GC-MS analysis of Bioactive Components of *Andrographis Alata* (Vahl) Nees (Acanthaceae)

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ABSTRACT

Introduction and Aim: The purpose of the present investigation was carried out to determine the various bioactive components by the ethanol extract of whole plant of *Andrographis alata* using GC-MS analysis.

Materials and Methods: The chemical composition of the ethanol extract of whole plant of *A. alata* was investigated using GC-MS (GC Clarius 500 perklin Elmer) analyser equipped with mass selective detector TQ Quadrupole mass spectrometer with national institute of standard and technology (NIST) library.

Results: Nineteen compounds were identified. The prevailing compounds were Alpha-1-rhamnopyranose, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, 9,12- Octadecadienoic acid (Z,Z)-, Oleic acid, Octadecanoic acid, n-Tetracosanol-1, Phytonadione, Ergosta-4,6,8(14),22-tetraen-3-one, Lupeol, 4H-1-Benzopyran-4-one,5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-, 4H-1-Benzopyran-4-one,5-hydroxy-6,7-dimethyl-2-phenyl-, Squalene, Cholestan-3-ol,2-methylene,(3β,5α)-, 4H-1-Benzopyran-4-one,5-(acetyloxy)-2-[3-(acetyloxy)-4-methoxyphenyl]-7-methoxy-, 4H-1-Benzopyran-4-one,5,7-dihydroxy-2-(3,4,5-trimethoxyphenyl)-, Anthroquinone,7-methoxy-2-methyl-1,4,5-trihydroxy-, Butylphosphonic acid, butyl 4-(2-phenylprop-2-yl)phenyl ester, Stigmasterol.

Conclusion: From the result it could be concluded that the whole plant of *A. alata* contains various bioactive compounds which have various medicinal properties.

Key Words: GC-MS, *A.alata*, Ethanol extract, Bioactive compounds.

INTRODUCTION

Medicinal plants and herbs have various bioactive compounds with therapeutic potential and the properties found in the traditional medicinal plants and their constituents offer exciting opportunity to develop them into novel plants. India is blessed with different medicinal practices like Ayurveda, Siddha and Unani. Plants have use in folk medicine and traditional system of medicine. Traditional medicine makes use of theories, beliefs and experience that helps to maintain health and also to protect from various ailments. Plants consist of various phyto pharmaceuticals which have led to its use in medicinal fields (1). Worldwide, India is a richest biodiversity country and it has a 45,000 plant species. In India, around 20,000 medicinal plants have been recorded recently. 500 traditional communities cure the different disease using medicinal herbs. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (2).

Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemotherapeutants, these are non-phytotoxic, more systemic and easily biodegradable (3-5)

GC-MS is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and

volatile essential oil, fatty acids, lipids and alkaloids

The aim of the present study is to identify the phytocomponents of this plant and subjecting the ethanol extract of the whole plant to Gas chromatography-Mass Spectrum analysis. This work will help to identify the compounds, which may be used to cure many diseases.

About 21 species of *Andrographis* are reported to occur in India (6-8) Several other species of *Andrographis* are used as substitutes and adulterants of *A. paniculata* the most important among these being *A. alata* and *A.lineata*(9).

Andrographis Wallish ex Nees belongs tothe family Acanthaceae. The genus *Andrographis* wallish ex Nees is comprised of annual herbs or small shrubs, including about 40 species, distributed in the tropical Asia (10). *A. alata* (Vahl) Nees commonly known as"Periyanangai" is distributed in south-west India, Sri Lanka, Tamilnadu, Kerala, AndhraPradesh at altitudes ranging from 100m-3000m(11).

The plant is used as stomachic, intermittent fevers, malaria, alternative, snake bite, antisnake venom, appetizer, activating taste, indigestion, skin diseases, worms, poisonous bites, giving strength fever, diarrhoea, childhood diseases, ascitis and impurities to blood. (12,13,7&9).It is used in snake-bites, constipation,skin diseases and lung diseases. These plants are also claimed to possess snake- bites.Leaf paste is locally applied for skin diseases in cattle (9)

MATERIALS AND METHODS

Plant Material

The plant specimens for the present study were collected from Kalakad foot hills. The plants were identified with the help of "Flora of the presidency of Madras" (14) and "Flora of Tamil Nadu Carnatic" (15) and the binomials of the plants were as given in the "Flora of Tamilnadu" (16). The materials were also identified and authenticated by Dr. P. Jayaraman, Director, PARC, Chennai-45 and Dr. V. Chelladurai (Rtd.), Research officer, Medicinal plant Survey Unit, Government Siddha Medical College, Palayamkottai.

