Chemical composition analysis, antioxidant and antibacterial activity evaluation of essential oil of *Atalantia monophylla* Correa

Ramaraj Thirugnanasampandan, Ramya Gunasekar, Madhusudhanan Gogulramnath

Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India

Submitted: 16-09-2014 Revised: 03-11-2014 Published: 02-06-2015

ABSTRACT

Background: *Atalantia monophylla* Correa. a small tree belongs to the family Rutaceae. It is distributed throughout India and in Tamil Nadu the species is commonly seen in foothills of dry vegetation. **Objective:** The aim was to hydrodistillate and analyze the chemical composition of essential oil from the fresh leaves of *A. monophylla* Correa. collected in two different seasons (December, 2013 and May, 2014) and to evaluate antioxidant and antibacterial activities of isolated essential oil. **Materials and Methods:** Chemical composition of isolated essential oil was analyzed by gas chromatography, gas chromatography coupled with mass spectrometry. Antioxidant activity of oil was assessed using five different antioxidant test systems. Antibacterial activity of oil was tested against six pathogenic bacteria by broth dilution method. **Results:** Essential oil obtained from the leaves collected during May, 2014 had shown more compounds. Antioxidant activity of oil was moderate when compared with positive control. Minimum inhibitory concentration value of oil was ranges between 139.32 ± 0.001 and 541.11 ± 0.003 μg/mL against all the tested bacteria. **Conclusion:** Result clearly indicates essential oil collected during May, 2014 showed more bioactive compounds.

Key words: Antibacterial activity, Antioxidant, *Atalantia monophylla*, Essential oil, Gas chromatography coupled with mass spectrometry

Access this article online

Website: www.phcogres.com

DOI: 10.4103/0974-8490.152009

Quick Response Code:

INTRODUCTION

Atalantia monophylla Correa. a small tree belongs to the family *Rutaceae*. It is distributed throughout India and in Tamil Nadu the species is commonly seen in foothills of dry vegetation. The tribes (Pulayar) of Thadagai hills are using leaves to treat swellings and act as insect repellent. Atalaphyllinine, atalantin, dehydroatalantin, cycloepiatalantin and atalaphylline 3, 5-dimethyl ether have been reported from root bark.[1-3] Antimicrobial activity of essential oil isolated from the leaves was reported.[4] Bioactivity guided isolation of pyropheophorbide from leaves showed antiviral activity against herpes simplex virus type 2.[5] Antiallergic acridine alkaloids, cycloatalaphylline-A, citrrusinine-I, buxifoliadine-E, junosine and yukocitrine were reported from roots.[6] Various solvent extracts of leaves showed antifeedant, larvicidal and pupicidal activities against *Helicoverpa armigera* and ovicidal activity against *Spodoptera litura*.[7-8] Chemical composition of essential isolated from the leaves was reported in the literature.[9] Based on the phytochemical importance of *A. monophylla*, the present study was aimed to carry out the antioxidant and antibacterial activities of essential oil isolated from the leaves collected from Thadagai hills, Tamil Nadu, India.

Address for correspondence:
Dr. Ramaraj Thirugnanasampandan, Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore - 641 029, Tamil Nadu, India.
E-mail: rtsampandan@yahoo.com

MATERIALS AND METHODS

Collection of plant material and essential oil extraction

Fresh leaves of *A. monophylla* Correa. was collected from the boundaries of Thadagai hills (Anamalai Hills), Western ghats, South India during December, 2013 (winter) and May, 2014 (summer). 500 g of leaves was hydrodistilled for about 3 h and the extracted oil was collected. The collected essential oil was treated with sodium sulfate, tightly sealed and stored at 4°C until further use.

Chemical composition analysis

Gas chromatography analysis

Gas chromatography (GC) analysis was carried out using Varian 3800 GC equipped with mass selective detector coupled to front injector type 1079. The chromatograph was fit with DB-5 column (30 m × 0.25 mm). The injector temperature was set at 280°C and the oven temperature was initially maintained at 45°C then programmed to 300°C at the rate of 10°C/min and finally held at 200°C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 mL/min. The percentage of composition of the essential oil was calculated by the GC peak areas.

