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Phytochemical Analysis of *Ammannia multiflora*

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ABSTRACT

The plants of genus *Ammannia* are widely used in traditional Chinese medicinal system but no detailed phytochemical investigations on *A. multiflora* have been carried so far. The aim of present study was to carry out chemical investigation of *A. multiflora*. In a preliminary screening, glycosides, flavonoids, carbohydrates, steroids, phenols and sterols were detected but alkaloids were absent. Further, plant material was successively extracted with hexane, chloroform and methanol and the chloroform extract was subjected for chromatographic separation over Vacuum Liquid Chromatography (VLC). The non-polar VLC fractions of chloroform extract along with hexane extract were later analyzed with GC-MS which resulted in the characterization of fourteen constituents being reported for the first time from *A. multiflora*. Some of the constituents were potentially active and could be used in future development of anti-microbial agents.

Key words: *A. multiflora*, Lythraceae, phytomolecules, vacuum liquid chromatography, GC-MS

INTRODUCTION

The emerging and re-emerging infections and spread of deadly drug resistant strains of disease causing organisms has made a challenge to the global public health in terms of their treatment. Plants have been source of potential therapeutic agents ever since mankind has evolved (Tatke and Jaiswal, 2011). According to WHO, about 80% of the world's population is using plants as primary medicinal aid. The medicines based on herbal formulations usually have lesser side effects and better compatibility with human body than modern medicines. This makes the herbal medicines an ideal remedy for treatment of the diseases (Kamboj, 2000; Karim *et al*., 2011). Twenty five percent of drugs prescribed by Western pharmacists comprise of elements that are of plant origin. Studies aiming at the development of rapidly propagating methods for medicinal plants is an indication that demand for these plant is increasing (Nalawade and Tsay, 2004; Huang *et al*., 2000; Khan *et al*., 2004; Malik *et al*., 2007; Banu and Bari, 2007; Jamil *et al*., 2007; Gantait *et al*., 2010; Satyavani *et al*., 2011). Hence, it will be of immense importance to develop alternative antibiotic drugs from plants (Bele *et al*., 2009). The random screening of plants and their constituents for drug discovery has been many times expensive failure. Hence, in this capital intensive world, scientists are now concentrating mainly on focused screening of plants (Valler and Green, 2000). Presently, in modern drug discovery programme, screening of folk and traditional medicines is being used as a productive approach (Fabricant and Farnsworth, 2001). Another way to discover new pharmacologically active compounds is the result of their crude

extracts when tested for *in vitro* activity. In this way many active constituent have been isolated and identified from medicinal plants for various types of remedies.

The genus *Ammannia* (Family-Lythraceae) is commonly called as red stems (Caton *et al.*, 1997) and many species of this genus are being used in traditional medicinal system. *A. baccifera* is commonly called 'blistering Ammannia' or 'jungli mehendi'. It is used in traditional Chinese medicine to cure human female infertility (Huang *et al.*, 1995). It is reported to have antipyretic and antidiuretic (Joanofarc *et al.*, 2003), antimicrobial (Dash *et al.*, 2008), antirheumatic, anticancer (Uddin *et al.*, 2011) and rubefacient activities. *A. baccifera* is also used in the treatment of skin diseases (Parekh and Chanda, 2007). Recently, antisteroidogenic activity in female albino mice ovaries has been reported in ethanolic extract of *A. baccifera* (Dhanapal *et al.*, 2005). *Ammannia multiflora* (syn. *A. parviflora*, *Suffrenia dichotomy*) is commonly called as 'many flowered Ammannia'. It is nearly cosmopolitan in distribution especially in America, Africa, Asia, Australia and Europe. In India it grows in marshy places along the banks of rivers and rivulets and cultivated paddy fields (Singh and Khanuja, 2006). It is an erect herb with numerous four angled branches. The plant is 15-43 cm tall, leaves narrow-oblong to lanceolate, 5-50 mm long, 1.5-8 mm wide and slightly reddish in color. Flowers pink to purple. From literature review, there is only one report on the isolation of rhamnetin and 3-rhamnosyl glucoside from *A. multiflora* (Balraj and Nagarajan, 1981). Recently methanol extract of *A. multiflora* roots showed significant *in vitro* antimalarial activity (IC_{50} = 18 μg mL^{-1}) (Simonsen *et al.*, 2001). Therefore, detailed phytochemical investigation using chromatographic and spectroscopic techniques were performed. The present study was aimed at preliminary phytochemical investigation of *A. multiflora*.