Preparation of Extract

The powders of the plant (30 gm) were extracted with ethanol at room temperature for 48h. The extract was filtered and concentrated under reduced pressure in a rotary evaporator. The extract was subjected to GC-MS analysis.

GC-MS Analysis

GC MS analysis of ethanol extract was performed with GC Clarius 500 Perkin Elmer System and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a BR-5MS column (30 mmx0.25 mm ID x 0.25 µm df, composed of 5% Diphenyl/ 95% Dimethyl poly siloxane). For GC-MS detection and electron ionization system with an ionizing energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2 µl was employed (split ratio of 50:1). Injection temperature 280oC; ion source temperature 200oC. GC oven temperature started at 80ºC and holding for 2min and it was raised to 160ºC at the rate of 20ºC/min, without holding. Holding was allowed at 285ºC for 5 min with program rate of 5ºC/min. The injector and detector temperatures were set at 250ºC and 280ºC respectively. The mass spectrum of compounds in samples was obtained by electron ionization at 70 eV and the detector was operated in scan mode from 50-500amu (atomic mass units). A scan interval of 0.5seconds and fragments from 45 to 450 Da was maintained. The total running time of GC programme and MS programme were 36minutes.

Identification of Components

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns and mass spectra of WILEY. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the component stored in the NIST library .The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. The GC-MS analysis of *Andrographis alata* whole plants revealed the presence of nineteen compounds. The identification of the phytochemical compounds was

confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) biological activities, the structure of the compound and peak area in percentage are presented in Table (1&2) and Fig 1. The first compound identified with less retention time in *Andrographis alata* (10.03 min) was Alpha-1-rhamnopyranose whereas Stigmasterol was the last compound which took longest retention time (34.29 min) to identify. The other compounds with intermediate retention time like 3,7,11,15-Tetramethyl-2-hexadecen-1-ol(12.53 min), n-Hexadecanoic acid(14.12 min), 9,12- Octadecadienoic acid (Z,Z)-(16.51 min), Oleic acid(16.59 min),Octadecanoic acid(16.94 min), n-Tetracosanol-1(17.59 min), Phytonadione(19.93 min), Ergosta-4,6,8(14),22-tetraen-3-one(21.56 min), Lupeol(23.34 min), 4H-1-Benzopyran-4-one,5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-(24.96 min), 4H-1-Benzopyran-4-one,5-hydroxy-6,7-dimethyl-2-phenyl-(26.03 min), Squalene(26.34 min), Cholestan-3-ol,2-methylene, (3β,5α)-(26.55min), 4H-1-Benzopyran-4-one,5-(acetyloxy)-2-[3-(acetyloxy)-4-methoxyphenyl]-7-methoxy-(27.84min),4H-1-Benzopyran-4-one,5,7-dihydroxy-2-(3,4,5-trimethoxyphenyl) (28.58min),Anthroquinone,7-methoxy-2-methyl-1,4,5-trihydroxy-(29.26 min), Butylphosphonic acid, butyl 4-(2-phenylprop-2-yl)phenyl ester(33.67 min)

Table 1: Phytocompounds identified in the *Andrographis alata*(Vahl) Nees by GC-MS

S.No	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.	10.03	Alpha-1-rhamnopyranose	C6H12O5	164	0.65
2.	12.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	1.87
3.		n-Hexadecanoic acid	C16H32O2	256	5.94
4.	16.51	9,12- Octadecadienoic acid (Z,Z)-	C18H32O2	280	1.80
5.	16.59	Oleic acid	C18H34O2	282	4.14
6.	16.94	Octadecanoic acid	C18H36O2	284	0.93
7.	17.59	n-Tetracosanol-1	C24H50O	354	3.87
8.	19.93	Phytonadione	C31H46O2	450	3.90
9.	21.56	Ergosta-4,6,8(14),22-tetraen-3-one	C28H40O	392	0.75
10.	23.34	Lupeol	C30H50O	426	4.20
11.	24.96	4H-1-Benzopyran-4-one,5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-	C16H12O5	284	17.40
12	26.03	4H-1-Benzopyran-4-one,5-hydroxy-6,7-dimethyl-2-phenyl-	C17H14O5	298	7.93
13.	26.34	Squalene	C30H50	410	0.47
14.	26.55	Cholestan-3-ol,2-methylene,(3β,5α)-	C28H48O	400	4.93
15.	27.84	4H-1-Benzopyran-4-one,5-(acetyloxy)-2-[3-(acetyloxy)-4-methoxyphenyl]-7-methoxy-	C21H18O8	398	11.51
16.	28.58	4H-1-Benzopyran-4-one,5,7-dihydroxy-2-(3,4,5-trimethoxyphenyl)-	C18H16O7	344	1.74
17.	29.26	Anthroquinone,7-methoxy-2-methyl-1,4,5-trihydroxy-	C16H12O6	300	10.83
18.	33.67	Butylphosphonic acid, butyl 4-(2-phenylprop-2-yl)phenyl ester	C23H33O3P	388	13.87
19.	34.29	Stigmasterol	C29H48O	412	3.26

