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Phytochemical studies on *Allamanda cathartica* L. using GC-MS

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ABSTRACT

Objective: To explore the phytochemical constituents present in *Allamanda cathartica* (*A. cathartica*) L. using GC-MS. **Methods:** 20 g of the powdered leaf and stem sample of *A. cathartica* was equilibrated with 200 d/m of *A. cathartica* ethanol for 24 h, separately. The volume of the supernatant was later reduced by heating to 2 d/m. The concentrated ethanolic extracts were further subjected to GC-MS analysis. **Results:** The GC-MS analyses determined the presence of 28 different phytochemical compounds in the ethanolic leaf extract of *A. cathartica*. The major phytoconstituents were 9,12,15-octadecatrienoic acid (Z,Z,Z)- (16.39%), *n*-hexadecanoic acid (14.08%), 3-O-methyl-d-glucose (11.03%) and 9,12,15-octadecatrienoic acid ethyl ester (Z,Z,Z)- (10.58%). The ethanolic stem extract of *A. cathartica* showed the presence of 26 different bioactive compounds. The major ones are 3-O-methyl-d-glucose (29.86%), 2-furancarboxaldehyde 5-(hydroxymethyl)- (14.87%), *n*-hexadecanoic acid (9.13%) and 9,12,15-octadecatrienoic acid (Z,Z,Z)- (7.34%). **Conclusions:** This study helps to predict the formula and structure of biomolecules which can be used as drugs and further investigation may lead to the development of drug formulation.

1. Introduction

Medicinal plants form the backbone of traditional medicine in the last few decades with intense pharmacological studies. They are regarded as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. In developing countries, it is estimated that about 80% of the population really depends on traditional medicine for their primary healthcare. There arises a need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies[1]. Plants are rich sources of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties[2]. Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant[3].

The family Apocynaceae consists of several important medicinal plants with wide range of biological activities and interesting phytochemical constituents. *Allamanda cathartica* L (*A. cathartica*). commonly known as the Yellow Bell, Golden Trumpet or The Buttercup flower is a genus of tropical shrubs and vines belonging to the family Apocynaceae. Hailing from tropical America, Brazil in particular, this genus was named after 18th century Swiss Botanist Dr. Frederic Allamand, who sent its seeds to Linnaeus, the Swedish Botanist. The word 'cathartica' means purgative[4]. Because of its rapid growth, pruning is often necessary, which can expose gardeners to the toxic sap that causes dermatitis symptoms of rash, blisters, and itch. Although incidence is much less, plant parts are toxic if ingested. All parts contain the toxic iridoid lactone, allamandin[5]. The bark, latex and the infusion of its leaves in small doses is cathartic. The decoction of its bark is a hydragogue. In Guyana, its latex is employed as a purgative and for relieving colics. It has also been implicated in the treatment of malaria and jaundice. There has been no report stating the use of *A. cathartica* for medicinal purposes in

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India[6]. Moreover, all the plant parts are reported to be poisonous and hence the plant has not been extensively used in medicine. In the last few years GC-MS has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non plant species[7-9]. Therefore, the present study was aimed to explore the phytochemical constituents of *A. cathartica* using GC-MS analysis.

2. Materials and methods

2.1. Collection and preparation of plant material

Healthy, disease free plants of *A. cathartica* L. were collected from the natural habitats of Tiruchirappalli district, Tamil Nadu, India. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The stem and leaves of *A. cathartica* were shade dried separately and ground into fine powder using mortar and pestle. The powdered materials were stored in air tight polythene bags until use.

2.2. Plant sample extraction

20 g of the powdered leaf and stem sample of *A. cathartica* was equilibrated with 200 d/m of AR ethanol for 24 h. The volume of the supernatant was later reduced by careful heating to 2 d/m. The concentrated ethanolic extracts of the leaf and stem samples of *A. cathartica* were analyzed using the Clarus 500 GC-MS (Perkin Elmer) equipment at the Indian Institute of Crop Processing Technology, Thanjavur, Tamilnadu, India.

2.3. GC-MS analysis

The Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 (100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1 μm df) and the components were separated using Helium as carrier gas at a constant flow of 1 mL/min. The 2 μL sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC extraction process, the oven was maintained at a temperature of 110 ℃ with 2 minutes holding. The injector temperature was set at 250 ℃ (mass analyser). The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200 ℃; Source temperature: 200 ℃). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da.