MATERIALS AND METHODS

Plant material: The whole plant of *A. multiflora* was collected from the Lucknow district of Uttar Pradesh, India (November, 2009) and identified by one of us (DCS) and Dr. S.C. Singh, Scientist, Botany Division, Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. A voucher specimen (No. -9455) has been deposited at the CIMAP herbarium.

Preparation of extracts: The shade dried plant material was stored at room temperature following good storage practices. The 100 g pulverized plant material of *A. multiflora* (whole plant) was extracted overnight thrice successively with hexane, chloroform and methanol. The combined extracts were separately dried over rotavapor (BUCHI, Switzerland) under vacuum at 40°C which afforded hexane extract (HE, 1.5 g), chloroform extract (CE, 1.8 g) and methanol extract (ME, 9.5 g).

Phytochemical screening: The preliminary phytochemical screening of MeOH extract of *A. multiflora* was done to assess the presence of alkaloids, saponins, phenols, carbohydrates, steroids, flavonoids and glycosides. The tests were performed using standard procedures (Sofowora, 1993; Trease and Evans, 1989; Akinjogunla *et al.*, 2010; Ganesh and Vennila, 2011).

Separation of chloroform extract over VLC: The vacuum liquid chromatography was first mentioned by Coll *et al.* (1977) for separation of diterpenes but no experimental details describing the method were reported. Subsequently, Targett *et al.* (1979) described an elaborate set up of VLC and demonstrated its effectiveness for the separation of a standard dye mixture and a mixture

Table 1: Elution of VLC column of Chloroform Extract (CE) and pooling of fractions

Solvent system	Pooled fractions	Sample code
100% Hexane	1	CE-1
100% Hexane	4-6	CE-2
35% Hexane : 65% $CHCl_3$	17-22	CE-3
97% $CHCl_3$: 3% MeOH	84-93	CE-4

of (+)-fenchone and (+)-camphor. The VLC has been proved useful for separation of mixture of natural products as well as mixtures resulting from synthetic operations (Pelletier *et al.*, 1986). The VLC technique is a preparative layer chromatographic separation where flow of solvent is activated by vacuum. The achievement of excellent separation is due to fine particle size of stationary phase (silica) which provides very large number of theoretical plates.

In brief, the sintered glass funnel was used as VLC column (3.5×4.5 cm) and tightly packed with silica gel H (particle size ~10 μM, 4 g). To check the uniform packing of VLC column 100 mL of hexane was run through it and later, VLC column was completely dried under vacuum. The chloroform extract was (1.5 g) was dissolved in minimum amount of MeOH and applied uniformly with the help of a pipette on the top of VLC column. Once the extract was uniformly loaded, the column was completely dried under vacuum to get rid of polar solvent (MeOH). Elution of the VLC column was carried out with solvents of increasing polarity viz. hexane, chloroform, methanol in various proportions and fractions were collected. TLC plates (silica gel $60F_{254}$, Merck) were first examined under UV illumination at 254 and 365 nm and then sprayed with vanillin-sulphuric acid (1: 5, w/v) solution in ethanol followed by heating at 95°C for 5 min. A total of 93 fractions of 50 mL each were collected and pooled in to four major fractions (CE-1, CE-2, CE-3 and CE-4) on the basis of their TLC profile (Table 1).

