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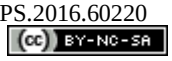
ISSN 2231-3354 

Antioxidant activity and determination of bioactive compounds by GC-MS in fruit methanol extracts -a comparative analysis of three *Atalantia* species from south India

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ABSTRACT

The members of genus *Atalantia* are widely used in Traditional, Folk and Ayurvedic systems of Medicine; of which three species are found in south India namely *A. monophylla*, *A. racemosa*, *A. wightii*. The fruits of these plants are picked and widely consumed by the tribal people. The present study aims to test the anti-oxidant potential of the methanol extracts of fruits and also to identify the bioactive compounds present in the extracts through GC/MS analysis. Among the three fruits tested for the antioxidant activity using the DPPH method, the crude methanolic extracts of *A. monophylla* shows higher potential. The IC_{50} value calculated was found to be 336.84 µg/ml for *A. monophylla*, 348.75 µg/ml for *A. racemosa* and 375.64 µg/ml for *A. wightii*. Ascorbic acid used as standard had an IC_{50} value of 103.03 µg/ml. Total antioxidant capacity was expressed as ascorbic acid equivalent and was calculated using the Phosphomolybdenum method. The methanol extract of *A. monophylla*, *A. racemosa* and *A. wightii* determined by phosphomolybdenum method exhibited significant antioxidant activity of 686mg/g, 592 mg/g and 655mg/g ascorbic acid equivalent respectively. Also the bioactive compounds in the Methanol extracts were evaluated using GC/MS.

INTRODUCTION

The genus *Atalantia* Correa (Family: Rutaceae) comprises of about 11 very closely related species (Swingle and Reece, 1967); of which three species are found in south India. *Atalantia monophylla* (Roxb.) DC. commonly called as wild lime is commonly seen in the dry evergreen forests of south India from the coastal regions to about 600m altitude. *Atalantia racemosa* Wight. (Bombay Atalantia) is seen in the evergreen forests of Peninsular India and Srilanka from about 150m to about 1000m. It is considered as a replacement taxon for *A. monophylla*. *Atalantia wightii* is endemic to shola forests of Western Ghats and is commonly referred to as Nilgiri Atalantia. It is distributed from 100m to about 1700m.

The members of the genus *Atalantia* are important plants in the understory layer of the forests. They are commonly found in the disturbed areas such as roadside, forest edges etc. and also along the sides of the streams and periphery of other water bodies where sufficient sunlight is available.

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It is a source of food for larvae of several pollinators and also a source of food for seed dispersal agents (Vanitharani *et al.*, 2011; Rajasekhar *et al.*, 2006; Storz and Kunz, 1999; Punekar, 2002). Also the leaves of *A. monophylla* are an important source of food for the Asiatic elephant (*Elephas maximus*) (Mohapatra *et al*., 2013). Fruit, leaves and roots of the members of genus *Atalantia* are used for medicinal purposes. Traditional medicinal practitioners use the leaves of *A. monophylla* for the treatment of rheumatoid pain and glandular swelling. A decoction of the leaves is often applied for itching and other skin complaints (Panda, 2004) and also in treatment of dysentery (Jain, 1991). The leaves and roots are used as blood purifier and roots are given in cough and dysentery. Berries yield warm oil, which is valuable application in chronic rheumatism, paralytic limb and as stimulant (Krithikar and Basu, 1993; Panda, 2004). Fruit juice is anti-bilous and the fruit juice is consumed to treat stomach disorder by Kani tribals (Khare, 2007; John de britto and Mahesh, 2007). The juice pressed out from fruits heals endemic Cachexia (pitao) (Jain, 1991). Root is antispasmodic (Panda, 2004), antiseptic and stimulant and is used in treatment of snake bite (Jain, 1991; Krithikar and Basu, 1993) and antispasmodic pills are made from root (Jain, 1991; Manilal, 2003).

