Research Article

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ANALYSIS OF ESSENTIAL OIL CONSTITUENTS OF THREE *ANNONA* SPECIES GROWING IN KERALA, INDIA

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ABSTRACT

The volatile chemical composition of leaf essential oils of three *Annona* species (*Annona cherimola, Annona muricata* and *Annona squamosa*) from the different regions of Kerala, South Indian was determined using gas chromatography-mass spectrometry analysis. A total of 41 constituents belonging to monoterpenoids, sesquiterpenoids and diterpenoids were identified. Monoterpenes and sesquiterpenoids were the major class of volatile compounds in most of the *Annona* species examined. The study led to the identification of major compounds as germacrene D (23.5%), bicyclogermacrene (14.6%) and β-caryophyllene (11.7%) in *A. cherimola*, α-pinene (13.3%), β-caryophyllene (11.2%) and β-pinene (10.1%) in *A. muricata*, and β-caryophyllene (11.9%) and α-pinene (8.2%) in *A. squamosa* respectively. The examined essential oils showed that β-caryophyllene is the common constituent identified in all the three species and other constituents are more specific for each species under study. Present investigation reports, a comparison of essential oil compositions of three *Annona* species from Kerala. Results of this study prove that essential oils examined have considerable dissimilarity in chemical composition with previously reported leaf essential oil compositions from other regions. A chemotaxonomic analysis of these essential oils based on the distribution of compounds has revealed an efficient method to differentiate *Annona* species unambiguously.

Keywords: *Annona* species, Essential oil, Volatile compounds, Chemotaxonomic analysis.

INTRODUCTION

Genus *Annona* represents a rich source of secondary metabolites and its essential oils having great therapeutic importance. *Annona* is a flowering plant comprising of around 166 species of mostly neotropical trees and shrubs. The plants belonging to the genus *Annona* are also rich in phenolic compounds and have immense therapeutic potentials. The different parts of the plant including leaf, bark, stem, and root are used in traditional medicine to treat various disorders like diabetics, hypercholesterolemia, hypertension, and cancer. *Annona* species especially *Annona cherimola, Annona glabra, Annona muricata, Annona reticulata* and *Annona squamosa* are extensively used in traditional medicines against several human ailments and diseases especially cancer and parasitic infections[1]. It is also considered a good source of natural antioxidants for various diseases[2]. The fruits of these plants are used as a natural medicine for arthritis, diarrhoea, dysentery, fever, malaria, parasites, rheumatism, skin rashes and worms. *Annona* fruits are rich in the phenolic compound and other bioactive phytoconstituents. Leaves of *Annona* plants are utilised to treat diabetes, headaches, and insomnia. The seeds of these plants are believed to possess anthelmintic activities against external and internal worms and parasites[3]. Antiproliferative activity of *Annona reticulata* roots on human cancer cell lines was also reported in literature[4]. A review showed the chemical composition of essential oils of various plant parts taken from numerous species belonging to different Annonaceae, the main compounds are usually monoterpene hydrocarbons in fruit and seed oils, sesquiterpene hydrocarbons in leaf oils, and oxygenated sesquiterpenes in bark and root oils[5]. Reports showed that most of the constituents identified are common, such as α-pinene, β-pinene, spathulenol, β-elemene, β-caryophyllene and caryophyllene oxide. Other components seemed to be more specific to each *Annona* species.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Annona* species from various strategic locations across Kerala state in South India were collected. Voucher specimens were kept in the herbarium of the Department of Botany, Mar Ivanios College, Thiruvananthapuram, India.

Isolation of essential oils

Fresh leaves (250 g each) of the *Annona* species (*A. cherimola, A. muricata* and *A. squamosa*) were hydro - distilled using a Clevenger-type apparatus for 4 h. The essential oils collected were dried over anhydrous Na_2SO_4 and kept at 4 ^{0}C until analysed.

GC/MS analysis

The GC/MS analysis were performed on a Hewlett Packard 6890 Gas Chromatograph (Hewlett-Packard, USA) fitted with an HP-5 (Phenyl-dimethyl polysiloxane (5:95), 30 m x 0.32 mm, i.d., 0.25-µm film thickness) capillary column, coupled with a mass detector (Model 5973). GC-MS operation conditions: Injector temperature, 220 ^{0}C; transfer line, 240 ^{0}C; oven temperature Programme, 60-250 ^{0}C (3 ^{o}C/min); carrier gas, He at 1.4 mL/min. Mass spectra: Electron Impact (EI^+) mode, 70 eV with a mass rangeof 40 to 450 m/z; ion source temperature, 240 ^{0}C.

