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Research Article

ISOLATION OF SOME POTENTIAL PHYTOCOMPOUNDS FROM *ADHATODA VASICA* THROUGH GAS CHROMATOGRAPHY-MASS SPECTROSCOPY ANALYSIS

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ABSTRACT

Objective: The aim of this study is to screen the medicinal compounds present in the leaves, shoots, and flowers of *Adhatoda vasica* by gas chromatography-mass spectroscopy (GC-MS) analysis.

Methods: Plant leaves, shoots, and flowers were collected, washed, shade dried, and powdered. Methanol extracts of all plant parts were prepared by soxhlation method. All the plant part extracts were analyzed for the identification of phytocompounds present in plant parts using GC-MS and matched by the National Institute of Standards and Technology library.

Results: A wide range of fatty acids and the heterocyclic compound was identified which is responsible for antibacterial, antifungal, anti-inflammatory, and antimycotic activity.

Conclusion: The study concludes that *A. vasica* have many important important biologically compounds so it can be recommended as a plant of pharmaceutical importance.

Keywords: *Adhatoda vasica*, Medicinal plant, Screen, Phytocompounds, Gas chromatography-mass spectroscopy.

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INTRODUCTION

India is the biggest producer of medicinal plants with the vast geographical area and is also known as the botanical garden of the world. Medicinal plants have several medicinal important compounds such as flavonoids, alkaloids, essential oils, peptides, phenols, and unsaturated long chain fatty acids, which show antibacterial, antifungal, and antiviral activities [1]. These phytochemicals have the important role of protection against diseases. The major advantage of plant-based natural drugs over synthetic drug is that natural drugs are safe, have fewer side effects and are biodegradable. According to the World Health Organization, around 80% of the world's population use plants for their primary health care [2].

Adhatoda vasica is a highly valued Indian medicinal plant belongs to Acanthaceae family. It is also known as *Justicia adhatoda* (Linn.), Vasaka and Malabar nut. *A. vasica* is an erect, terrestrial, and perennial shrub. The leaves are dark green above, and pale yellow below and the flowers are small irregular zygomorphic, bisexual, and hypogynous arranged in a pedunculated spike [3]. The plant leaves, flowers, fruit, and roots are used extensively in the treatment of asthma, cold cough, bronchitis and tuberculosis, joint pain, lumbar pain, sprains, eczema, malaria, rheumatism, swellings, and venereal diseases [4,5].

Plant leaves are anti-inflammatory and effective in skin disorders and cardiotonic [6]. *A. vasica* also decrease the viscosity of mucous to assist with expectoration and causes thinning of sputum and phlegm and asthma.

In Southeast Asia, various preparations of leaves are used for curing bleeding, hemorrhage, skin diseases, wounds, headache, snake-bites, and leprosy [7,8]. The yellow leaves are used for a cough in North India and thus used as an ingredient in cough syrups as an expectorant and antispasmodic [9].

The plant has certain components such as alkaloids, terpenes, triterpenes, phytosterols, flavonoids, essential oils, hydrocarbon, and fatty acids. These components in *A. vasica* are responsible for antimicrobial, antiasthmatic antitubercular activity, and wound-healing activity.

Gas chromatography-mass spectrometry (GC-MS) is a combination of two different analytical techniques, Gas Chromatography (GC). GC is a technique used to analyze drugs, present in a sample. There are a few reports available in literature on the extraction of phytochemical constitutes in various solvent and analysis of bioactive compound present in *A. vasica* plant parts using GC-MS. Thus, the present research is aimed to investigate phytochemical analysis and the chemical composition of the selected solvent extract of GC-MS study.

METHODS

Collection of plant materials

A. vasica plant was collected from Amer, Jaipur district, Rajasthan, and India. The plant was identified by herbarium, University of Rajasthan, Jaipur, and preserved in the laboratory of JECRC University, Jaipur.

Preparation of plant materials

Plant parts were placed in the laboratory and washed in running tap water to remove dust. Then, plant parts (stems, leaves, and flowers) were cut into pieces, shade-dried in a dust free environment and pulverized to a powder using a grinder separately for extraction. The dry powder was preserved in airtight polythene cover.

Preparation of samples

The dried powdered samples of plant parts were extracted in Soxhlet extraction unit [10,11]. Extraction was done in water and 80% methanol for 24 hrs on heating mental. The plant extracts are separately filtered using Whatman No. 1 filter paper. The filtrate of methanol extracts was

then evaporated by heating at water bath at 60°C. The obtained extracts were stored for GC-MS analysis (Fig. 1).

Interpretation of mass spectrum

The plant extracts were subjected to GC-MS analysis. For this 1 mg/ml concentration of the extracted samples is prepared in methanol. The preparation was kept in a sterile glass vial and analyzed for GC-MS.

