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Chemical composition and antibacterial activity of the essential oil from the aerial parts of *Aristolochia tagala*

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Abstract: The chemical composition of the essential oil obtained by hydrodistillation of the aerial parts of *Aristolochia tagala* was analysed using Gas Cromatography-Mass Spectrometry method. Thirty seven compounds in the oil were identified. The major components were the sesquiterpenes, namely, β-caryophyllene (13.8%), α-humulene (6.5%), germacrene-A (4.3%), bicyclogermacrene (4.2%) and caryophyllene oxide (3.9%). The oil showed moderate antibacterial activity against the tested Gram-positive and Gram-negative bacteria.

Keywords: *Aristolochia tagala*, Aristolochiaceae, essential oil composition, β-caryophyllene, α-humulene, germacrene-A, antibacterial activity, GC, GC-MS.

INTRODUCTION

Plants of Aristolochiaceae are scramblers, shrubs, or rhizomatous herbs, discussed under seven genera and 410 species (Mabberley, 1990). *Aristolochia*, Asarum and Thottea are the three Indian genera reported (Hooker,1980). Aristolochia includes twenty seven species in India of which *Aristolochia bracteolata, A. indica* and *A. tagala* are useful in medicine.

Aristolochia tagala (Cham) are large climbing shrubs with stout, grooved stems, found in the Himalayas from Nepal, eastwards to Sikkim and in the Decan peninsula on the Western Ghats of India. Leaves are ovate, acuminate, deeply cordate at base, flowers numerous in loose slightly hairly racemes, greenish yellow, perianth upto 7cm long, pale green with a globose base and curved tube, oblique mouth and straight lip, capsules 2 to 5 cm long, oblong-ellipsoid, transversely rigid and glabrous. The roots of *Aristolochia tagala* are frequently used to adulterate *Aristolochia indica* for use in medicine (Anonymous, 1985).

The phytochemical constituents isolated from roots of *A. tagala* were aristolochic acids-A and C, 7-hydroxy aristolochic acid-A and allantoin (Ding et al.,1981). The leaves contained 4, 7-dimethyl- 6-methoxy-1- tertralone (Yu et al.,1984).

Characterisation of the essential oils of some species of *Aristolochia* like *A. elegans, A. argentina, A. longa, A.ovalifolia* (Sagrero-Nieves, 1994) A. brevipis, (Sagrero-Nieves, 1997) *A. indica* (Krishna Rao, 1935) and *A. asclepiadifolia* (Sagrego - Nieves 1993) were reported. Most of them contain sesquiterpenoids as the major fraction that included β-caryophyllene, cadinanes, aristolanes, germacranes and bicyclogermacranes (Wu et al., 2004).

No reports on the isolation and characterisation of the essential oil of *A. tagala* are available and this paper reports the chemical composition and antibacterial activity of the essential oil of *A. tagala*.

MATERIALS AND METHODS

Plant materials: Fresh aerial parts of *A. tagala* were collected from Wynad, Kerala. The collected material was identified and certified by the Botanical Survey of India, Coimbatore.

Oil isolation: Fresh plantl parts of *A. tagala* (500g) were chopped and hydrodistilled using a Clevenger type apparatus for 4 h. The oil was collected, dried over anhydrous sodium sulphate and stored at 4^0 C until it was analysed.

Gas Chromatography- Mass Spectrometry analysis: The Gas Chromatography (GC) analysis (Sabulal et al, 2009) of the oil was carried out on a Nucon 5765 series gas chromatograph fitted with SE-30 (10% Chromosorb-W) packed stainless steel column (2 m x 2 mm) with Flame Ionisation Detector (FID) using nitrogen as carrier gas at flow rate of 40 mL/min. Oven programme: 80°-150°C (8 °C/min.), 150°-230 °C (5 °C/min), 230 °C (10 min), injector temperature 220 °C; detector temperature 250 °C. Relative percentage of the components were calculated from the peak area-percent report of the volatiles from GC/ FID data (Table-1).

GC- MS analysis (Anil John et al, 2008) of the oil was carried out by splitless injection of 1.0 μL of the oil on a Hewlett Packard 6890 gas chromatograph fitted with a cross-linked 5% PH ME siloxane HP-5 MS capillary column, (30 m x 0.32 mm, 0.25 µm) coating thickness, coupled with a model 5973 series mass selective detector using the following operation conditions: injector temperature 220 °C; transfer line 290°C; oven temperature programme 60-246 °C (3°C/min); carrier gas – helium at 1.4 mL/min. Mass spectra: Electron Impact (EI) mode at 70 eV with a mass range of 40-450 m/z, ion source temperature 250 °C. Relative retention indices (RRI) of constituents (Table-1) were determined on the HP-5 MS column using C_5-C_{30} straight chain alkanes as standards (Van den Dool and Ktarz, 1963). Individual components of the oil were identified by Wiley 275 L database matching and by comparison of retention times and mass spectra of constituents with published data (Adams, 2001) and by comparison of their RRIs (Davies, 1990; Adams, 2001).

