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Comparative GC-MS Profile and Antioxidant Activity of *Annona Glabra* and *Annona Reticulata* Leaf Essential Oil from South India

Sonia Mol Joseph[1], Amala Dev A. R[2]
[1,2]PG & Research Department of Chemistry, Mar Ivanios College (Autonomous), Thiruvananthapuram, Kerala

***Corresponding Author: Sonia Mol Joseph**

ABSTRACT: This study reports the comparison of chemical composition and antioxidant activity of leaf essential oil from two *Annona* species - *Annona glabra* and *Annona reticulata* by GC-MS method. Out of the forty-one compounds identified from essential oil analysis comprising 80.5% to 96.1% from *A. reticulata* and *A. glabra*. In *A. glabra* leaf oil, 27 compounds were identified whereas in *A. reticulata* leaf oil, 30 compounds were identified. Essential oil of *A. glabra* was dominated by diterpenoids whereas that of *A. reticulata* by sesquiterpenoids. E-nerolidol (26.9%) and spathulenol (16.7%) were the major constituents of *A. glabra*. But in *A. reticulata*, β-elemene (17.8%) and E-phytol (15.3%) were identified at higher concentrations. Diterpenoids (76.3%) and sesquiterpene hydrocarbons (43.7%) were the major class of compounds in *A. glabra* and *A. reticulata* respectively. Elemol (8.7%), β-Pinene (7.4%), α-Pinene (6.9%) and β-Elemene (5.3%) were some of the main compositions of *A. glabra* leaves whereas β-Caryophyllene (7.9%), α-Pinene (7.7%), Spathulenol (5.5%) and β-Pinene (5.1%) were the major constituents in *A. reticulata* leaves. Antioxidant activity was not detected by the DPPH assay for both the oils evaluated. Results of the GC-MS analysis supported the fact that these essential oils may become useful in the research of new therapeutic agents of promising clinical use.

KEYWORDS: Essential oil, *Annona* species, Diterpenoids, Sesquiterpenoids, Therapeutic agents

INTRODUCTION

Plants belonging to genus *Annona* represent a number of species which are abundant in secondary metabolites and essential oils of high medicinal value in healing and remedial therapies. *Annona* is a flowering plant comprising approximately 166 species of mostly neotropical and afrotropical trees and shrubs. Among a wide variety of aromatic plants which are known for their therapeutic applications, the plants included in genus *Annona* have abundant phenolic compounds and have various applications with immense nutraceutical and therapeutic potentials. All *Annona* species especially *Annona sqamosa, Annona glabra, Annona reticulate, Annona muricata,* and *Annona cherimola* are widely used in conventional medicines for treatment of human illness and diseases, specifically for cancer and parasitic ailments. The different parts of the tree including leaf, bark and root are used in traditional medicine to treat conditions such as diabetics, hypercholesterolemia, hypertension, gastrointestinal diseases etc. *Annona* species is farmed in various parts of India mainly for its fruit which is edible. The parts of these plants form a crucial ingredient in various traditional medicines popular in tropical regions. It is appraised as a source of natural antioxidants for various diseases[1]. The fruit of these plants have wide spread applications in preparation of various natural medicines for treatment of diseases like diarrhea, arthritis, fever, dysentery, malaria, skin rashes, worms and rheumatism. It is also popular as a supplement to stimulate production of mother's milk after giving birth to a child. It is reported to have anthelmintic properties too[2]. Predominant components identified in Annonacea family are Monoterpene hydrocarbons found in fruit and seed oils, oxygenated sesquiterpenes diterpenoids which are constituents of bark and root oils and sesquiterpene hydrocarbons which are found in leaf oils. Major constituents found such as limonene, α-pinene, β-caryophyllene, β-pinene, p-cymene and caryophyllene oxide are generally found in all *Annona* genus[3].