GC-MS analysis for organic constituents: The Hexane Extracts (HE) and four pooled VLC fractions (CE-1 to CE-4) of chloroform extract were analyzed using a Perkin-Elmer Turbo Mass Spectrometer instrument using a PE-Wax column (60×0.32 mm i.d., film thickness 0.25 μm). The carrier gas was helium. Temperature programming was 5 min at 70°C, rising at 2°C min to 120°C and 3°C min to 240°C. MS were recorded at 70 eV in the range of 40-400 amu with scan rate 1s and inter scan delay 0.1s. Compounds were identified by computer matching of the mass spectral fragmentation patterns of peaks with the Wiley and NIST libraries mass spectral database.

RESULTS AND DISCUSSION

There is only one report on the isolation of free rhamnetin and 3-rhamnosyl glucoside from *A. multiflora* (Balraj and Nagarajan, 1981). The roots of *A. multiflora* have shown significant antiplasmodial activity (Simonsen *et al.*, 2001). Therefore, under a drug discovery programme, detailed phytochemical investigation of *A. multiflora* using chromatographic and spectroscopic techniques was performed. In this study, the preliminary phytochemical screening of *A. multiflora* MeOH extract showed the presence of many bioactive class of compounds like flavonoids, steroids, terpenes and phenols which are widely used in modern medicines (Table 2). However, alkaloids were absent in the plant.

Further, chloroform extract of *A. multiflora* was resolved on VLC which afforded four pooled fractions, CE-1, CE-2, CE-3 and CE-4. Further, hexane extract (HE) and four VLC fractions of chloroform extract (CE-1 to CE-4) were analyzed by GC-MS and the results have been summarized

Table 2: Qualitative analysis of phytochemical components

Phytochemical components	Result on methanolic extract of *A. multiflora*
Flavonoids	+
Alkaloids	-
Reducing sugars	+
Saponins	+
Phenols	+
Steroids	+
Glycosides	+
Sterols	+

+: Preseuce, -: Absence

Table 3: GC-MS analysis of hexane extract (HE) and VLC fractions (CE-1 to CE-4) of chloroform extract of *A. multiflora*

Sample code	R. T.	Constituent identified	M. F.	M. Wt.
HE	47.326	11-Tridecen-1-ol	$C_{13}H_{26}O$	198
	51.526	Hexadecanoic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256
CE-1	22.850	1,1-dibutoxybutane	$C_{12}H_{26}O_2$	202
	34.926	1-butoxy-2-ethyl-1-hexene	$C_{12}H_{24}O$	184
	50.276	14-methyl-pentadecanoic acid-methyl ester	$C_{17}H_{34}O_2$	270
	56.001	9,12- octadecadienoicacid-methyl ester	$C_{19}H_{34}O_2$	294
	56.201	9,12,15-octadecatrienoic acid methyl ester (ZZZ)	$C_{19}H_{32}O_2$	292
	78.002	Octacosane	$C_{28}H_{58}$	394
CE-2	56.626	3,7,11,15-tetramethyl	$C_{20}H_{40}O$	296
		2-hexadecen-1-ol (Phytol)		
CE-3	56.351	9,12,15-Octadecatrienoic acid methyl ester (ZZZ)	$C_{19}H_{32}O_2$	292
CE-4	34.551	(E)-2-Decenal	$C_{10}H_{18}O$	154
	37.826	Tetradecane	$C_{14}H_{30}$	198
	45.626	3-Tetradecene (Z)	$C_{14}H_{28}$	196
	52.701	2,4-Bis(1,1-dimethyl ethyl)-phenol	$C_{14}H_{22}O$	206
	59.151	1-Hexadecanol	$C_{16}H_{16}O_2$	240

in Table 3. The identified compounds (Fig. 1) are 11-Tridecen-1-ol and hexadecanoic acid (Palmitic acid) from hexane extract while 1,1-dibutoxybutane; 1-butoxy-2-ethyl-1-hexene; 14-methyl-pentadecanoic acid-methyl ester; 9,12- octadecadienoicacid-methyl ester; 9,12,15-octadecatrienoic acid methyl ester (ZZZ); Octacosane; 3,7,11,15-tetramethyl-2-hexadecene-1-ol (Phytol); (E)-2-Decenal; Tetradecane; 3-Tetradecene(Z); 2,4-Bis(1,1-dimethyl ethyl)-phenol and 1-Hexadecanol from the four pooled VLC fractions (CE-1 to CE-4) of chloroform extract.