Identification of components

The essential oil components were identified by comparison of their retention indices (*RI*s) on an HP-5 column calculated using standard series of C_8-C_{30} hydrocarbons (Aldrich Chemical

Company, USA), by Wiley 275. L and NIST 11 database matching and by literature comparison[6].

RESULTS AND DISCUSSION

Essential oil analysis through GC-MS led to the identification of 41 compounds comprising 80.5 to 92.8% of total essential oil compositions (Table 1). Sesquiterpenes and sesquiterpenoids were the major constituents in all three species viz. *A. cherimola, A. muricata* and *A. squamosa*. In *A. muricata* mono- and sesquiterpenoids were almost equally distributed. Whereas in *A. cherimola* and *A. squamosa*, sesquiterpene hydrocarbons dominated over sesquiterpenoids. The presence of aliphatic hydrocarbons was negligible in all the *Annona* species studied.

Major compounds identified in *A. cherimola* were germacrene D (23.5%), bicyclogermacrene (14.6%) and β-caryophyllene (11.7%) respectively while in *A. muricata*, α-pinene (13.3%), β-caryophyllene (11.2%) and β-pinene (10.1%) were the major compounds. But in *A. squamosa*, β-caryophyllene (11.9%) and α-pinene (8.2%) were the major constituents obtained.

The present study reports for the first time, a comprehensive account of the components of volatile oil from three Annona species collected from Kerala, India.

Table 1: Essential oil compositions of three *Annona* species growing in Kerala

S. No.	Compounds	RI_{Lit}	RI_{Cal}	Relative contents (%)		
				*AC	*AM	*AS
1	α-pinene	932	944	1.3	13.3	8.2
2	β-pinene	974	974	5.2	11.6	6.5
3	myrcene	988	984	0.3	1.2	-
4	limonene	1024	1019	-	0.9	-
5	E-β-ocimene	1044	1038	0.3	1.6	1.2
6	bicycloelemene	1324	1323	1.1	-	-
7	δ-elemene	1338	1327	2.1	-	-
8	α-cubebene	1348	1338	0.8	-	-
9	α-copaene	1374	1365	0.8	-	1.2
10	β-bourbonene	1387	1373	-	-	1.8
11	β-elemene	1390	1382	4.2	3.6	1.8
12	Z-caryophyllene	1408	1393	-	-	3.1
13	β-caryophyllene	1417	1407	11.7	11.2	11.9
14	aromadendrene	1439	1425	0.5	-	2.1
15	α-humulene	1454	1443	2.9	1.4	2.7
16	γ-muurolene	1479	1465	2.1	-	2.5
17	germacrene D	1486	1469	23.5	-	-
18	β-selinene	1490	1476	-	1.1	1.7
19	viridiflorene	1496	1479	2.2	-	2.3
20	α-selinene	1498	1483	-	1.1	1.8
21	bicyclogermacrene	1500	1482	14.6	-	-
22	α-muurolene	1500	1488	-	2.2	0.9
23	δ-amorphene	1512	1508	-	2.5	4.9
24	γ-cadinene	1513	1502	1.0	-	3.2
25	1,10-diepicubenol	1519	1615	-	0.8	-
26	Trans cadina,1-4-dien	1534	1521	-	-	1.8
27	elemol	1548	1543	1.9	-	1.0
28	spathulenol	1578	1570	3.4	1.3	1.2
29	caryophyllene oxide	1583	1568	0.9	6.8	1.5
30	globulol	1590	1576	-	-	1.7
31	isospathulenol	1632	1622	-	-	1.0
32	caryophylla-4(12)8(13)-dien-5-ol	1640	1624	-	1.0	-
33	epi-α-cadinol	1640	1631	-	3.3	7.3
34	epi-α-muurolol	1642	1633	-	2.6	-
35	α-muurolol	1646	1637	-	1.0	-
36	β-eudesmol	1649	1643	1.1	-	-
37	α-cadinol	1652	1644	1.2	7.3	3.6
38	hexadecanoic acid	1960	1963	-	1.7	-
39	(E-E) geranyl linalool	2027	2017	-	-	3.6
40	E-phytol	2105	2104	-	4.6	-
41	octadecanoic acid	2161	2154	-	1.2	-
Monoterpene hydrocarbons				7.1	27.1	15.9
Oxygenated monoterpenes				-	-	-
Sesquiterpene hydrocarbons				77.2	24.0	43.7
Oxygenated sesquiterpenes				8.5	25.2	17.3
Diterpenoids				-	4.6	3.6
Aliphatic compounds				-	2.9	-
Total identified (%)				**92.8**	**83.8**	**80.5**

*[AC: *A. cherimola*, AM: *A. muricata*, AS: *A. squamosa*]