Gas chromatography/mass spectrometry analysis

Gas chromatography coupled with mass spectroscopy was performed using Varian 3800 GC equipped with Varian 1200 L single quadrupole mass spectrometer. The GC conditions were the same as reported for GC analysis and the same column was used. The mass spectrometer operated in the electron impact mode at 70 eV. Ion source and transfer line temperature were maintained at 250°C. The compounds were identified based on the comparison of their retention indices, retention time and mass spectra.[10]

Antioxidant activity

1,1-Diphenyl-2-Picrylhydrazyl free radical scavenging activity

Different concentrations of test sample mixed individually with 0.1 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 50 mM tris-HCl buffer (pH 7.4). Reaction mixture was incubated at 37°C for 30 min and then absorbance was measured at 517 nm.[11] The percentage of DPPH free radical scavenging activity was calculated using the following equation: % Inhibition = $[(A_B - A_A)/A_B] \times 100$, where A_B, absorption of blank sample, A_A, absorption of test sample.

Metal chelating activity

Briefly, 2 mM $FeCl_2$ was added to different concentrations of test sample, and reaction was initiated by the addition of 5 mM ferrozine. The mixture was vigorously shaken and left to stand at room temperature for 10 min. Absorbance was measured at 562 nm after 10 min.[12] % Inhibition = $[(A_B - A_A)/A_B] \times 100$, where A_B, absorption of blank sample, A_A, absorption of test sample.

Hydroxyl radical scavenging activity

Reaction mixture includes 7.5 mM $FeSO_4$, 7.5 mM 1, 10-phenanthroline, 0.2 M phosphate buffer (pH 7.8), 30 mM H_2O_2 and test sample at different concentrations. The reaction was started by adding H_2O_2. After incubation at room temperature for 5 min, the absorbance of the mixture was read at 536 nm.[13] % Inhibition = $[(A_B - A_A)/A_B] \times 100$, where A_B, absorption of blank sample, A_A, absorption of test sample.

Prevention of deoxyribose degradation

Test sample of different concentrations was mixed with 20 mM deoxyribose, 0.1 M $NaPO_4$, 20 mM H_2O_2 and 50 mM $FeSO_4$. The reaction mixture was incubated for 60 min at 37°C. Then 2 mL of 10% ice-cold trichloroacetic acid was added, and 1 mL aliquot of the samples was added with 1 mL of 1% thiobarbituric acid (TBA). The TBA/sample mixture was heated in a water bath at 95°C for another 60 min and absorbance was read at 532 nm.[14] % Inhibition = $[(A_B - A_A)/A_B] \times 100$, where A_B, absorption of blank sample, A_A, absorption of test sample.

Inhibition of linoleic acid peroxidation

Briefly, 20 mM linoleic acid, 100 mM HCl (pH 7.5), 5 mM ascorbic acid were mixed with test sample and linoleic acid peroxidation was initiated by the addition of 4 mM $FeSO_4.7H_2O$, incubated for 60 min at 37°C and terminated by the addition of 2 mL of ice cold trichloroacetic acid (10% v/v). An amount of 1 mL of TBA (1% w/v in 50 mM NaOH) was added to 1 mL of the reaction mixture, followed by heating at 95°C for 60 min. The reaction sample was read at 532 nm.[15] The percentage of linoleic acid peroxidation inhibition activity was calculated using the following equation: % Inhibition = $[(A_B - A_A)/A_B] \times 100$, where A_B, absorption of blank sample, A_A, absorption of test sample.

Antibacterial activity

Bacterial strains

Clinical isolates of *Aeromonas hydrophila*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Proteus vulgaris* and *Staphylococcus aureus* were procured from Microbiology Laboratory of KMCH Hospital, Coimbatore, Tamil Nadu, India.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of essential oil was studied by broth microdilution method using 96-well microtiter plates.[16] Essential oil was dissolved in dimethylsulphoxide (DMSO) (1%) with the addition of Tween-80 (0.5%) and diluted in Muller Hinton Broth to get a concentration range of 25–225 μg/mL. The solution was then two-fold diluted in Muller Hinton Broth (100 μL), inoculated with bacterial strains and then incubated at 37°C for 24 h. The bacterial growth was measured as turbidity with a Cyberlab micro plate reader at 405 nm. The MIC was defined as the lowest concentration of the oil that inhibited the growth of the test bacteria. DMSO assayed as the negative control at a concentration of 1% did not inhibit any of the strains tested. All tests were assayed in triplicate in three independent experiments,

and median values were used for MICs calculation. Both gentamicin and ampicillin were served as a positive control.

Statistical analysis

The data obtained from the antioxidant and antibacterial studies were analyzed using SPSS (SPSS Inc. 16.00) for IC_{50} and MIC calculations.