Figure 1: GC-MS Chromatogram of *Andrographis alata* (Vahl) Nees

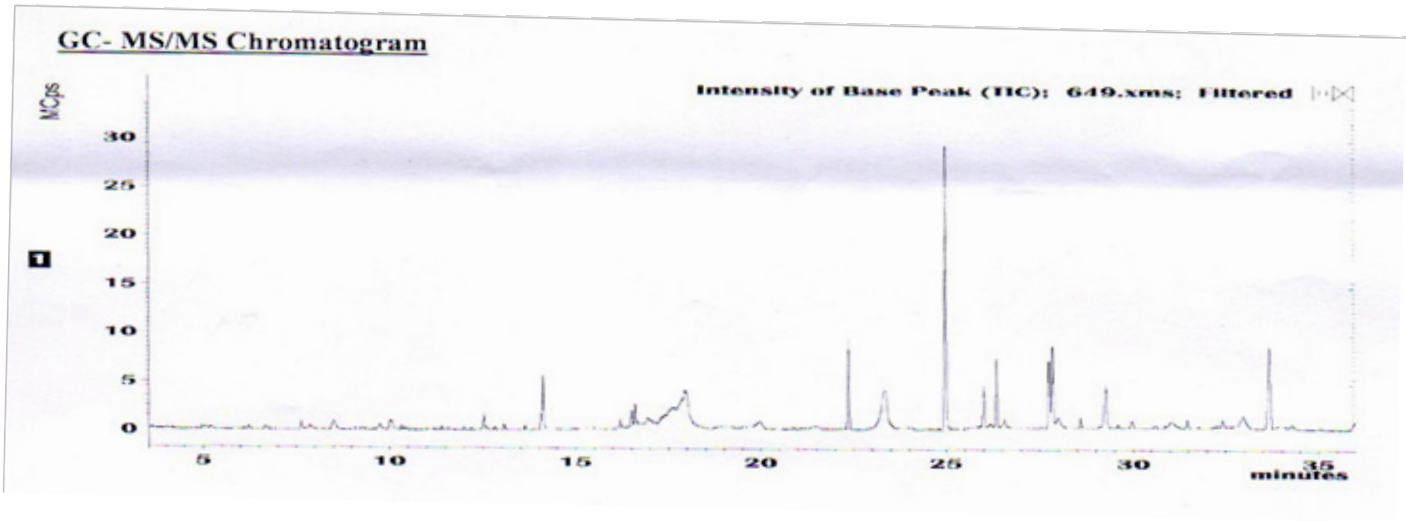
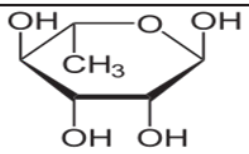
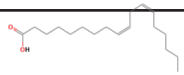
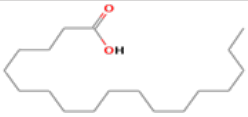
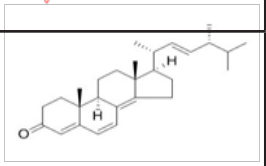
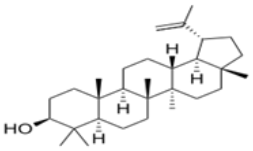
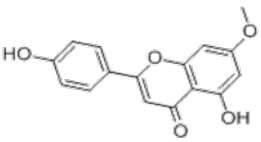
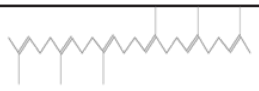
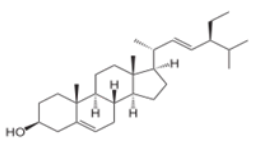


Table 2: Structure of the compounds identified in the *Andrographis alata*(Vahl)Nees by GC-MS