Annona reticulata L. popurlarly called as bullock's heart world-wide is a fast-growing, deciduous tree of high medicinal importance. Various phytoconstituents like tannins, glycosides, phenolics, acetogenins, alkaloids, carbohydrates, proteins, flavonoids, alkaloids, carbohydrates and proteins were enriched in *A. reticulata.* It also showed wide pharmacological activities such as analgesic, anthelmintic, wound healing anti-inflammatory, antipyretic, and cytotoxic effects[4]. Antiproliferative property of roots of *A. reticulata* on human cancer cell lines were also reported[5]. *A. reticulata* leaf extract showed cytotoxicity which is dose dependent in HT-29 Cell lines[6]. A new triterpenoid annonaretin A were isolated from *A. reticulata* leaf extract from Vietnam exhibited potent nitric oxide inhibition activity[7]. *A. glabra* also known as pond-apple or swamp-apple is a tropical lowland species found in freshwater or brackish water which was primarily considered as an environmental weed growing wild in countries like America and Asia. Earlier reports on *Annona* plants showed the presence of various phytochemical constituents such as terpenoids, saponins, tannins, anthraquinones, flavonoids, glycosides, steroids and acidic compounds. Pharmaceutical potential of various parts of *A. glabra* such as leaves, bark, fruits and seeds were utilized in traditional medicine for curing different diseases including cancer[8]. GC-MS analysis of *A. glabra* leaves grown in Egypt showed β-gurjunene (42.49%) as the major constituent[9]. And ent-kauran-19-al-17-oic acid and Cunabic acid isolated from *A. glabra* Linn supress the rapid reproduction and multiplication of human liver cancer (HLC) cell line SMMC-7721[10]. Annoglacins A and B, isolates derived from the fractionated ethanolic extracts of *A. glabra* leaves showed potent cytotoxic activity in human cancer cell lines[11]. Hexane extracted from stem bark of *A. glabra* showed antimicrobial, antifungal, insecticidal, sporicidal and cytotoxic activities[12]. The studies reported on four *Annona* species from Vietnam in 2013 revealed volatile compounds which showed that the leaf essential oil of *A. glabra* constituting α-cadinol (5.4%) and β-elemene (5.2%), β-caryophyllene (21.5%) germacrene D (17.7%) as significant compounds. However, β-elemene (5.9-16.6%), β-caryophyllene (8.3-14.9%), camphene (0.2-6.6%), α-copaene (2.0-7.3%), δ-cadinene (1.7-4.8%) and germacrene D (9.3-22.8%), β-bisabolene (0.4-10.2%), were significant constituents present in *A. reticulata* leaves. Considerable amounts of bicycloelemene (9.6% in steam and 6.1% in bark) and sabinene (11.2% in leaf and 2.7% in stem bark) were also identified[13]. Capillary GC and GC/MS analysis on hydrodistilled oil extracted from *A. reticulata* leaves found in Nigeria showed thirty nine components consisting mainly of 20 sesquiterpenes (52.9%), 18 monoterpenes (29.0%) and one aromatic ester (10.9%). (E,E)-farnesyl acetate (19.0%), ar-turmerone (12.0%), benzyl benzoate (10.9%) and γ-terpinene (7.4%) were the major constitutents reported[14].

Since antioxidant activity of essential oils is very pronounced, it hardly be attributed by the components alone but due to the complex chemical nature of natural products with different functionalities. The magnitude of antioxidant properties exhibited by various essential oils may responsible for the interaction of all the constituents present in the essential oil produces a synergistic effect. Since the antioxidant activity of *A.glabra* and *A.reticulata* as yet unexplored the main objective of this study was to evaluate the chemical composition and antioxidant activities of these essential oils.

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 Department of Botany, Mar Ivanios College, Thiruvananthapuram.

Isolation of essential oils: Fresh leaves (250 g each) of the *Annona* species (*A. glabra* and *A. reticulata*) were hydrodistilled using a Clevenger-type apparatus for 4 h. The essential oils collected were dried over anhydrous Na_2SO_4 and kept at 4^0C until analysed.

Antioxidant activity: Methanolic solution of DPPH was prepared at a concentration of 40 µg mL^{-1}. For evaluating the free radical scavenging assay, 2.7 mL of the stock DPPH solution were added in a test tube, followed by the addition of 0.3 mL each of diluted essential oils in methanol (300, 250, 200, 150, 100, 50 g mL^{-1}). In parallel, the control was prepared by adding all other reagents except the essential oil. After 60 min, readings were taken using a spectrophotometer at a wavelength of 510 nm[15]. Ascorbic acid was used as the standard for the antioxidant assay and the results were calculated as percentage inhibition of DPPH radical scavenging activity.

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^{o}C; transfer line, 240^{o}C; oven temperature programme, 60-250^{o}C (3^{o}C/min); carrier gas, He at 1.4 mL/min. Mass spectra: Electron Impact (EI^+) mode, 70 eV with a mass range of 40 to 450 m/z; ion source temperature, 240^{o}C.

Identification of components: The essential oil components were identified by comparison of their retention indices (*RIs*) on a 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[16].

RESULT AND DISCUSSION

The present study reports, a comparative phytochemical analysis of the volatile components of two *Annona* species collected from different geographical areas of Kerala. The distribution pattern of their components present in the essential oil of these two *Annona* species were analysed for their differentiation. Essential oils analysed through GC-MS resulted in recognising 41 compounds comprising 80.5 to 96.1% of the entire essential oil configuration (Table 1). Sesquiterpenoids were the significant components in *A. reticulata* while diterpenoids were in *A. glabra*. To a great extend in *A. glabra* both mono- and sesquiterpenoids were evenly distributed. Presence of aliphatic hydrocarbons was negligible in two *Annona* species studied. E-nerolidol (26.9%) and spathulenol (16.7%) respectively were the major constituents of *A. glabra*. But in *A. reticulata* β-elemene (17.8%) and E-phytol (15.3%) were found to be the major constituents.