Antibacterial assay of the oil: The antibacterial activity of the essential oil of *A. tagala* was tested by disc diffusion method (Berghe and Vlietnick,1991, Cappucino and Sherman, 1999) against two Gram-positive bacteria, Staphylococcus aureus and *S. albus* and two Gram-negative bacteria,.*Proteus vulgaris* and *Pseudomonas aeruginosa*. The culture medium used was Muller- Hinton agar medium at pH 7.2. The agar medium was poured into the plates to a uniform depth of 5 mm and allowed to solidify. Then the microbial suspensions were streaked over the surface of the media using a sterile cotton swab to ensure the confluent growth of the organism. Aliquots of 10 µL of samples at 1:2 dilution in acetone were aseptically applied onto the surface of the agar plates at well spaced intervals. These plates were incubated at 37 °C for 24 hours and the observed growth inhibition zones were measured. The control element used was gentamycin at a concentration of 1mg/mL .The activities are expressed in mm diameter of the inhibition zone including the disc diameter.

RESULTS AND DISCUSSION

Hydrodistillation of the aerial parts of *A. tagala* (500g) yielded 1.2 mL of brown coloured oil with a powerful earthy pleasant smell in 0.24% (v/w) yield. From the GC-FID and GC-MS analysis a total of 37 components constituting 75% of the total essential oil were identified. Sesquiterpenes were found to be the main constituent in the oil. Similar case was encountered for A. elegans (Bandoni et al, 1997). The major sesquiterpenes present were β-caryophyllene (13.8%), α-humulene (6.5%), germacrene-A (4.3%) and bicyclogermacrene (4.2%). The oil from the leaves of *A. elegans* and *A. longa* (De Pascual et al, 1983) also had β-caryophyllene and bicyclogermacrene as predominant components. The other significant sesquiterpenes present were caryophyllene oxide (3.9%), cyperene (2.8%), γ-gurjunene (2.2%), δ-cadinene (2.2%), spathulenol (2.1%) and β-selenene (1.3%). Some of these components were also present in the oil of *A. elegans* and *A. argentina* (Priestap et al, 2003). A study of sesquiterpenoid profile of the leaves of twelve *Aristolochia* species indicated the presence of bicyclogermacrene in all species (Silvia-Brando et al, 2006). The other major constituents present in the oil of *A.tagala* were the decanoic acids, namely, hexa, tetra and do-decanoic acid with composition of 10.3%, 4.7% and 2.5%; respectively. While *A. elegans* had octane derivatives in the oil, *A. argentina* had various isomers of undecatrienes. The compounds present in 0.1% yield were limonene, trans- β-ocimene,1-dodecene, bornyl formate, hexyl cyclohexane, bornyl acetate, δ-elemene, α-ylangene, α-farnesene and α-calacorene. The qualitative and quantitative analytical results are shown in Table-1. This is the first report of the isolation and characterization of the essential oil of *A. tagala*.

The antibacterial activity of the oil was tested by disc diffusion technique. It showed positive activity against the Gram-positive bacteria *Staphylococcus aureus* (13 mm) and *S. albus* (12 mm) and moderate activity against the Gram-negative bacteria *Pseudomonas aeruginosa* and *Proteus vulgaris* with inhibition zones of 7 mm and 6 mm, respectively against 7 mm of the control element gentamycin.

Table-1. Chemical composition of essential oil of *Aristolochia tagala*

S.No	Name of compound	RRI	Percentage
1	α-Cubebene	1340	0.4
2	α-Copaene	1365	1.9
3	β-Elemene	1383	2.1
4	Cyperene	1387	2.8
5	Tetradecane	1395	0.4
6	β-Caryophyllene	1410	13.8
7	β-Gurjunene	1419	0.2
8	Aromadendrene	1427	0.3
9	α-Humulene	1443	6.5
10	γ-Gurjunene	1463	2.2
11	β-Selinene	1474	1.3
12	Bicyclogermacrene	1486	4.2
13	Germacrene-A	1494	4.3
14	δ-Cadinene	1513	2.2
15	trans-Cadina 1(2), 4-diene	1521	0.3
16	Germacrene-B	1543	0.5
17	Spathulenol	1566	2.1
18	Caryophyllene oxide	1570	3.9
19	Dodecanoic acid	1579	4.7
20	1-Hexadecene	1585	1.6

21	Hexadecane	1592	1.0
22	Tetradecanoic acid	1773	2.5
23	1-Octadecene	1790	1.9
24	Octadecane	1798	0.7
25	Hexadecanoic acid	1979	10.3
26	1-Eicosene	1991	1.6
27	Eicosane	1997	0.4

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