Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Comparison of the volatile oil composition of three *Atalantia* species

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Publication Info

Paper received:
29 October 2011

Revised received:
03 April 2012

Re-revised received:
06 July 2012

Accepted:
08 August 2012

Abstract

The members of the genus *Atalantia* belonging to the family Rutaceae have many uses in traditional medicine. The aim of the present study was to investigate and compare the chemical composition of essential oils of three species of *Atalantia* namely *Atalantia monophylla* (Roxb.) DC., *Atalantia racemosa* Wight. and *Atalantia wightii* Tanaka. The extract percentage of the obtained essential oil was found to be 0.2, 0.17 and 0.31% in *A. monophylla*, *A. racemosa* and *A. wightii* respectively. The major compounds identified were α-Asarone (28.82%), Sabinene (13.19%), Eugenol methyl ether (12.71%), 1,2-Dimethoxy-4-(2-methoxyethenyl)benzene (11.63%) and β-Pinene (5.3%) in the essential oil of *A. monophylla*. In *A. racemosa,* T-Cadinol (11.08%), Caryophyllene oxide (9.78%), β-Caryophyllene (9.20%), Spathulenol (7.21%), β-Phellandrene (5.67%) and Decanal (4.01%) and in *A. wightii* β-Caryophyllene (16.37%), D-Limnonene (12.15%), Decanal (10.49%), β-Myrcene (7.67%), Tetradecanal (6.99%), Caryophyllene oxide (6.29%) and Hexadecylene oxide (5.87%) were the main constituents. Sesquiterpenes were the major class of compounds in *A. racemosa* and *A. wightii*, while in *A. monophylla* the essential oil was predominated by ether compounds. The results showed that GC/MS analysis of essential oils is a significant step in the bio-chemical profiling and bio-prospecting of *Atalantia* species.

Key words

Atalantia, Rutaceae, Essential oil, Sesquiterpenes

Introduction

The several closely related species of the genus *Atalantia* (Rutaceae) are found in India and other south east Asian countries. Morphology of the genus *Atalantia* resemble citrus in general aspects which are small to medium trees, spinous, bearing white fragrant flowers and small globose fruits that look like diminutive greenish-yellow limes or oranges (Ranade *et al.*, 2009). In South India, the genus is represented by three species namely *Atalantia monophylla* (Roxb.) DC., *Atalantia racemosa* Wight. and *Atalantia wightii* Tanaka. Both *A. monophylla* and *A. racemosa* are widely distributed in south India, while *A. wightii* is endemic to Shola forests of Western Ghats.

A. monophylla is commonly called as wild lime. Various parts of *A. monophylla* have been used in folk medicine for several purposes such as the treatment of rheumatoid pain and glandular swelling (Sankaranarayan *et al.*, 2010). An earlier work done by Manimaran *et al.* (2002) on essential oils of *A. monophylla* collected from Narrtha hills, Pudukkottai revealed methyl eugenol (36.46%), sabinene (24.89%) and elemicin (24.61%) as the major compounds. The root is believed to be antispasmodic (Kiritikar and Basu, 1999), whereas a decoction of the leaves is often applied for itching and other skin complaints (Panda, 2004). Hexane, chloroform and ethyl acetate extracts of *A. monophylla* shows antifeedant, larvicidal and pupicidal activities against *Helicoverpa armigera* (Baskar *et al.*, 2009). The chloroform extract of leaves has shown antiviral activity (Chansakaow *et al.*, 1996). Acridone alkaloids isolated from roots were reported to have anti-allergic activity (Chukaew *et al.*, 2008). Coumarins such as auraptene and 7-O-

geranylscopoletin were also isolated from the roots of *A. monophylla* (Chukaew *et al.*, 2008). The root bark contains tetratriterpenoid, atalantin, stigmasterol, xanthyletin, marmesin, sitosterol and acridone alkaloids. The leaves contain friedelin and epifriedlanol. Fruits contain alkaloid serverine. Besides its medicinal value *A. monophylla* leaves are used as flavoring agent, berries are used for making pickles, juice of berries are used for dyeing purpose and wood as timber. It is also useful as a rootstock for breeding new cultivars of Citrus Linn. (Guha Bakshi *et al.*, 1999). Kadars, Malasars and Muthuvan tribals of Anamalai hills use the whole plant of *A. wightii* to stupefy the fishes (Hosagoudar and Henry, 1996).