The chemical compositions of leaf essential oil of *A. squamosa* reported in 2005 showed that essential oil produced by hydro-distillation of plant leaves reveals eighteen compounds which account for 86% of the oil[7]. The leaf oil of *A. squamosa* was made up of monoterpene hydrocarbons (2.5%), sesquiterpene hydrocarbons (76.0%) and oxygenated sesquiterpenes (7.1%). β-Caryophyllene (23.0%), germacrene D (21.3%), bicyclogermacrene (8.5%) and β-elemene (7.8%) as the major constituents. Another study reported from South India has GC/MS analysis showing that β-cedrene (23%) and β-caryophyllene (14%) were the major compounds of the leaf oil of *A. squamosa*[8]. The leaf essential oil of the plant has demonstrated potent antimalarial activities[9,10].

GC/MS analysis of the leaf, peel, and fruit pulp oils of *A. muricata* L. showed the presence of 68 compounds of which 59 were identified. The main components of the leaf oil, in this case, were β-caryophyllene (31.4%) and other sesquiterpenes, while the fruit oil contained essentially aliphatic acids and esters, in particular methyl, (E)-2-hexenoate (39.8%)[11]. Another study reports the essential oil components of leaves of *A. muricata* L. from Benin obtained by steam distillation with a very low percentage. The analysis made by GC and GC/MS showed the presence of 80 compounds and the most abundant constituents were β-caryophyllene (13.6%), δ-cadinene (9.1%), epi-α-cadinol (8.4%), α-cadinol (8.3%)[12,13].

The volatile compounds identified from four species of *Annona* from Vietnam were reported in 2013. The oils were obtained from plant samples by steam distillation and subjected to GC and GC-MS analysis. The main compounds of *Annona glabra* L. were β-caryophyllene (21.5%) germacrene D (17.7%), α-cadinol (5.4%) and β-elemene (5.2%). *Annona squamosa* L., comprised mainly of α-pinene (1.0-11.9%), limonene (0.8-11.7%), β-cubebene (0.5-13.0%), β-caryophyllene (11.6-24.5%), spathulenol (0.8-9.0%), caryophyllene oxide (1.0-10.6%) and α-cadinol (3.3-7.8%). The significant constituents of *Annona muricata* L., were α-pinene (9.4%), β-pinene (20.6%), ρ-mentha-2, 4(8)-diene (9.8%), β-elemene (9.1%) and germacrene D (18.1%). However, camphene (0.2-6.6%), α-copaene (2.0-7.3%), β-elemene (5.9-16.6%), β-caryophyllene (8.3-14.9%), β-bisabolene (0.4-10.2%), δ-cadinene (1.7-4.8%) and germacrene D (9.3-22.8%) were the main compounds common to samples of *A. reticulata* L. There were significant amounts of sabinene (11.2% and 2.7%; leaf and stem bark) and bicycloelemene (9.6% and 6.1%; stem and bark) were also found[14].

Major constituents of the *A. squamosa* leaf essential oil from Brazil were identified as (E)-caryophyllene (27.4%), germacrene D (17.1%), bicyclogermacrene (10.8%), (Z)-caryophyllene (7.3%), β-elemene (6.2%) and α-humulene (5.7%)[7]. The volatile constituents of essential oil obtained by steam distillation of *A. squamosa* L. bark from Maharashtra and India were identified. Six major components were 1H-Cycloprop(e)azulene (3.46%), germacrene D (11.44%), bisabolene (4.48%), caryophyllene oxide (29.38%), bisabolene epoxide (3.64%) and kaur-16-ene (19.13%). The oil was also screened for its antimicrobial activity, which exhibited significant antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus*[15]. Volatile components of soursop from Cuba were isolated by simultaneous steam distillation/solvent extraction and GC/MS analysis. Forty-one compounds were identified in this study, methyl 3-phenyl-2-propenoate, hexade-canoic acid, methyl (E)-2-hexenoate and methyl 2-hydroxy-4-methyl valerate were the major compounds[16,17].

CONCLUSION

In the present study, the wide array of volatile chemical structures in *Annona* species and their distribution pattern were utilized for their differentiation. The present investigation reports for the first-time essential oil compositions of three *Annona* species from Kerala. The examined oil showed considerable dissimilarity in chemical composition with previously reported leaf essential oil compositions from other regions. Chemotaxonomy based on the distribution of compounds with distinct carbon skeletons can be used as an efficient supporting tool in differentiating *Annona* species unambiguously. The study also aimed to scientifically validate essential oil components of three major species of *Annona* which leads to an enormous scope for future investigations into the therapeutic potential of the plants as a promising drug candidate for treating various human ailments.

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