In addition to its medicinal value various parts of *A. monophylla* is used for several other purposes. Juice of berries is used for dyeing purposes. Wood is a good source of timber. It is also useful as a rootstock for breeding new cultivars of *Citrus* Linn (Guhabhakshi *et al.,* 1999). Decoction of leaves of *A. racemosa* is used in the treatment of bronchitis, asthma and cough. The leaves are chewed to get relief from bronchitis. Leaf powder is used as blood purifier and decoction of the leaf is given every night as blood purifier. One tea spoon powder is given in cough and sour throat for 6 hours. Also a tea spoon of root powder is given every night as blood purifier (Pullaiah, 2006). The Kurichia tribes are using the leaf juice of *A. racemosa* Wight var. racemosa internally to treat acidity (Deviprasad *et al.,* 2013). The ripe and unripe fruits are eaten by Paliyars (Arinathan *et al.,* 2007). Also the fruits are pickled and eaten by several tribal groups such as Kurumba gounder, Sadaya gounder, Ariyan, Muthuvan and Kattunaikkan (Das *et al.,* 2013). Kadars, Malasars and Muthuvan tribals of Anamalai hills use the whole plant of *A. wightii* to stupefy the fishes (Hosagoudar and Henry, 1996). Earlier works in *A. wightii* revealed the presence of Coumarins (Umbeliferone and geranyl umbeliferone) (Banerji *et al.,* 1982) and few triterpenes (lupeol, lupenone, epi-friedelinol) (Desai *et al.,* 1977). The fruits of the members of the genus *Atalantia* are picked and widely consumed by the tribal people. Therefore the present study aims to test the anti-oxidant potential of the methanol extracts of fruits and also to identify the bioactive compounds present in the extracts through GC/MS analysis.

MATERIALS AND METHODS

Collection of plant material

The fruits and leaves were collected from different places in south India. *A. monophylla* samples were collected from Nagamalai hills of Madurai district, Tamil Nadu. *A. racemosa* and *A. wightii* were collected from Meghamalai hills, Theni, Tamil Nadu and the shola forests near Vaguvarai estate, Munnar, Kerala respectively (Fig. 1). The herbarium of the collected specimens were prepared and identified at Botanical Survey of India, Coimbatore. The herbarium specimen was deposited at the herbarium of department of Plant science, Madurai Kamaraj University. The samples were then shade dried and the moisture content of each sample was calculated.

DPPH radical scavenging assay

The DPPH reacts with methanol or ethanol to yield a purple color DPPH radical (DPPH). The presence of antioxidants which include polyphenolics and flavonoids in the sample will scavenge the formed DPPH radical and there by a decreased color will be absorbed which is spectrophotometrically measured at 517nm. To 0.5 ml of DPPH solution, add 2ml of the extract (100-1000µg/ml) and the reaction mixture is vortexed for 10s and allow standing at room temperature for 30 minutes. The absorbance is recorded at 517 nm by using UV-Vis spectrophotometer. Compare with the 75% ethanol which acts as control solution. Ascorbic acid is used as reference antioxidant compound.

The percentage of DPPH radical scavenging activity is expressed as

[1 – (Test sample absorbance/Blank sample absorbance] ×100(%).

Phosphomolybdenum method

Antioxidant present in the sample reduce the Mo(VI) to Mo(V) which then react with the phosphate group sodium phosphate to form a green colored Mo(V) – Phosphate complex (Phosphomolybdenum complex) in an acetic medium. This complex is then spectrophotometrically measured at 695 nm. The tubes containing 0.2 ml of extract (100-1000µg/ml) is mixed with 1.8 ml of distilled water, 2ml of Phosphomolybdenum reagent solution. Incubate it at 95ºC for 90 minutes. The mixture is closed to room temperature and the absorbance is measured at 695 nm against reagent blank. The antioxidant capacity is expressed as Ascorbic Acid Equivalent (AAE).