RESULTS

Chemical composition of essential oil

Hydrodistillation of 500 g of leaves (collected during May 2014) yielded 350 μL of yellow colored essential oil with a fragrance of citrus. A total of 36 compounds constituting 88.13% were identified with sabinene (23.81%) as major compound followed by trans-asarone (19.55%), β-pinene (13.35%) and myrcene (10.39%) [Table 1]. Interestingly the essential oil isolated from leaves collected during winter (December, 2013) season showed 23 compounds (72.25%) with trans isoeugenol (23.73%) as a major one [Table 2].

Antioxidant activity of essential oil

Essential oil collected during summer season (May, 2014) was used for antioxidant studies. Concentration range of 250 μg/mL showed 69.04% of DPPH free radical scavenging with IC_{50} value of 198.97 ± 0.002 μg/mL. Ferrous ion chelation and hydroxyl radical scavenging activity of the oil was high at higher concentration, and IC_{50} value was recorded as 204.78 ± 0.002 and 199.35 ± 0.003 μg/mL respectively. Fenton reaction induced deoxyribose degradation was prevented by the essential oil in a dose dependent manner with IC_{50} value of 176.54 ± 0.002 μg/mL and percentage of inhibition of linoleic acid peroxidation was calculated as 66.30 at 250 μg/mL with IC_{50} value of 219.15 ± 0.002 μg/mL [Table 3]. In all the test system studied, the activity of oil was less observed when compared with butylated hydroxytoluene (47.10 ± 0.001 μg/mL).

Antibacterial activity of oil

The MIC values of *A. monophylla* essential oil against all the tested bacterial strains were recorded in [Table 4]. The antibacterial activity was strong against *A. hydrophila* followed by *P. mirabilis*, *P. aeuroginosa, P. vulgaris* and *E. coli* (MIC < 350 μg/mL). The oil showed the least activity against both *K. pneumoniae* and *S. aureus* (MIC > 500 μg/mL).

DISCUSSION

The result of this study clearly indicates leaf material collected during summer season (May, 2014) had shown more compounds of biological interest and it's assumed that high temperature with low water content and other environmental factors might have induced relatively high secondary metabolite production. Earlier reports on essential oil of *A. monophylla* collected from Narrtha and Nagamalai hills (Tamil Nadu, South India) reported that methyl eugenol and asarone were major compounds.[9,17] However in the present investigation sabinene has been identified as a major compound. Though similar compound was reported in the previous studies, the percentage composition has been varied. This variation might be due to soil, climate, availability of water, geographical location, environmental factors, season of material collection, etc.[18]

Essential oil isolated from the summer season had more bioactive compounds, so it was used for further studies. Antioxidant activity of oil was concentration dependent while increasing concentration percentage of free radical scavenging was also increased. When compared with

Table 1: Chemical composition of essential oil of *A. monophylla* collected during May, 2014

RI*	Compound name	Percentage
924	α-Thujene	0.20
932	α-Pinene	1.36
946	Camphene	0.12
969	Sabinene	23.81
974	β-Pinene	13.35
988	Myrcene	10.39
1001	Carene <δ-2->	0.45
1002	α-Phellandrene	0.66
1022	Ortho-cymene	0.13
1024	Limonene	0.84
1025	β-Phellandrene	3.32
1032	*cis* β-Ocimene	0.14
1054	γ-Terpiene	0.85
1086	Terpinolene	0.19
1095	Linalool	0.05
1119	Mentha-2,8-dien-1-ol <trans-p->	0.19
1174	Terpinen-4-ol	1.99
1186	α-Terpineol	0.06
1187	1-Dodecene	0.26
1266	1-Decanol	0.69
1387	β-Bourbonene	0.03
1403	Methyl eugenol	2.45
1408	*cis*-caryophyllene	2.68
1409	α-Gurjunene	0.25
1431	β–Gurjunene	0.07
1432	*trans*-α-Bergamotene	0.07
1436	β-Humulene	0.57
1492	δ-Selinene	0.21
1505	β-Bisabolene	0.27
1521	β-Sesquiphellandrene	0.99
1555	Elemicin	0.20
1675	*trans*-asarone	19.55
1577	Spathulenol	0.26
1582	Caryophyllene oxide	0.31
1697	2-Pentadecanone	0.16
1942	Phytol	0.10
Total identified		88.13

*RI=Retention index obtained on DB-5 column, *A. monophylla=Atalantia monophylla*