Nerolidol (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol) is a sesquiterpene alcohol availiable in several plants that has widespread applications in both cosmetic and non-cosmetic industries. It is approved as a food flavouring agent by Food and Drug Administration, USA. Chemically nerodinol exists in two geometrical forms and its pharmacological studies have validated it as a significant drug constituent having wide spread applications in the areas of agriculture and medicine[17]. β-Elemene is reported to have antitumor and anti-inflammatory properties. It is an elemene sesquiterpene compound which provokes powerful apoptosis in a bewildering array of various human cancer cells. β-elemene which is a monomeric active component derived from *Curcuma wenyujin,* a conventional Chinese medicine, has clinically proven antitumor activities and is in use for last 20 years in China[18]. Therefore an essential oil containing such an effective ingredient is of great importance in tumor therapy. Another major compound found is spathulenol, which is a volatile sesquiterpenoid commonly used as an anaesthetic and vasodilator agent. Similarly E-phytol is a diterpene alcohol commonly found in aromatic plants.

The present study presents a novel report on essential oil constitutions and antioxidant activity of two *Annona* species, of which, *A. glabra* is endemic to the Coastal areas of Kerala. The essential oil analysed showed remarkable variations in chemical composition with formerly examined leaf essential oil compositions from different regions. *In-vitro* antioxidant activity was not evidenced for both the oils evaluated by DPPH assay. Despite the essential oils tested in this study are not showing significant antioxidant activity, many essential oil have shown antioxidant potential. Chemotaxonomy considering the allocation of compounds with definite carbon skeletons has provided a scientific framework in demarcation of various *Annona* species without any paradox. The study is also aimed to scientifically validate essential oil components of two different species of *Annona* which has an enormous scope for future investigations into the phytochemistry and phytopharmcology aspects of the plant species.

CONCLUSION

In this study, phytochemical profiles of leaf essential oils of *A. glabra* and *A. reticulata* were compared. Sesquiterpenoids, E-nerolidol and spathulenol were found to be the major constituents of *A. glabra* leaves. But in *A. reticulata* leaf oil both sesquitepenes (β-elemene) and diterpenoid (E-phytol) were detected as major compounds. Out of 41 compounds identified only 14 compounds were found to be common to both plant species under investigation. That clearly indicates noticeable variability of leaf essential oil compositions of both the species. The result of the present study highlights essential oil constituents of *Annona* species which are rich in sesquiterpenes and diterpenoids. Further studies are in progress for scientific validation of these essential oils.

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Table 1:

Sl No.	Compounds	RI $_{lit}$	RI $_{cal}$	Relative contents (%)	
				A. glabra	*A. reticulata*
1	□-pinene	932	944	6.9	7.7
2	□-pinene	974	974	7.4	5.1
3	myrcene	988	984	0.7	-
4	limonene	1024	1019	0.7	0.6
5	E-□-ocimene	1044	1038	2.1	0.7
6	linalool	1096	1100	2.0	-
7	bicycloelemene	1324	1323	-	1.0
8	□-cubebene	1348	1338	0.6	-
9	□-copaene	1374	1365	1.1	3.0
10	□-bourbonene	1387	1373	-	2.5
11	□-cubebene	1387	1378	1.3	0.8
12	□-elemene	1390	1382	5.3	17.8
13	□-caryophyllene	1417	1407	1.3	7.9
14	□-copaene	1432	1417	-	1.5
15	□-humulene	1454	1443	0.4	1.2
16	□-muurolene	1479	1465	-	1.3

17	germacrene D	1486	1469	-	4.8
18	□-selinene	1490	1476	1.4	-
19	□-selinene	1498	1483	1.0	-
20	bicyclogermacrene	1500	1482	-	1.4
21	□-muurolene	1500	1488	-	2.9
22	□-amorphene	1512	1508	0.5	0.6
23	1,10-diepicubenol	1519	1615	-	0.6
24	trans calamenene	1522	1510	0.4	-
25	elemol	1548	1543	8.7	-
26	E- nerolidol	1563	1563	26.9	0.6
27	spathulenol	1578	1570	16.7	5.5
28	caryophyllene oxide	1583	1568	-	2.4
29	globulol	1590	1576	0.6	0.6
30	cis-dihydromayurone	1595	1599	-	0.6
31	isospathulenol	1632	1622	1.7	0.7
32	epi- □-muurolol	1642	1633	-	1.6
33	□-muurolol	1646	1637	0.4	2.6
34	□-eudesmol	1649	1643	1.5	-
35	□-cadinol	1652	1644	-	2.9
36	selin-11-en-4-□-ol	1659	1647	3.0	-
37	Intermedol	1666	1660	1.4	-
38	Z-nerolidyl acetate	1677	1664	1.4	-
39	8-□-11-elemodiol	1747	1712	0.7	0.6
40	5E,9E-farnesyl acetone	1913	1902	-	0.7
41	E-phytol	2105	2104	-	15.3
Monoterpene hydrocarbons				17.8	15.9
Oxygenated monoterpenes				2.0	-
Sesquiterpene hydrocarbons				19.8	43.7
Oxygenated sesquiterpenes				13.3	17.3
Diterpenoids				76.3	3.6
Total identified (%)				**96.1**	**80.5**