The compounds characterized by GC-MS are being reported for the first time from this plant and many of them are bioactive. The palmitic acid has been isolated from many plants and posses antibacterial and cholesterolaemic effects, selective toxicity to human leukemic cells. It also has shown *in vivo* antitumor activity in mice by making a target to DNA topoisomerase I (Harada *et al*., 2002; Saxena *et al*., 2007). Further, palmitic acid and its ester derivatives have antitubercular activity (Saikia *et al*., 2010). On the other hand the second bioactive phytomolecule, phytol has been reported from many plants including *Calotropis procera* (Ait.) and may find its use in treatment of rheumatoid arthritis and inflammation (Okiei *et al*., 2009). The third antibacterial constituent, 2, 4-bis (1, 1-dimethylethyl)-phenol may find use in eye infections (Ogunlesi *et al*., 2010). With these bioactive constituents, *A. multiflora* may be used in the

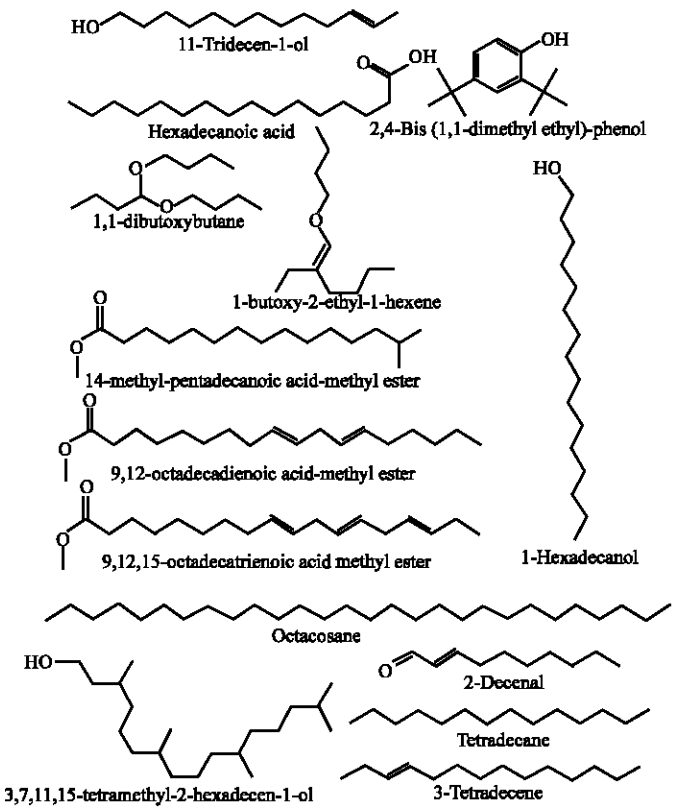

Fig. 1: Structure of compounds characterized by GC-MS

development of future antibacterial and antifungal herbal formulations. Further attempts to isolate and characterize other bioactive phytomolecules from *A. multiflora* using modern isolation techniques are in process.

CONCLUSION

The present GC-MS study revealed the presence of medicinally useful constituents in *A. multiflora*. Further, phytochemical screening of *A. multiflora* indicated presence of glycosides, flavonoids, carbohydrates, steroids, phenols and sterols. Since the methanolic extract of roots of *A. multiflora* have shown potent *in vitro* antimalarial activity (IC_{50} = 18 μg mL^{-1}), the activity guided fractionation and characterization of bioactive phytomolecules are necessary for future study. Thus, the plant studied here can be seen as potential source of new useful drugs.

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