Table 2: Chemical composition of essential oil of *A. monophylla* collected during December, 2013

RI*	Compound name	Percentage
932	α-pinene	0.86
1008	Carene <δ-3->	7.76
1014	α-terpinene	0.36
1020	Para-cymene	1.40
1022	Ortho-cymene	1.29
1024	Limonene	1.30
1095	Linalool	0.83
1108	1,3 8-p-menthatriene	0.75
1174	Terpinen-4-ol	0.91
1201	Decanal	5.9
1210	Nonadienal < (2E, 4E) ->	11.26
1232	Thymol methylether	0.57
1323	Methyl decanoate	0.61
1403	Methyl eugenol	2.76
1408	*cis*-caryophyllene	1.45
1409	α-gurjunene	0.85
1448	*trans*-Isoeugenol	23.73
1484	Germacrene D	3.38
1555	Elemicin	2.78
1568	*cis* Isoelemicin	2.54
1574	Germacrene D-4-ol	0.33
1582	Caryophyllene oxide	0.32
1652	α-cadinol	0.31
Total identified		72.25

*RI=Retention index obtained on DB-5 column, *A. monophylla=Atalantia monophylla*

Table 3: Antioxidant activity of essential of *A. monophylla*

Assay	IC_{50}* (μg/mL)
DPPH free radical scavenging	198.97±0.002
Metal ion chelation	204.78±0.003
Hydroxyl radical scavenging	199.35±0.003
Prevention of deoxyribose degradation	176.54±0.002
Inhibition of linoleic acid peroxidation	219.15±0.002

IC_{50}=Inhibitory concentration values were expressed as the mean±SD of three replicates, SD=Standard deviation; *A. monophylla=Atalantia monophylla*; DPPH=1,1-diphenyl-2-picrylhydrazyl

Table 4: Antibacterial activity of *A. monophylla* essential oil

Test bacteria	MIC* (μg/mL)		
	Oil	Ampicillin	Gentamicin
Aeromonas hydrophila	139.32±0.001	13.2±0.001	10.8±0.001
Escherichia coli	328.14±0.003	14.1±0.002	9.89±0.001
Klebsiella pneumoniae	516.73±0.002	13.8±0.001	12.5±0.001
Pseudomonas aeruginosa	203.84±0.002	14.7±0.001	11.18±0.001
Proteus mirabilis	189.57±0.003	16.4±0.001	13.1±0.002
Proteus vulgaris	233.73±0.002	15.9±0.01	12.3±0.001
Staphylococcus aureus	541.11±0.003	12.8±0.002	11.9±0.001

*MIC=Minimum inhibitory concentration values were expressed as the mean±SD of three replicates; *A. monophylla=Atalantia monophylla*; SD=Standard deviation

positive control antioxidant activity of oil was less however the oil showed considerable free radical scavenging activity. Antioxidant activity of oil may be related to the presence of the monoterpene hydrocarbon, sabinene. Similar result was reported by Valente *et al*.[19] where sabinene and essential oil of *Oenanthe crocata* rich in sabinene and *trans* β-ocimene showed considerable free radical scavenging activity. Meanwhile other monoterpene hydrocarbons such β-pinene, myrcene, limonene, β-phellandrene and an ether, asarone could also be taken in to account.

Essential oils as antimicrobial agents in food systems may be considered as additional intrinsic determinant to increase the safety and shelf life of foods.[20] Pirbalouti *et al*.[21] has reported that monoterpenes are disrupting the microbial cytoplasmic membrane and leads to loss in the high impermeability of the membranes for protons and larger ions. Since the main constituent of *A. monophylla* oil is monoterpenes so we assumed that observed antibacterial activity might be due to sabinene, β-pinene, myrcene, β-phellandrene and asarone. Meanwhile synergistic effect of other compounds also need to be considered.[22]

CONCLUSION

Leaf essential oil of *A. monophylla* collected during May, 2014 showed more bioactive compounds when compared with oil collected during December, 2013 and could be used as a natural supplement and preservative in food, crude drug and phytopharmaceutical preparations.

ACKNOWLEDGMENTS

This work was financially supported by Department of Science and Technology-Science and Engineering Research Board (DST-SERB) New Delhi, India under grant no. SB/FT/LS-230/2012; date 02.05.2013.