GC/MS analysis of plant extracts

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 Rtx®-5MS (30m X 0.25 mm i.d. X 0.25 um film thickness). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 260 °C, the ion-source temperature was 230 °C, the oven temperature was programmed from 100 °C (isothermal for 3 min), with an increase of 10 °C/min to 250 °C, then 20 °C/min to 380 °C, ending with a 19 min isothermal at 380 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 650 Da. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The components were identified based on the library search carried out using NIST and WILEY library.

RESULTS

DPPH radical scavenging activity

DPPH radical scavenging activity was tested to analyze the antioxidant potential of the methanol extracts of fruits of *Atalantia* species. Vitamin C (Ascorbic acid) was used as the standard and was compared with the sample for calculating the anti oxidant activity. Among the three fruits tested for the antioxidant activity using the DPPH method, the crude methanolic extracts of *A. monophylla* shows higher potential. All the three extracts show similar results. The percentage inhibition of methanolic extract of fruits was found to be 82.55%, 72.4% and 80.04 at 1000µg concentration in *A. monophylla, A. racemosa* and *A. wightii* respectively (Fig. 2). Ascorbate used as standard showed 94.3 % inhibition at 1000µg concentration. The IC_{50} value calculated was found to be 336.84 µg/ml for *A. monophylla*, 348.75 µg/ml for *A. racemosa* and 375.64 µg/ml for *A. wightii*.

Ascorbic acid used as standard had an IC_{50} value of 103.03 µg/ml (Table 1).

Table 1: IC_{50} values of DPPH radical scavenging activity of Methanol extracts of three species of *Atalantia* compared with the standard (Vitamin C).

Plant Name	IC_{50} (µg/ml)
A. monophylla	336.84 ± 44.71
A. racemosa	348.75 ± 11.19
A. wightii	375.64 ± 15.17
Vitamin C	103.03 ± 32.067

Phosphomolybdenum method

Total antioxidant capacity was expressed as ascorbic acid equivalent and was calculated using the Phosphomolybdenum method. The methanol extract of *A. monophylla*, *A. racemosa* and *A. wightii* exhibited significant antioxidant activity of 686mg/g, 592 mg/g and 655mg/g respectively (Table 2). The fruit of *A. racemosa* showed the maximum antioxidant activity among the three species.

Table 2: Total antioxidant capacity of three species of *Atalantia* by Phosphomolybdenum method.

Plant Name	Ascorbic acid equivalent
A. monophylla	686 mg/g
A. racemosa	592 mg/g
A. wightii	655 mg/g

GC-MS analysis of methanol extract of the three *Atalantia* species

The bioactive compounds in the Methanol extract of *A. monophylla* were evaluated using GC/MS. The analysis was done using Shimadzu QP-2010 plus with thermal desorption system TD 20, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. Twenty seven compounds were identified from the mass spectra obtained. 1,3,4,5-Tetrahydroxy-cyclohexanecarboxylic acid was the major compound identified. In *A. racemosa* also 27 compounds were identified and n-Hexadecanoic acid was the major compound. In *A. wightii* 18 compounds were identified. β-Sitosterol was the major component in the methanolic extract of *A. wightii*. β-Sitosterol and Stigmasterol was present in all the three species (Table 3-5).

β-sitosterol is a very good for reducing the cholesterol levels in the blood and is sometimes used in treating hypercholesterolemia. The way of action is that β-Sitosterol inhibits cholesterol absorption in the intestinal absorption by competing thereby reducing the serum cholesterol levels (Matsuoka *et al.*, 2008). It also shows anti-inflammatory and antipyretic activities (Gupta *et al.*, 1980). Stigmasterol is used as a precursor for the manufacture of semisynthetic progesterone (Sundararaman and Djerassi, 1997). It is reported to be useful in prevention of ovarian, prostate, breast, and colon cancers (Ghosh *et al.*, 2011). Stigmasterol also possesses anti-osteoarthritis and cholesterol lowering activity (Chen *et al.*, 2012).

Table 3: Compounds identified from the methanol extract of *A. monophylla* fruit using GC-MS.

