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PHYTOCHEMICAL PROFILING AND GCMS STUDY OF *ADHATODA VASICA* LEAVES

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ABSTRACT

*Adhatoda vasica (*AV), a popular Indian medicinal plant, is a rich source of polyphenolic compounds especially flavonoids which are responsible for strong anti-oxidant properties that helps in the therapy of various respiratory diseases. In the present investigation, phytochemical screening and gas chromatography mass spectrometry analysis of AV leaves was carried out to evaluate the chemical composition in different solvent extraction which could be useful in future experimental studies. Phytochemical analysis confirmed the presence of alkaloids, flavaones, flavanoids, phytosterols, fixed oils, saponins, phenolic compounds, tannins, carbohydrates and glycosides in the ethanolic extract comparing to the other extracts. Interestingly, ten anti-inflammatory compounds like terpene alcohol, diterpene, linolenic acid, alkaloid, vitamin, steroid and sesquiterpene oxide were identified through GC- MS analysis in both the ethanol and methanol extract. In conclusion, the ethanol extract is more effective for extracting major active compounds and for therapeutic applications.

KEYWORDS : ***Adhatoda vasica*, GCMS, Vasicolinone, Phytochemicals**

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INTRODUCTION

The plant AV and its component vasicine and its derivatives are extensively being used for bronchodilatory/mucolytic preparations since history till date. With herbal renaissance happening all over the globe, medicinal herbs are staging a phenomenal comeback. Ethno-botanical information from India estimates that more than 6000 higher plant species forming about 40 % of the higher plant diversity are used in its codified and folk healthcare traditions[1].AV belong to the family *Acanthaceae*. It is a shrub found in most part of the Tamil Nadu. The plant is used extensively in the treatment of asthma, cough, bronchitis and tuberculosis, joint pain, lumber pain, sprains, eczema, malaria, rheumatism, swellings, venereal diseases, as an anti–hyperglycemic, anti–diarrhoeal, anti–convulsant and cytotoxic[2-7]. Quinazoline alkaloids present in the leaves are established as active principles. In the indigenous food preparations, AV leaves were made into a decoction with pepper and dried ginger. But the modern medicine searched its active ingredients and found out that vasicine, oxyvascicine and vasicinone are the alkaloids present in vasaka, the active ingredients for expelling sputum from the body[8]. Bromhexine, a synthetic derivative of the alkaloid vasicine found the market in the treatment of respiratory disorders.The plant is used as an ingredient of numerous popular formulations including cough syrups used in combination with ginger and tulsi where it exerts its action as an expectorant and antispasmodic[9]. Bisolvon, a branded drug containing vasaka as an ingredient is used to clear the airways by decreasing the mucus secretions and opening the air passages[10]. There are various herbal formulations, viz. Kada, Fermiforte, Spirote available for the treatment of various kinds of respiratory disorders[11-14].There are a few reports in the literature on the selection of solvent for the extraction of phytochemical constitutes and GCMS analysis of AV leaves. Thus, the present study is the investigation of suitability of extraction solvent through phytochemical qualitative analysis and the chemical composition of selected solvent extract by GCMS study.

MATERIALS AND METHODS

(i) Collection & authentication of plant material

The leaves of AV (*Acanthaceae)* were collected from the herbal garden, Tamil University, Thanjavur, India and authenticated by Professor Jagadeesan, Head, Department of Environmental and Herbal Sciences, where a voucher specimen was submitted.

(ii) Qualitative screening of phytochemicals in various solvent extracts of AV leaves

The leaves of AV was dried at 40º C in hot air oven, crushed by hand and ground into coarse powder (40 mesh size) using an laboratory mill. The powder was extracted with solvents like petroleum ether, methanol, chloroform, acetone, ethanol and water for 12 hours at room temperature followed by filtration through the whatman no.1 filter paper. Phytochemical screening of plant extracts was done following the standard procedure[15-16]. All the prepared plant leaf extracts were subjected to preliminary phytochemical screening for the presence of alkaloids, carbohydrates, glycosides, phytosterols, fixed oils, fat, saponins, phenolic compounds, tannins, flavones, flavanoids, proteins, amino acids, gums and mucilages by standard methods.