GC-MS was recorded in a GC-MS-2010 Shimadzu instrument operating in EI mode at 70 ev. A Restek-5MS column (30 m × 0.25 mm × 0.25 µm) was used. The oven temperature program was 60° raised to 280°C at 5°C/min and held for 2 minutes, then 250-280°C at 10°C/min and held for 14 minutes at 280°C. The injector temperature was 290°C with normal injection mode. The flow rate of carrier gas, helium was 1.00 ml/min. A scan interval of 0.5 seconds with a scan range of 41-600 total GC running time was 30 minutes. The identification of compounds was confirmed by comparing the mass spectral data with data obtained from the literature.

RESULTS

Identification of components

Identification of phytocompounds was based on the principles of retention time, molecular formula, molecular weight (MW), and concentration (peak area %). It is done to determine some compounds present in plants having any medicinal value. The GC mass spectrum of the sample was interpreted using the database of National Institute of Standards and Technology (NIST) having more than 2,00,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The names of compounds with MW and structure of the test materials were ascertained [12].

***A. vasica* leaf extract**

Fig. 2 shown in the GC/MS analysis of leaf extracts of *A. vasica* revealed the existence of several compounds. The major compounds identified in *A. vasica* leaf in terms of area percentage are 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (26.76), 9,12,15-octadecatrienoic acid, (Z,Z,Z)-, linolenic acid (19.44), hexadecanoic acid, methyl ester (16.82), cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] methyl]-, methyl ester (9.69), methyl (Z)-5,11,14,17-eicosatetraenoate(6.03), and binaphthyl sulfone (2.16).

***A. vasica* shoot extract**

Fig. 3 shown in the major compounds identified in *A. vasica* shoot in terms of area percentage are 9,12-octadecadienoic acid, methyl ester (39.59), n-hexadecanoic acid (25.86), tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy- (10.39), mannitol, 1,3,4,5-tetra-O-methyl-, diacetate, D- (4.64), methanone, [4-(dimethylamino)phenyl]phenyl- (3.64), undecane, 6-cyclohexyl- (2.02), linoleic acid trimethylsilyl ester (1.41), and 1-dimethyl(3-chloropropyl) silyloxyoctane (1.46).

Fig. 1: Samples for gas chromatography-mass spectrometry analysis

***A. vasica* flower extract**

Fig. 4 show in the similarly, the major compounds identified in *A. vasica* flower in terms of area percentage are n-hexadecanoic acid (28.47), 9,12-octadecadienoic acid (Z,Z)- (17.68), 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (13.98), octadecanoic acid (11.77), dibutyl phthalate (6.67), hexadecanoic acid, methyl ester (6.53), and 9,12-octadecadienoic acid (Z,Z)-, methyl ester (5.02).

DISCUSSION

In the phytochemical screening of methanol extract of *A. vasica* leaves, shoot, and flower powder showed positive results for most of the phytochemical constituents. According to the results, most of the compounds identified, are medicinally very important. For medical practitioners, the role of phytochemical compounds is helpful for the cure of many infections. The most of the compounds identified in this study belong to a fatty acid. Many fatty acids are known to have antibacterial and antifungal properties [13]. Pentadecanoic acid, tetradecanoic acid, hexadecanoic acid, octadecanoic acid, and oleic acids are among the fatty acids known to have potential antibacterial and antifungal activity [14,15]. The activity of compounds was identified from Dr. Duke's phytochemical and ethnobotanical database [16] and given in Tables 1-3.

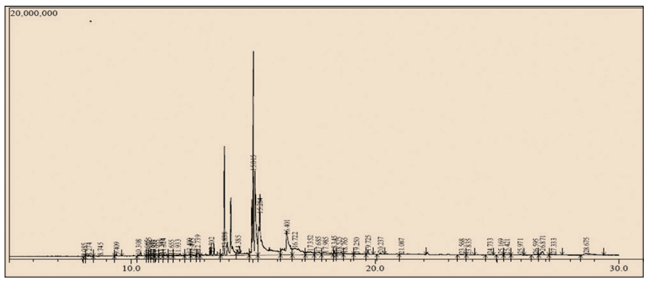


Fig. 2: Gas chromatography-mass spectrometry chromatogram of *Adhatoda* vasica leaf extract

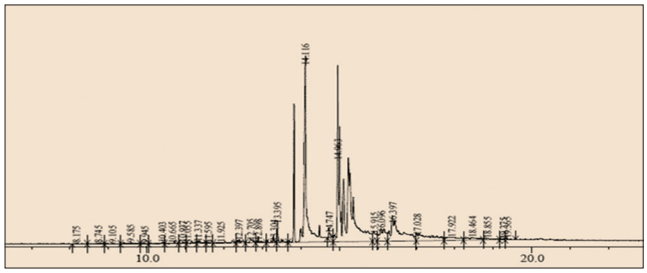


Fig. 3: Gas chromatography-mass spectrometry chromatogram of *Adhatoda vasica* shoot extract