2.4. Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique, WILEY8 and FAME having more than 65 000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The name, molecular weight, molecular formula and structure of the component of the test material was ascertained[10]. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC MS solution ver. 2.53.

3. Results

The results pertaining to GC-MS analysis leads to the identification of number of compounds from the GC fractions of the ethanolic extract of *A. cathartica* L stem and leaves. These compounds were identified through mass spectrometry attached with GC. The various components present in the leaf and stem of *A. cathartica* that were detected by the GC-MS are shown in Table 1 and 2. Glycerin, Hexanoic acid, ethyl ester, propane,1,1,3-triethoxy-, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, decanoic acid,ethyl ester, *n*-hexadecanoic acid, hexadecanoic acid,ethyl ester, tetradecanoic acid, 9,12-octadecadienoic acid (Z,Z)-, 9,12,15-octadecatrienoic acid,(Z,Z,Z)-, 9,12-octadecadienoic acid,ethyl ester, octadecanoic acid,ethyl ester and 2-phenanthrenecarboxaldehyde, 1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-7-hydroxy-2,4b,8,8-tetramethyl were commonly present in the stem and leaves extracts of *A. cathartica*. Thymine, heptanediamide,N,N′-di-benzoyloxy, 2-furancarboxaldehyde, 5(hydroxymethyl)-, à-D-glucopyranoside, O-à-D-glucopyranosyl-(1.fwdarw.3)-à-D-fructofuranosyl, D-glucopyranoside, dodecanoic acid, ethyl à-d-glucopyranoside, 3-O-methyl-d-glucose, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 6,7-dimethylthieno (2,3-b) quinolin-3-ylamine, phytol and oleic acid were present only in stem extracts of *A. cathartica*. The leaves extracts of *A. cathartica* showed their uniqueness by the presence of octanoic acid, ethyl ester, benzoic acid, 2-hydroxy-, methyl ester, 1-deoxy-d-mannitol, beta-L-arabinopyranoside-methyl, 3-O-methyl-d-glucose, 11-oxatetracyclo[4.2.1.1(2,5).1(7,10)]undec-3-ene,9-methoxy-9-methyl-, cyclobuta(1,2:3,4) dicyclooctene-1,7(2H,6bH)-dione,dodecahydro-,(6aà,6bà,12aà,12bà)-, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, pentadecanoic acid, cis,cis,cis-7,10,13-hexadecatrienal, phytol, podocarpa-1,12-diene-ë14, à-acetic acid, 7-hydroxy-8,13-dimethyl-3-oxo-,ë-lactone, nonadecanoic acid, ethyl ester, vitamin E and squalene. The GC-MS spectrum confirmed the presence of various components with different retention times as

Table 1.
Compounds identified in the ethanolic leaf extract of *A. cathartica* by GC-MS.