(iii) Chemical components identification through GCMS

Sample preparation:

The AV leaves (25 grams) powder was soaked in 40 ml of ethanol and 40 ml methanol and kept for overnight soaking. The sample was filtered and concentrated through nitrogen flushing to 1 ml. 2 µl of prepared sample was injected into the GC-MS instrument.

Equipment: GC Clarus 500 Perkin Elmer, Carrier gas: 1ml per min, Split: 10:1, Detector:

Mass detector Turbo mass gold-Perkin Elmer,Software: Turbomass 5.2, Sample injected: 2µl, Column: Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25mm x 0.25µm df , Oven temperature Programme: 110° C with 2 min hold ,Up to 200° C at the rate of 10 ° C/min without hold, Up to 280 ° C at the rate of 5° C / min with 9 min hold, Injector temperature 250° C, Total GC running time 36 min, Inlet line temperature 200°C, Source temperature 200°C Electron energy:70 eV, Mass scan (m/z): 45-450,Solvent Delay: 0-2 min, Total MS running time: 36 min[17]

Interpretation of mass spectrum GC-MS

In the MS Programme, NIST Version 2.0 library database of National Institute Standard and Technology (NIST) having more than 2,00,000 patterns was used for identifying the chemical components of the AV leaves. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

The preliminary phytochemical analysis of crude extracts of leaves of AV revealed the presence of various phytochemicals such as alkaloids, flavonoids, phenols, steroids and tannins (Table 1). Comparing the extraction solvents, alkaloids and flavanoids were present in chloroform, methanol, ethanol and water extracts, phenols were present in the ethanol and water leaf extract. Phytosterols were present in choloroform, acetone and ethanol extracts of leaf powder but absent in water extract. Ethanolic leaf extract showed the presence of all the phytochemicals when compared with the other extracts. The preliminary phytochemical studies received pronounced importance, because the AV possesses varied composition of secondary metabolites.

Table 1

Result of phytochemical investigation of various extracts of AV leaf powder

Plant constituent test/ reagent used	EXTRACTS					
	PE	ME	CE	AE	EE	WE
Alkaloids						
a) Mayer 's reagent	-	+	+	-	+	+
b) Dragendroff's reagent	-	+	+	-	+	+
c) Hager 's reagent	-	+	+	-	+	+
d) Wagner 's reagent	-	+	+	-	+	+
Carbohydrates & Glycosides						
a) Molich's Reagent	-	-	-	-	+	+
b) Fehling Solution	-	-	-	-	+	+
c) Barfoed's Test	-	-	-	-	+	+
d) Benedict's Reagent	-	-	-	-	+	+
e) Libermann-Burchard's Test	-	+	-	-	+	+
f) Legal's Test	-	-	-	-	+	+
g) Borntrager 's Test	-	-	-	-	+	+
Phytosterols						
a) Libermann's Sterol Test	+	-	-	+	+	-
b) Libermann – Burchard Test	+	+	-	+	+	-
Fixed oil & Fats						
a) Spot Test	+	-	-	-	-	-
b) Saponification Test	+	-	-	-	-	-
Saponins						
a) Foam Test	-	+	-	-	+	+
b) Haemolysis Test	-	+	-	-	+	+
Phenolic compounds & Tannis						
a) with Ferric Chloride Solution	-	+	-	-	-	+

b) with Gelatin Solution	-	-	-	-	-	+
c) with Lead acetate Solution	-	-	-	+	+	-
d) with Aqueous bromine Solution	-	-	-	-	+	+
Proteins & Amino acids						
a) Millon's Reagent	-	+	-	-	+	+
b) Biuret Test	-	+	-	-	+	+
c) with Ninhydrin Reagent	-	+	-	-	+	+
Gums & Mucilages						
a) Alcoholic Precipitation test	-	-	-	-	-	+
b) Molisch's Test	-	-	-	-	-	+
Flavones & Flavonoids						
a) with Aq.NaOH	-	+	+	-	+	+
b) with Con.H2SO4	-	+	+	-	+	+
c) with Mg.+ HCl	-	+	+	-	+	+

+ : Positive, - : Negative, PE - Petroleum ether Extract, ME – Methanol Extract, CE – Chloroform Extract, AE – Acetone Extract, EE – Ethanolic Extract, WE – Water Extract

The GC/MS spectral results and comparison of results with library search successfully enabled the identification of the eighteen compounds belonging to the various groups like aminoacids, ester, terpene alcohol, diterpene, linolenic acid, alkaloid, vitamin, steroid, sesquiterpene oxide and nitrogen compounds. The active principles with their retention time (RT), compound name, molecular formula, activity are given in the Table 2. The GCMS chromatogram of both ethanolic and methanolic extracts was given in figure 1 & 2.