Sl. No	Retention time	Area percentage	Name of compound	Molecular formulae
1	5.58	0.39	2,3-Dihydro-benzofuran	C_8H_8O
2	5.86	11.40	5-Hydrxoymethylfurfural	$C_6H_6O_3$
3	6.85	8.91	p-Vinylguaiacol	$C_9H_{10}O_2$
4	8.20	3.24	4-Hydroxy-3-methoxy-benzaldehyde	$C_8H_8O_3$
5	9.85	0.24	Guaiacylacetone	$C_{10}H_{12}O_3$
6	9.97	0.62	5(Hydroxymethyl)spiro[2.4]heptan-5-ol	$C_8H_{14}O_2$
7	10.22	1.04	3,5-Dimethoxy-4-hydroxycinnamaldehyde	$C_{11}H_{12}O_4$
8	10.57	0.40	Sinapinaldehyde	$C_{11}H_{12}O_4$
9	10.68	0.89	m-Anisic acid	$C_8H_8O_4$
10	11.25	0.59	1-Piperidino-1-cyclopentene	$C_{10}H_{17}N$
11	12.14	40.83	1,3,4,5-Tetrahydroxy-cyclohexanecarboxylic acid	$C_7H_{12}O_6$
12	12.40	4.09	(E)-Conipheryl alcohol	$C_{10}H_{12}O_3$
13	14.00	0.30	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
14	14.45	2.34	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
15	15.05	0.31	Juvabione	$C_{16}H_{26}O_3$
16	15.62	0.14	Bornyl isovalerate	$C_{15}H_{26}O_2$
17	16.11	1.15	9-Hexadecenoic acid	$C_{16}H_{30}O_2$
18	16.30	0.36	Octadecanoic acid	$C_{18}H_{36}O_2$
19	16.81	9.77	Scyllo-Inositol	$C_6H_{12}O_6$
20	18.04	7.64	Butyl benzyl phthalate	$C_{19}H_{20}O_4$
21	19.81	0.28	o-Phthalic acid	$C_8H_6O_4$
22	25.27	0.24	3-Bromocholest-5-ene	$C_{27}H_{45}Br$
23	25.55	1.60	Stigmasterol	$C_{29}H_{48}O$
24	25.97	2.55	β-Sitosterol	$C_{29}H_{50}O$
25	27.03	0.20	Stigmasterol	$C_{29}H_{48}O$
26	27.14	0.17	Brassicasterol	$C_{28}H_{46}O$
27	27.38	0.28	γ-Sitosterol	$C_{29}H_{50}O$

Table 4: Compounds identified from the methanol extract of *A. racemosa* fruit using GC/MS.