Phytochemical profiling has become a valuable tool for identifying the chemotypes, infra-specific races, validating the traditional uses and bio-prospecting of medicinal plants (Zheng *et al.*, 1992; Stephen and James, 1996; Guha Bakshi *et al.*, 1999). Earlier studies were focused on essential oils derived from the leaves of only *A. monophylla* through hydro distillation, but limited to only ten major compounds (Manimaran *et al.*, 2002). Also the volatile oil constituents of *A. racemosa* and *A. wightii* were not reported earlier. The present study reports the comparative account of volatile oil composition of three species of *Atalantia* namely *A. monophylla*, *A. racemosa* and *A. wightii*.

Materials and Methods

Isolation of essential oil: The essential oils were isolated from 200 g of fresh leaf sample collected from the different places of South India. *A. monophylla* leaves were collected from Nagamalai hills of Madurai district, Tamil Nadu. The leaves of *A. racemosa* and *A. wightii* were collected from Meghamalai hills, Theni, Tamil Nadu and the Shola forests near Vaguvarai estate, Munnar, Kerala respectively. The plants were collected during the month of March 2011. The herbarium of the collected specimens were prepared and identified at Botanical Survey of India, Coimbatore. The herbarium specimens were submitted at the herbarium of Department of Plant Science, Madurai Kamaraj University. The leaves were first washed with tap water and then dried under fan for 10 min. It was then weighed and chopped to small pieces, after that it was hydrodistilled in a Clevenger type apparatus for 3 hr in 200 ml water at 100ºC temperature. The essential oil was carefully collected in a screw cap bottle and dried over anhydrous sodium sulphate. Essential oil was stored at -20° C for further analysis.

GC/MS analysis of essential oil : A Shimadzu QP-2010 plus with thermal desorption system TD 20 was used to obtain the chromatograms. The name and specification of the column used is AB-Innowax (60 m X 0.25 mm X film thickness-0.25 m). The temperature was programmed from 50°C with 5 minute initial hold to 280°C at 4° C min^{-1} and a final hold for 5 min at 280°C. The injector and detector temperature were set at 220 and 240°C respectively and the split ratio was 1/60. Helium was used as the carrier gas and the ionizing voltage used was 70 eV. The components were identified based on the library search carried out using NIST and WILEY library.

Results and Discussion

The extract percentage of the obtained essential oil isolated from *A. monophylla* was found to be 0.2%. Twenty nine compounds were identified from the essential oil of *A. monophylla*. The samples were predominated by ethers which constitute about 54.96% of the compounds (Fig. 1). The monoterpenes constitute about 27.2%, the sesquiterpenes 13.17% and aldehydes 2.68%. The major compounds identified were α-Asarone (28.82%), Sabinene (13.19%), Eugenol methyl ether (12.71%), 1,2-Dimethoxy-4-(2-methoxyethenyl)benzene (11.63%) and β-Pinene (5.3%). Ten compounds were identified in an earlier work done by Manimaran *et al.* (2002) on essential oil from *A. monophylla* leaves collected from Narrtha hills, Pudukkottai and the major compounds identified in that study were Methyl eugenol (36.46%), Sabinene (24.89%) and Elemicin (24.61%). Even though similar compounds have been identified in the present study, there is significant variation in the compounds identified as well as in their concentration. The difference in chemical constitution may be probably due to different environmental and genetic factors, different chemotypes and the nutritional status of the plants as well as other factors that can influence the oil composition (Ozcan and Chalchat, 2002). The main compound present in the essential oil collected from Nagamalai hills was Asarone which has neuroprotective effect and Hypolipidemic activity etc (Limon *et al.*, 2009).