Table 2

Chemical compounds identified in ethanol and methanol extracts of the AV leaves

No	Retention Time	Compounds in the Ethanol / Methanol extract	Molecular Formula	Compound Nature	*Activity
1.	6.13[E], 7.23[M]	Benzenemethanol, à-[1-(methylamino)ethyl]-	$C_{10}H_{15}NO$	Amino compound	Antimicrobial
2.	7.38[M]	Cyclopentaneethanamine, N,à-dimethyl-	$C_9H_{19}N$	Amino compound	Antimicrobial
3.	8.53[E], 8.59[M]	Pseudoephedrine, (+)-	$C_{10}H_{15}NO$	Alkaloid	Antimicrobial, Anti-inflammatory
4.	11.05[E], 11.04[M]	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Terpene alcohol	Antimicrobial, Anti-inflammatory
5.	11.91[E] ,11.90[M]	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	$C_6H_7N_3O_2$	Amino compound	Antimicrobial
6.	12.79[E]	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid	Antioxidant, Hypo-cholesterolemic, Hemolytic 5-Alpha reductase inhibitor
7.	14.18[E], 14.17[M]	1-Eicosanol	$C_{20}H_{42}O$	Alcoholic compound	Antimicrobial
8.	14.23[E], 14.23[M]	Phytol	$C_{20}H_{40}O$	Diterpene	Antimicrobial, Anti-inflammatory
9.	14.93[E], 15.08[M]	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	Linolenic acid ester	Antiinflammatory, Hypocholesterolemic, Antihistaminic.
10.	16.61[E]	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	Unsaturated fatty acid	Anticholestrol
11.	18.92[E], 18.90[M]	Ethanethioic acid, S-[2-(dimethylamino)ethyl] ester	$C_6H_{13}NOS$	Ester compound	Antimicrobial
12.	23.18[E], 23.18[M]	Pyrrolo[2,1-b]quinazolin-9(1H)-one, 3-[2-(dimethylamino)phenyl]-2,3-dihydro-	$C_{19}H_{19}N_3O$	Alkaloid	Antimicrobial, Anti-inflammatory
13.	23.68[E], 23.60[M]	Squalene	$C_{30}H_{50}$	Triterpene	Antibacterial, Antioxidant, Immunostimulant
14.	27.86[E], 27.81[M]	Vitamin E	$C_{29}H_{50}O_2$	Vitamin compound	Antiinflammatory,Antioxidant, Vasodilator, Antibronchitic,
15.	29.68[E]	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-	$C_{22}H_{32}O_2$	Steroid	Anti-inflammatory, Antimicrobial

		one, 3-hydroxy-, (3á,17á)-		compound	Antiasthma
16.	30.75 E	trans-Z-à-Bisabolene epoxide	$C_{15}H_2O$	Sesquiterpene oxide	Anti-tumor, Anti-inflammatory
17.	31.37 E, 31.31 M	5à-Androstan-16-one, cyclic ethylene mercaptole	$C_{21}H_{34}S_2$	Steroid	Antimicrobial Anti-inflammatory Antiasthma
18.	32.37 E, 32.30 M	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	$C_{15}H_{26}O$	Sesquiterpene alcohol	Antiinflammatory

Note: E – Ethanol extract, M – Methanol extract. *Source: Dr.Duke's Phytochemical and Ethnobotanical Databases

The activity of compounds were identified from Dr. Duke's Phytochemical and Ethnobotanical database[16]. The anti-inflammatory compounds identified in the ethanol and methanol extracts were Benzenemethanol, à-[1-(methylamino)ethyl]- , Pseudoephedrine, (+)-, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, Phytol, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, Pyrrolo[2,1-b]quinazolin-9(1H)-one, 3-[2-(dimethylamino)phenyl]-2,3-dihydro-, Vitamin E, Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-, trans-Z-à-Bisabolene epoxide, 5à-Androstan-16-one, Cyclic ethylene mercaptole and 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-.