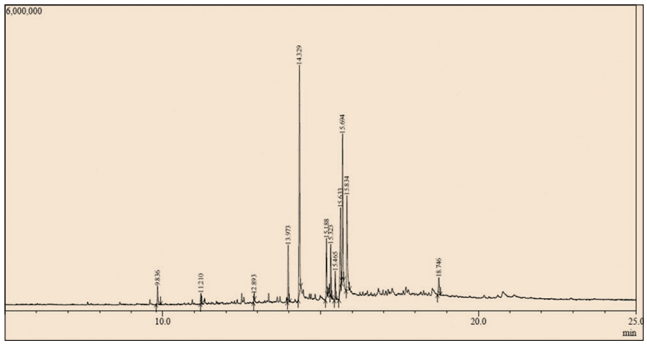


Fig. 4: Gas chromatography-mass spectrometry chromatogram of *Adhatoda vasica* flower extract

CONCLUSION

A. vasica plant and its parts are used for the treatment of asthma, bronchitis, bio-insecticides, and skin diseases. There are few reports on the isolation and identification of bioactive compounds from *A. vasica.* In this study methanolic extract of *A. vasica* leaf, shoot, and flowers were analyzed for the presence of active bioactive compounds by GC-MS analysis. The major compound identified by GC-MS belongs

Table 1: Phytocomponents identified in the methanol leaf extracts of *A. vasica*

S. No.	RT	Area	Area %	Compound name
1.	11.933	1204336	0.59	2,4-heptadiene, 2,4-dimethyl-
2.	12.739	1413434	0.69	2-propenoic acid, 3-(4-hydroxyphenyl)-, methyl ester
3.	13.302	5517844	2.70	2-pentadecanone, 6,10,14-trimethyl-
4.	13.838	34404595	16.86	Hexadecanoic acid, methyl ester
5.	15.015	54755840	26.82	9,12,15-octadecatrienoic acid, methyl ester, (Z, Z, Z)-
6.	15.284	39767291	19.49	9,12,15-octadecatrienoic acid, (Z, Z, Z)-, Linolenic acid
7.	16.401	19816699	9.69	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] methyl]-, methyl ester
8.	16.722	12339833	6.03	Methyl (Z)-5,11,14,17-eicosatetraenoate
9.	17.352	4428361	2.16	Binaphthyl sulfone
10.	17.685	2913202	1.42	5,5-dimethyl-1,3-dioxane-2-ethanol, tert-butyldimethylsilyl ether
11.	17.985	3525626	1.72	1,3-cyclohexanedione, 5-isopropyl
12.	18.525	2155917	1.05	Cis-4,7,10,13,16,19-docosahexaenoic acid, trimethylsilyl ester
13.	18.765	1698100	0.83	Binaphthyl sulfone
14.	19.250	1619081	0.79	Diazoprogesterone
15.	19.725	1079474	0.53	Pyrrolo[2,1-b] quinazolin-9 (1H)-one, 3-[2-(dimethylamino) phenyl]-2,3-dihydro-
16.	21.087	1017568	0.50	Dodecanoic acid, 1,2,3-propanetriyl ester
17.	23.568	461550	0.35	Stigmasta-3,5-dien-7-one
18.	26.871	1834751	0.90	2-(6-Chloro-benzo[1,3]dioxol-5-ylmethylsulfanyl)-9H-1,3,4,9-tetraaza-fluorene
19.	28.675	2814052	1.38	Gamma-sitosterol

RT: Retention time, *A. vasica: Adhatoda vasica*

Table 2: Phytocomponents identified in the methanol shoot extracts of *A. vasica*

S. No.	RT	Area	Area %	Compound name
1.	12.397	1142521	0.50	Methyl tetradecanoate
2.	12.705	1314765	0.57	Tetradecanoic acid
3.	12.898	1040958	0.45	Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide
4.	13.304	1174574	0.51	2-pentadecanone, 6,10,14-trimethyl-
5.	13.395	2811456	1.22	2-propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester
6.	14.116	59518139	25.86	n-hexadecanoic acid
7.	14.747	1107599	0.68	Heptadecanoic acid
8.	14.963	91112275	39.59	9,12-octadecadienoic acid, methyl ester
9.	15.915	3356623	1.46	1-dimethyl (3-chloropropyl) silyloxyoctane
10.	16.096	8375018	3.64	Methanone, [4-(dimethylamino) phenyl] phenyl-
11.	16.397	23919977	10.39	Tricyclo [20.8.0.0 (7,16)]triacontane, 1 (22),7 (16)-diepoxy-
12.	17.028	10679999	4.64	Mannitol, 1,3,4,5-tetra-O-methyl-, diacetate, D-
13.	17.922	4643559	2.02	Undecane, 6-cyclohexyl-
14.	18.464	3242496	1.41	Linoleic acid trimethylsilyl ester
15.	18.855	1462856	0.80	7-hexadecenoic acid, methyl ester, (Z)-
16.	25.185	997590	0.43	Octadecanoic acid, 4-methoxy-, methyl ester
17.	25.518	1914437	0.83	Cholestane-3. beta.,5. beta.-diol
18.	26.523	1446286	0.63	5-Cholestene-3-ol, 24-methyl-
19.	28.635	2693012	1.17	Gamma-sitosterol