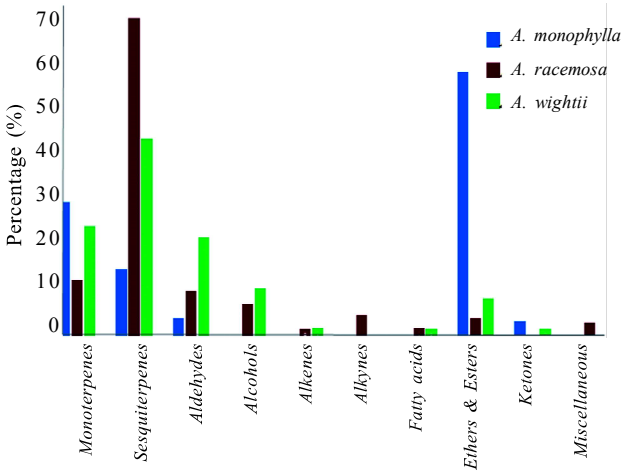

Fig. 1 : Comparison of various classes of compounds present in the three *Atalantia* species

A total of 65 compounds were identified from the essential oil of *A. racemosa*. The extract percentage of essential oil from this plant was found to be 0.17%. The predominant class of compounds were sesquiterpenes which constitute around 66.52%. Other major classes of compounds present in the essential oil were monoterpenes (10.78%), aldehydes (8.42%), alcohols (5.46%) and alkynes (3.23%) (Fig. 1). The major components identified were T-Cadinol (11.08%), Caryophyllene oxide (9.78%), β- Caryophyllene (9.20%), Spathulenol (7.21%), β-Phellandrene (5.67%) and Decanal (4.01%).

Whereas in *A. wighlii* the essential oil was predominated by sesquiterpenes (40.94%), followed by monoterpenes (22.25%), aldehyde (19.93%), alcohol (8.85%), etc (Fig. 1). The extract percentage of the essential oil obtained was highest in *A. wightii* compared to the other two and was found to be 0.31%. The chromatogram obtained was analysed and a total of 64 compounds were identified. The major compounds identified were β-Caryophyllene (16.37%), D-Limnonene (12.15%), Decanal (10.49%), β-Myrcene (7.67%), Tetradecanal (6.99%), Caryophyllene oxide (6.29%) and Hexadecylene oxide (5.87%).

Decanal, Bicyclogermacrene, Caryophyllene oxide, T-Cadinol, α-Cadinol, β-Myrcene, β-Phellandrene, α-Caryophyllene, β-Caryophyllene and β-Elemene were identified in all the three species. Decanal is an important compound of family Rutaceae and is used in fragrances and flavoring. Comparison of the compounds revealed the presence of sesquiterpenes as the predominant class of compounds in both *A. racemosa* and *A. wightii*, while in *A. monophylla* the essential oil was predominated by ether compounds. The dominant sesquiterpene present in all the three essential oils was β-Caryophyllene. The oils of all the three species contains considerable amount of monoterpenes represented by β-Myrcene and β- Phellandrene. The medicinal properties shown by the essential oils may be due to the combined effect of these bioactive compounds present. The results showed that GC/MS analysis of essential oils is a significant step in the bio-chemical profiling of *Atalantia* species. In plants containing essential oils, the chemical composition is more important than morphological characters from the taxonomic point of view (Grayer *et al.*, 1996). Further investigations based on chemical composition of plants collected from various geographical locations will become a valuable tool for identifying the various chemotypes, validating the traditional uses and bio-prospecting.

Acknowledgments

The authors thank UGC (MRP & CAS), DBT (IPLS) and DST (PURSE) for partial financial assistance through research grants. Authors also thank Mr. Ajai Kumar of Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi for extending help in GC/MS analysis. The first author thanks Madurai Kamaraj University for the award of University Stipendiary Fellowship.

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