S. No	RT	Name of the compound	Molecular Formula	MW	Peak Area%	Compound Nature	Uses
1	3.69	Glycerin	$C_3H_8O_3$	92	2.29	Alcohol	Flavor
2	4.01	Hexanoic acid, ethyl ester	$C_8H_{16}O_2$	144	0.23	Ester	Antifungal, flavor
3	4.99	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	0.14	Ether	Flavor
4	6.17	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	0.17	Flavonoid fraction	**
5	6.67	Octanoic acid, ethyl ester	$C_{10}H_{20}O_2$	172	0.07	Ester	Anticandidal, antifungal, pesticide
6	6.89	Benzoic acid, 2-hydroxy-, methyl ester	$C_8H_8O_3$	152	0.06	Aromatic compound	Allergenic, anesthetic, antibacterial, antipyretic, antisalmonella, antiseptic, antifungal, antiyeast, insectifuge
7	9.41	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200	0.09	Ester	Nematicide, pesticide
8	11.62	1-deoxy-d-mannitol	$C_6H_{14}O_5$	166	3.88	Alcohol	Allergenic, analgesic, anthelmintic, antioxidant, antispasmodic, anti-inflammatory, diuretic, laxative, nephrotoxic, sweetener
9	12.58	Beta-L-arabinopyranoside, methyl	$C_6H_{12}O_5$	164	2.01	Sugar moiety	**
10	13.85	3-O-methyl-d-glucose	$C_7H_{14}O_6$	194	11.03	Sugar moiety	**
11	14.27	oxatetracyclo[4.2.1.1(2,5).1(7,10)]undec-3-ene, 9-methoxy-9-methyl-	$C_{12}H_{16}O_2$	192	0.37	Hydrocarbon	**
12	14.33	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.36	Fatty acid	**
13	14.86	Cyclobuta(1,2:3,4)dicyclooctene-1,7(2H,6bH)-dione, dodecahydro-, (6aà,6bà,12aà,12bà)-	$C_{16}H_{24}O_2$	248	0.86	Hydrocarbon	**
14	15.58	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.61	Terpene alcohol	**
15	15.81	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.25	Fatty acid	Antioxidant
16	16.27	cis,cis,cis-7,10,13-hexadecatrienal	$C_{16}H_{26}O$	234	0.95	Alcohol	**
17	17.42	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256	14.08	Palmitic acid	**
18	17.75	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	3.40	Ester	**
19	19.81	Phytol	$C_{20}H_{40}O$	296	5.66	Diterpene	Anticancer
20	20.25	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	16.39	Linolenic acid	**
21	20.42	9,12-octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	2.62	Linoleic acid	**
22	20.52	9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	10.58	Ester	**
23	20.91	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	1.65	Ester	**
24	22.80	Podocarpa-1,12-diene-ë14,à-acetic acid, 7-hydroxy-8,13-dimethyl-3-oxo-, ë-lactone	$C_{21}H_{26}O_3$	326	1.04	Lactone compound	**
25	23.28	2-phenanthrenecarboxaldehyde, 1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-7-hydroxy-2,4b,8,8-tetramethyl-	$C_{19}H_{30}O_2$	290	8.44	Aromatic compound	**
26	24.00	Nonadecanoic acid, ethyl ester	$C_{21}H_{42}O_2$	326	0.53	Ester	**
27	26.09	Vitamin E	$C_{29}H_{50}O_2$	430	4.63	Vitamin E	**
28	31.27	Squalene	$C_{30}H_{50}$	410	6.60	Triterpene	Antibacterial, antioxidant, antitumor, anticancer, immunostimulant, pesticide, chemopreventive

Table 2.
Compounds identified in the ethanolic stem extract of *A. cathartica* by GC-MS.

RT	Name of the compound	Molecular formula	Molecular weight	Peak area%	Compound nature	Uses
3.72	Glycerin	$C_3H_8O_3$	92	2.90	Alcohol	Flavor
4.01	Hexanoic acid, ethyl ester	$C_8H_{16}O_2$	144	0.53	Ester	Antifungal, flavor
4.99	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	0.68	Ether	Flavor
5.29	Thymine	$C_5H_6N_2O_2$	126	2.60	DNA base	**
6.16	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	1.67	Flavonoid fraction	**
6.45	Heptanediamide, N,N′-di-benzoyloxy-	$C_{21}H_{22}N_2O_6$	398	1.35	Alkaloid	**
6.67	Octanoic acid, ethyl ester	$C_{10}H_{20}O_2$	172	0.16	Ester	Anticandidal, antifungal, pesticidal
7.06	2-furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	14.87	Aldehyde	**
8.36	à-D-Glucopyranoside, O-à -D-glucopyranosyl-(1.fwdarw.3)-à -D-fructofuranosyl	$C_{18}H_{32}O_{16}$	504	0.52	Sugar	**
9.40	Decanoic acid, ethyl ester	$C_{12}H_{24}O_2$	200	0.32	Ester	Nematicide, pesticide
10.78	-D-glucopyranoside, O-à -D-glucopyranosyl-(1.fwdarw.3)-à -D-fructofuranosyl	$C_{18}H_{32}O_{16}$	504	2.42	Sugar	**
11.62	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.40	Fatty acid	**
12.66	Ethyl à-d-glucopyranoside	$C_8H_{16}O_6$	208	5.88	Sugar	**
14.17	3-O-methyl-d-glucose	$C_7H_{14}O_6$	194	29.86	Sugar	**
14.33	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.23	Fatty acid	**
15.57	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.77	Terpene alcohol	**
17.39	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256	9.13	Palmitic acid	**
17.73	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.57	Ester	**
18.65	6,7-dimethylthieno(2,3-b)quinolin-3-ylamine	$C_{13}H_{12}N_2S$	228	2.73	Alkaloid	**
19.79	Phytol	$C_{20}H_{40}O$	296	0.86	Diterpene	Anticancer
20.09	9,12-octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	3.49	Linoleic acid	**
20.19	9,12,15-octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	7.34	Linolenic acid	**
20.39	9,12-Octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	1.83	Ester	**
20.49	Oleic acid	$C_{18}H_{34}O_2$	282	4.00	Fatty acid	5-alpha-reductase inhibitor, allergenic, anti-inflammatory, anticancer, insectifuge
20.87	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.49	Ester	**
23.23	2-phenanthrenecarboxaldehyde, 1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-7-hydroxy-2,4b,8,8-tetramethyl-	$C_{19}H_{30}O_2$	290	2.41	Aromatic compound	**

