene, and germacrene B and showed considerable variations in amounts due to season of collection.[34] This tree distributed abundantly across India, and the volatile components vary across the various geographical locations. The literature also revealed that the volatile component and content in the plant varies from region to region. However, to date, there are no reports of isolation of volatile compounds from leaves of *A. marmelos* from Shivalik range foothills region. This study focused to analyze the volatile components in the oil isolated from the leaves of *A. marmelos* to compare these results with the previous ones obtained for the same species but collected from different geographical regions. This study will, therefore, provide an additional data regarding the region selection to be taken into account while harvesting the volatile oil from the plant.

MATERIAL AND METHODS

Collection and Authentication of *A. marmelos* Plant

The leaves of *A. marmelos* were collected from the foothills of Shivalik range (around Chandigarh), India, in the month of September. The plant material was authenticated by Dr. Sujata Bhattacharya, Assistant Professor, School of Biological and Environmental Sciences, Shoolini University, Solan. Voucher specimens of the plant (SUBMS/89) have been deposited in the School of Biological and Environmental Sciences, Shoolini University, Solan.

2.2 Isolation of Volatile Oil

The volatile oil was isolated from fresh leaves of *A. marmelos* by hydrodistillation for 4 h in a Clevenger-type apparatus. The percentage yield of volatile oil (calculated per weight of fresh leaves) was 0.91 (v/m). The obtained oil was dried over anhydrous sodium sulfate and stored at −20°C. Further, 1 µL of the oil was analyzed using gas chromatography-mass spectrometry (GC-MS).

GC/MS Analysis

GC analysis was performed in a Thermo Scientific TSQ 8000 gas chromatograph - Mass Spectrometer, fitted with MS TSQ 8000 detector and column TG-5MS (30 m × 0.25 mm × 0.25 µm). The temperature program was 60°C (5 min) and then rose to 280°C (at 4°C/min). Injector and detector temperatures were both 250°C. Carrier gas (helium) at 1 mL/min, and injection volume was 1.0 µL. The injector and detector were held at 250°C and 240°C, respectively. Components were quantified as area percentage of total volatiles from electronic integration (EZChrom V. 6.7 software) with mass-selective detector operated in electron impact mode (70 eV) with interface at 230°C. Components were identified as far as possible from the best match to their mass spectrum in the NIST library, and confirmed in many compounds by their relative retention indices. In addition, the mass spectra from the literature were also compared with the current data.

RESULTS AND DISCUSSION

The percentage of the volatile oil from fresh leaves of *A. marmelos* (L.) Correa was 0.91% (v/w). GC-MS analysis of oil revealed the presence of 13 components and these identified components represent more than 85% of the total oil composition, and the most abundant constituents of the oil were limonene (a relatively stable terpene) (67.83%), caryophyllene (8.76%), α-Ocimene(4.84%), cubedol (3.29%), humulene (2.84%), myrcene (2.60%), and α-Copaene (2.53%). The GC-MS oil chromatogram is presented shown in Figure 1. In addition, the details of chemical composition along with the retention time and peak of the components are mentioned in Table 1.

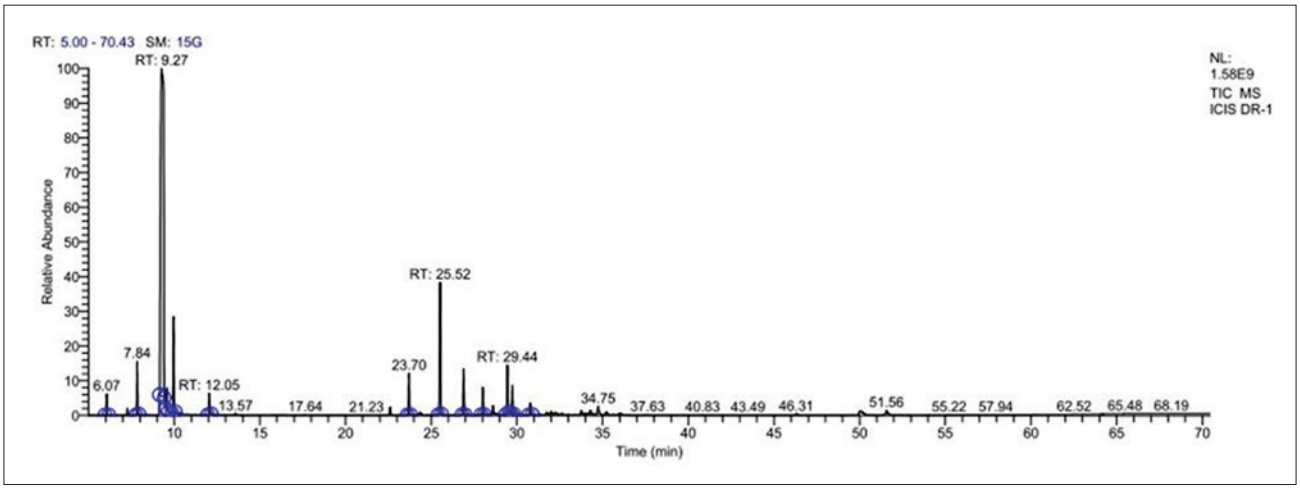

Figure 1: Gas chromatography-mass spectrometry chromatogram of oil from *Aegle marmelos*