REFERENCES

1. Basa SC. Atalaphyllinine, a new acridone base from *Atalantia monophylla*. Phytochemistry 1975;14:835-6.
2. Dreyer DL, Bennett RD, Basa SC. Limonoids from *Atalantia monophylla* isolation and structure. Tetrahedron 1976;32:2367-73.
3. Kulkarni GH, Sabata BK. An acridone alkaloid from the root bark of *Atalantia monophylla*. Phytochemistry 1981;20:867-8.
4. Prasad YR. Chemical investigation and antimicrobial efficacy of the volatile leaf oil of *Atalantia monophylla* Corr. Prafuemerie Kosmetik 1988;69:418-9.
5. Chansakaow S, Ruangrungsi N, Ishikawa T. Isolation of pyropheophorbide a from the leaves of *Atalantia monophylla* (ROXB.) CORR. (*Rutaceae*) as a possible antiviral active principle against herpes simplex virus type 2. Chem Pharm Bull (Tokyo) 1996;44:1415-7.
6. Chukaew A, Ponglimanont C, Karalai C, Tewtrakul S. Potential anti-allergic acridone alkaloids from the roots of *Atalantia monophylla*. Phytochemistry 2008;69:2616-20.
7. Baskar K, Kingsley S, Vendan SE, Paulraj MG, Duraipandiyan V, Ignacimuthu S. Antifeedant, larvicidal and pupicidal activities of

Atalantia monophylla (L) Correa against *Helicoverpa armigera* Hubner (*Lepidoptera*: *Noctuidae*). Chemosphere 2009;75:355-9.

8. Baskar K, Muthu C, Raj GA, Kingsley S, Ignacimuthu S. Ovicidal activity of *Atalantia monophylla* (L) Correa against *Spodoptera litura* Fab. (*Lepidoptera*: Noctuidae). Asian Pac J Trop Biomed 2012;2:987-91.
9. Das AK, Swamy PS. Comparison of the volatile oil composition of three *Atalantia* species. J Environ Biol 2013;34:569-71.
10. Adams RP. Identification of Essential Oil Components by Gas Chromatography and Mass Spectrometry. 3rd ed. USA: Allured Publication Corporation; 1995.
11. Blois MS. Antioxidant determination by the use of a stable free radical. Nature 1958;181:1199-200.
12. Dinis TC, Maderia VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994;315:161-9.
13. Zhao GR, Xiang ZJ, Ye TX, Yuan YJ, Guo ZX. Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*. Food Chem 2006;99:767-74.
14. Halliwell B, Gutteridge JM, Aruoma OI. The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Anal Biochem 1987;165:215-9.
15. Choi B, Kang S, Ha Y, Park G, Ackman RG. Conjugated linoleic acid as a supplemental nutrient for common carp (*Cyprinus carpio*). Food Sci Biotechnol 2002;11:457-61.
16. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard. 8th ed. Wayne, PA: CLSI; 2009.
17. Manimaran S, Sathya S, Tamizhmani T, Subburaju T, Chinnaswamy K, Nanjan MJ, Suresh B. Phytochemical investigation on leaf volatile oil of *Atalantia monophylla* Correa. Indian Perfum 2002;46:341-2.
18. Tamilarasi T, Thirugnanasampandan R. Antioxidant evaluation of essential oil and RAPD analysis of *in vitro* regenerated *Blumea mollis* (D. Don) Merr. Acta Physiol Plant 2014;36:1593-8.
19. Valente J, Zuzarte M, Gonçalves MJ, Lopes MC, Cavaleiro C, Salgueiro L, *et al.* Antifungal, antioxidant and anti-inflammatory activities of *Oenanthe crocata* L. essential oil. Food Chem Toxicol 2013;62:349-54.
20. Salgueiro L, Martins AP, Correia H. Raw materials: The importance of quality and safety a review. Flavour Fragr J 2010;25:253-71.
21. Pirbalouti AG, Hossayni I, Shirmardi HA. Essential oil variation, antioxidant and antibacterial activity of mountain fennel (*Zaravschanica membranacea* (Boiss.) M. Pimen.). Ind Crops Prod 2013;50:443-8.
22. Lopes-Lutz D, Alviano DS, Alviano CS, Kolodziejczyk PP. Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. Phytochemistry 2008;69:1732-8.

Cite this article as: Thirugnanasampandan R, Gunasekar R, Gogulramnath M. Chemical composition analysis, antioxidant and antibacterial activity evaluation of essential oil of *Atalantia monophylla* Correa. Phcog Res 2015;7:S52-6.

Source of Support: Nil. C**onflict of Interest:** None declared.