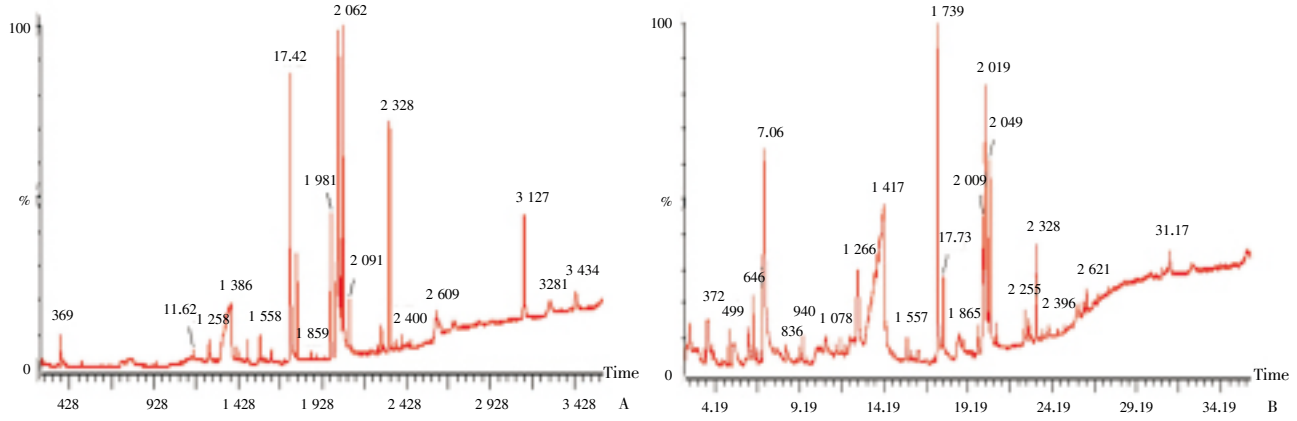

Figure 1. GC-MS chromatogram of ethanolic leaf extract of *A. cathartica*.

illustrated in Figure 1A and B. The biological activities listed are based on Dr. Duke's phytochemical and ethnobotanical databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

4. Discussion

The prediction of the biological activities by applying the Duke's databases was confirmed with previous observations and supplemented the traditional usage of the *A. cathartica*[11-18]. By interpreting these compounds, it is found that *A. cathartica* possess various therapeutic application. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in *A. cathartica*. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. The presence of phytochemicals have been shown to possess antifungal, anti-candidal, allergenic, anesthetic, antibacterial, antipyretic, antiseptic, insectifuge, analgesic, anthelmintic, antioxidant, antispasmodic, anti-inflammatory, diuretic, laxative, nephrotoxic, antitumor, anticancer, immunostimulant, chemopreventive and nematicide[19]. Hence, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of *A. cathartica*.

The present study helps to predict the formula and structure of biomolecules which can be used as drugs. This also enhances the traditional usage of *A. cathartica* which possess several known and unknown bioactive compounds. Further investigation may lead to the development of drug formulation.

Conflict of interest statement

We declare that we have no conflict of interest.

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