Table 1: Chemical composition of oil from Aegle marmelos

Compound	Molecular formula	Molecular weight	RT	Peak area	Area %	Peak height
α-Pinene	$C_{10}H_{16}$	136	6.07	258132699.31	0.87	96638444.74
α-Myrcene	$C_{10}H_{16}$	136	7.84	766949680.14	2.60	244467047.78
D-Limonene	$C_{10}H_{16}$	136	9.27	20027300263.24	67.83	1489696408.12
3-Carene	$C_{10}H_{16}$	136	9.58	295757737.68	1.00	97389522.34
α-Ocimene	$C_{10}H_{16}$	136	9.96	1429354935.57	4.84	436873860.69
Linalyl acetate	$C_{12}H_{20}O_2$	196	12.05	346055482.78	1.17	96365251.39
α-Copaene	$C_{15}H_{24}$	204	23.70	748429615.74	2.53	188830738.92
Caryophyllene	$C_{15}H_{24}$	204	25.52	2586668284.68	8.76	601078249.72
Humulene	$C_{15}H_{24}$	204	26.90	838253972.01	2.84	208760740.82
α-Cubebene	$C_{15}H_{24}$	204	28.01	502441420.58	1.70	124337651.42
Cubedol	$C_{15}H_{26}O$	222	29.44	972381746.95	3.29	220010051.46
Isoledene	$C_{15}H_{24}$	204	29.73	525530066.76	1.78	130309141.61
β-Bisabolol	$C_{15}H_{26}O$	222	30.78	230093873.05	0.78	52379882.30

Table 2: Tabular comparison of major components of volatile oil of the current study with the previous studies

Current study (%)	Pino *et al*.[31]	Satyal *et al*.[33]	Verma *et al*.[34]	Bhandari *et al*.[35]	Karawya *et al*.[36]	Raju *et al*.[38]
Limonene (67.83)	δ-cadinene (12.1%)	Limonene (64.1%)	α-phellandrene (35.8-49.8%)	α-phellandrene (20%)	α-phellandrene (27.5%)	α-phellandrene (39.2%)
Caryophyllene (8.76)	β-caryophyllene (10.0%)	(E)-β-ocimene (9.7%)	Limonene (24.7-34.3%)	Limonene (10%)	β-phellandrene (38%)	Limonene (26.8%)
		Germacrene B (4.7%)	(E)-β-ocimene (4.2-10.0%)	p-cymene (11.5%),	α-pinene (7.7%)	β-phellandrene (16.2%)
	α-pinene (0.1%)	(E)-caryophyllene (2.4%)	α-pinsene (6.0-8.0%)	Citronellal (10.0%)		α-pinene (6.6%)
			β-caryophyllene (7.5%)			

The volatile oil is characterized by higher percentages of monoterpenes which are in accord with previous reports from *A. marmelos* grown in various geographical locations across India.[35-38] The high content of monoterpenes in the leaves oil was further supported by study of Raju *et al*.[38] However, the concentration of α-pinene (6.6%) was quite different as compared to our current study, in which the percentage of α-pinene was 0.87% comparative data is presented in Table 2.

Further, the results obtained for this study were different from the leaves oil isolated from Cuba sources, which constituted mainly of sesquiterpenes;[31] however, our study showed the presence of monoterpenoids.

In addition to that, our study showed slightly higher concentration of limonene (67.8%) in comparis onto the essential oil isolated from Nepal sources which contain limonene (64.1%).[33] That proves the great influence of the climate conditions on the chemical composition of the essential oils, even if we consider the same species which was harvested from the same place, but in different consecutive years. It is possible that, during the vegetative stage, depending on the temperature, different metabolic reactions might take place.

The qualitative and quantitative variations between our results and the previous reports for the constituents of the leaves oil may be attributed to the difference in geographical location, climatic conditions and time of harvest. Which comes in accordance with Verma *et al*.[34] who reported that the growing location and time of harvest (season) had a close relation to the yield and quality of *A. marmelos* essential oil.

CONCLUSION

The results obtained revealed that the limonene content is higher in the leaves collected from the foothills of Shivalik region in comparison to the previously reported data. Furthermore, this study also demonstrated that the percentage and amount of volatile components vary from region to region within a country apart from chemotypic and seasonal

variations which were shown by previously reported studies. Therefore, this paper provides an additional region selection data to be taken into account while harvesting *A. marmelos* leaves from different geographical locations.

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