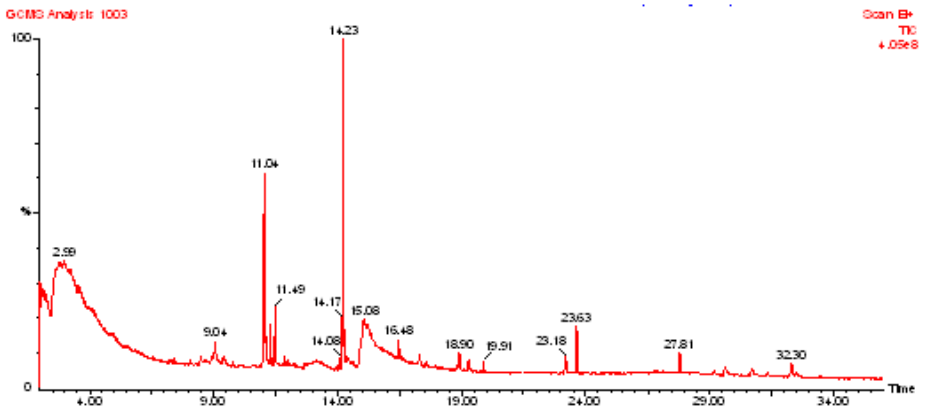

Figure 1
GCMS Chromotogram of methanolic extract of AV leaf powder

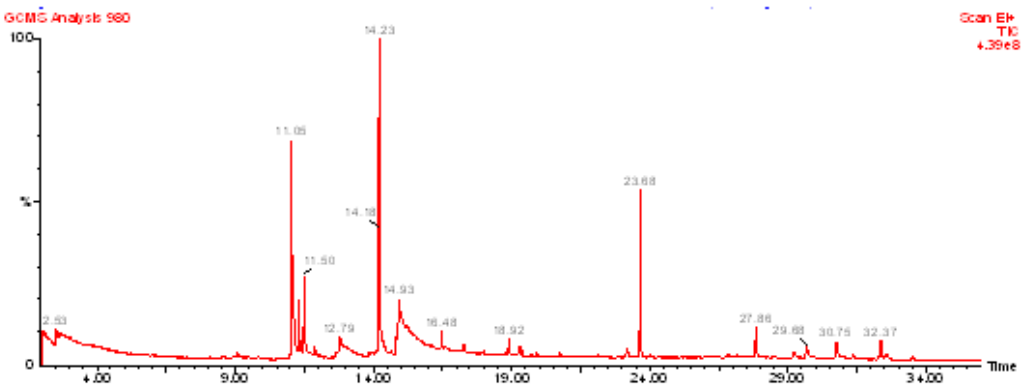

Figure 2
GCMS Chromotogram of ethanolic extract of AV leaf powder

DISCUSSION

In the phytochemical screening of different solvent extracts, the ethanolic extract of AV leaves powder showed positive results for most of the phytochemical constituents namely saponins, tannins, phenols, phytosterols, flavonoids, alkaloids, carbohydrates & glycosides, protein and aminoacids. The most suitable solvent for the extraction of active chemical ingredients from the AV leaves is alcoholic extracts.In the GCMS analysis, a compound named vasicolinone was identified, which is responsible for the small but persistent bronchodilatation[3] and a major alkaloid which is chiefly responsible for the expectorant action[18-19.] However, this may not be out of place to mention that the presence of vasicolinone (Pyrrolo[2,1-b]quinazolin-9(1H)-one, 3-[2-(dimethylamino)phenyl]-2,3-dihydro-) is the first report in the GCMS analysis of AV leaves. The presence of various bioactive compounds justifies the use of the plant leaves for various ailments by traditional practitioners[20].

CONCLUSION

The current study suggests that the active compounds were present in the ethanolic extract of AV leaves which are having the potent anti inflammatory and bronchodilator activity. It is concluded that the ethanol can be used for extracting active compounds from plants and incorporating into various medicinal/ food products. In addition, further research is necessary to identify and purify the active compounds responsible for therapeutic activity and animal study to evaluate the dosage of the identified chemical compounds.

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