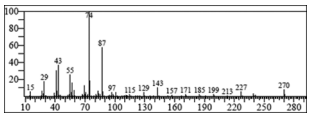
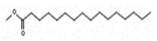
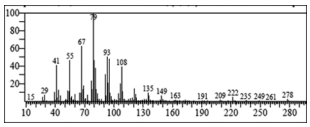
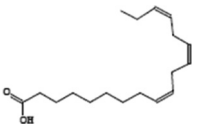
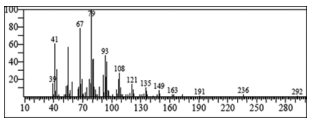
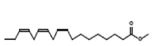
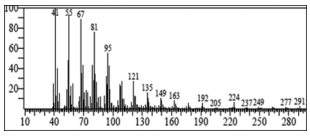
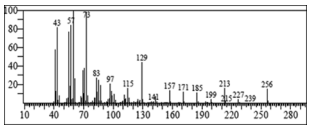
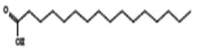
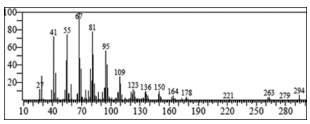
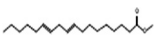
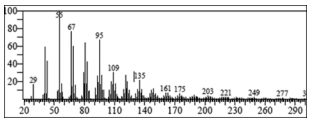
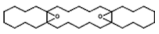
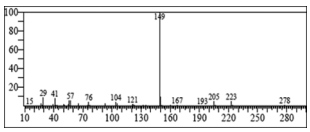
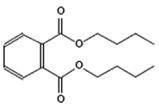
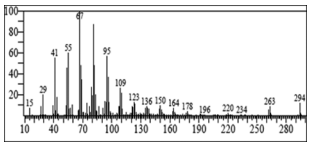
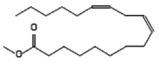
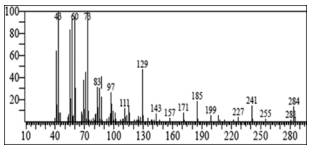
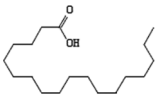
RT: Retention time, *A. vasica: Adhatoda vasica*

Table 3: Phytocomponents identified in the methanol extracts of *A. vasica* flower

S. No.	RT	Area	Area %	Compound name
1.	9.836	516043	2.21	Hydroquinone
2.	11.210	271287	1.16	Butylated hydroxytoluene
3.	12.893	163495	0.70	Tetradecanoic acid
4.	13.973	1525337	6.53	Hexadecanoic acid, methyl ester
5.	14.329	6651477	28.47	n-hexadecanoic acid
6.	15.188	1558643	6.67	Dibutyl phthalate
7.	15.323	1173754	5.02	9,12-octadecadienoic acid (Z, Z)-, methyl ester
8.	15.465	718923	3.08	9,12,15-octadecatrienoic acid, methyl ester
9.	15.633	2749691	11.77	Octadecanoic acid
10.	15.694	4130083	17.68	9,12-octadecadienoic acid (Z, Z)-
11.	15.834	3266777	13.98	9,12,15-octadecatrienoic acid, (Z, Z, Z)-
12.	18.746	634285	2.72	Bis (2-ethylhexyl) phthalate

RT: Retention time, *A. vasica: Adhatoda vasica*

Table 4: Major compound identified by GC-MS in *A. vasica*

S. No.	Name of compound	GC-MS chromatogram	Structure
1.	Hexadecanoic acid		
2.	9,12,15-octadecatrienoic acid, (Z, Z, Z)		
3.	9,12,15-octadecatrienoic acid		
4.	Cyclopropaneoctanoic acid		
5.	n-hexadecanoic acid		
6.	9,12-octadecadienoic acid		
7.	Tricyclo[20.8.0.0 (7,16)]triacontane		
8.	Dibutyl phthalate		
9.	9,12-octadecadienoic acid (Z, Z)-		
10.	Octadecanoic acid		

RT: Retention time, *A. vasica: Adhatoda vasica*, GC-MS: Gas chromatography-mass spectrometry

to fatty acids. These identified phytocompounds are presumed to be responsible for eliciting the traditional activity of *A. vasica* (Table 4).

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