Sl. No	R. Time	Area%	Name	Molecular formula
1	12.34	2.21	Tetradecanoic acid	$C_{14}H_{28}O_2$
2	13.39	0.67	Pentadecanoic acid	$C_{15}H_{30}O_2$
3	14.00	0.24	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
4	14.25	0.23	9-Hexadecenoic acid	$C_{16}H_{32}O_2$
5	14.52	36.94	n-Hexadecanoic acid	$C_{18}H_{32}O_2$
6	15.40	0.55	Eicosanoic acid	$C_{20}H_{40}O_2$
7	15.91	0.21	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$
8	16.19	16.08	Heptadecene-(8)-carbonic acid-(1)	$C_{18}H_{34}O_2$
9	16.37	3.96	Octadecanoic acid	$C_{18}H_{36}O_2$
10	16.54	0.65	1,4-Didecylbenzene	C26H46
11	16.90	0.47	Linoleic acid	$C_{18}H_{32}O_2$
12	17.36	0.37	Heptadecyl heptafluorobutyrate	$C_{21}H_{35}F_7O_2$
13	17.98	0.21	n-Hexadecylaniline	$C_{22}H_{39}N$
14	18.24	0.25	1-Chlorotetradecane	$C_{14}H_{29}Cl$
15	19.15	0.45	1-Decanol, 2-hexyl	$C_{16}H_{34}O$
16	19.81	0.93	n-Octyl phthalate	$C_{24}H_{38}O_4$
17	24.39	2.77	1-Octacosanol	$C_{28}H_{58}O$
18	24.71	0.12	Cholesta-3,5-diene	$C_{27}H_{44}$
19	25.37	0.41	(3β,22E)-3-Methoxystigmasta-5,22-diene	$C_{30}H_{50}O$
20	25.67	0.41	(22E)-Stigmasta-4,6,22-trien-3-yl acetate	$C_{31}H_{48}O_2$
21	25.83	10.52	Stigmasterol	$C_{29}H_{48}O$
22	26.05	1.34	Cholesta-4,6-dien-3-ol	$C_{27}H_{44}O$
23	26.20	17.38	β- Sitosterol	$C_{29}H_{50}O$
24	26.44	0.19	22,23-Dibromostigmasterol acetate	$C_{31}H_{50}Br_2O_2$
25	26.96	0.31	Campesterol	$C_{28}H_{48}O$
26	27.14	1.69	Stigmasta-5,22-dien-3-ol	$C_{31}H_{50}O_2$
27	28.39	0.37	Stigmast-4-en-3-one	$C_{29}H_{48}O$

Fig. 1: Habit of three species of *Atalantia* in south India.

Table 5: Compounds identified from the methanol extract of *A. wightii* fruit using GC/MS.

Sl. No	Retention time	Area percentage	Name of compound	Molecular formulae
1	14.05	10.15	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
2	14.48	12.20	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
3	15.72	2.82	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$
4	15.77	1.66	Methyl 10-undecenoate	$C_{12}H_{22}O_2$
5	15.94	2.56	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$
6	16.21	3.54	5-Isopropyl-1,3-cyclohexanedione	$C_9H_{14}O_2$
7	16.34	0.87	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	$C_{12}H_{24}O_3$
8	17.42	3.43	7-Methoxy-8-(2-oxo-3-methylbutyl)coumarin	$C_{15}H_{16}O_4$
9	17.69	0.68	Undecanoic acid, methyl ester	$C_{12}H_{24}O_2$
10	18.11	0.87	(1R,4R,5R)-2-Benzyl-7,7-dimethyl-6,8-dioxa-2-azabicyclo[3.4.0]nonan-4-one	$C_{15}H_{19}NO_3$
11	19.84	0.70	Phthalic acid, ditridecyl ester	$C_{34}H_{58}O_4$
12	24.40	3.44	1,1-Dichloro-2-dodecanol	$C_{12}H_{24}Cl_2O$
13	25.60	4.08	22,23-Dibromostigmasterol acetate	$C_{31}H_{50}Br_2O_2$
14	25.82	16.14	Stigmasterol	$C_{29}H_{48}O$
15	25.96	2.89	Cholest-5-en-3-ol (3.beta.)-, tetradecanoate	$C_{41}H_{72}O_2$
17	26.19	30.97	β-Sitosterol	$C_{29}H_{50}O$
18	27.16	2.70	Stigmasterol	$C_{29}H_{48}O$

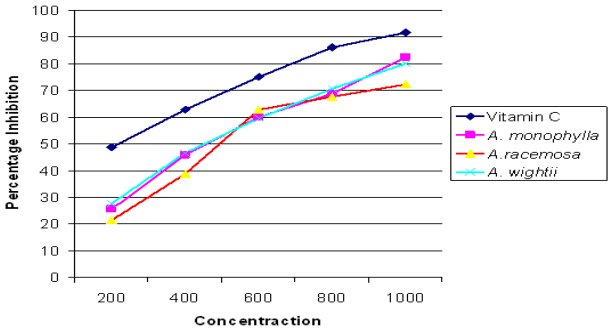

Fig. 2: DPPH radical scavenging activity of three species of *Atalantia* compared with the standard (Vitamin C).

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