S.NO	Name of the compound	Structure of the compound	Nature of the compound	Therapeutic activity
1.	Alpha-1-rhamno-pyranose		Sugar moiety	Antiviral activity, Analgesic activity, Anti-leishmanial activity, Anti-inflammatory, Anti-Hypoglycemic
2.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		Diterpene	Antidiabetic activity, Analgesic, Anti-inflammatory, Ulcerogenic, Antioxidant, Antibacterial, Anti-helmintic activity.
3.	n-Hexadecanoic acid		Palmitic acid	Analgesic, Anti-inflammatory, Ulcerogenic,Antihyperglycemic, antihyperlipidemic, Antioxidant activity
4.	9,12- Octadecadienoic acid (Z,Z)-		Linoleic acid	Analgesic, Anti-inflammatory, Ulcerogenic, Antioxidant
5.	Oleic acid		Fatty acids	Antioxidant, Bacteriocidal activity, Anticancer activity, Antimicrobial, Immunotherapeutic activity
6.	Octadecanoic acid		Stearic acid	Analgesic, Anti-inflammatory ,Ulcerogenic, Antiviral activity,Antioxidant activity,Antihyperglycemic, Antihyperlipidemic activities
7.	n-Tetracosanol-1		Fatty alcohol	Antibacterial activity, Anti-breast cancer activity, Antioxidant activity
8.	Phytonadione		Vitamin K	Anticoagulation therapy, Antioxidant activity
9.	Ergosta-4,6,8(14),22-tetraen-3-one			Anti proliferative activity, Hepatocellular/drug

10.	Lupeol		Triterpenoid	therapy, Diuretic activity
11.	4H-1-Benzopyran-4-one,5-hydroxy-2-(4-hydroxy-phenyl)-7-methoxy-		Flavonoid	Anti inflammatory , Anticancer activity, Anti proliferative activity
12.	4H-1-Benzopyran-4-one,5-hydroxy-6,7-dimethyl-2-phenyl-	Nil	Flavonoid	Anti-TB activity, Anti-cancer activity, Anti-healing activity, Antimicrobial activity
13.	Squalene		Triterpene	Anticancer activity, antimicrobial activity
14.	Cholestan-3-ol,2-methylene,(3β,5α)-	Nil	-	In vitro cytoprotective activity, Antioxidant activity, Antilipedemic activity, Anticancer activity
15.	4H-1-Benzopyran-4-one,5-(acetyloxy)-2-[3-(acetyloxy)-4-methoxyphenyl]-7-methoxy-	Nil	Flavonoid	Cytotoxic activity, Antiproliferative activity, Anticancer activity, Antioxidant activity
16.	4H-1-Ben zopyran-4-one,5,7-dihydroxy-2-(3,4,5-trimethoxyphenyl)-	Nil	Flavonoid	Antimitotic activity, Anticancer, Antimicrobial activities
17.	Anthroquinone,7-methoxy-2-methyl-1,4,5-trihydroxy-	Nil	-	Anti-viral, Anticancer, Anti-leishmanial , anti-arthritic, Anti-inflammatory activities
18.	Butylphosphonic acid, butyl 4-(2-phenylprop-2-yl)phenyl ester	Nil	Glyceride esters of polyethylene glycols	Anti-inflammatory, Termiticidal activities,
19.	Stigmasterol		Unsaturated plant sterol	Immunomodulatory activity, Antiproliferative , Apoptotic, Antimutagenic , Antiulcerogenic, Antitumor activities

DISCUSSION

One of the ongoing problems scientist and medical workers face in the fight against infectious diseases is the development of resistance to the agents used to control them. There has been a remarkable progress in the prevention, control and even eradication of infectious diseases with improved hygiene and development of antimicrobials and vaccines. In recent times, in addition morphological markers, anatomical, cytological, biochemical, and molecular markers are also being used to categorize the organisms (17). GC-MS is a valuable tool for reliable identification for phytocompounds(18&19). In this study 19 compounds have been identified from the ethanolic extract of the whole plant of *Andrographis alata* by GC-MS analysis.The therapeutically important active principle andrographoloide was observed in the aerial parts of *A.paniculata* (20-22). Among the identified phytochemicals, 4H-1-Benzopyran-4-one,5-hydroxy-6,7-dimethyl-2-phenyl-,4H-1-Benzopyran-4-one,5-(acetyloxy)-2-[3-(acetyloxy)-4-methoxyphenyl]-7-methoxy-and 4H-1-Benzopyran-4-one,5,7-dihydroxy-2-(3,4,5-trimethoxyphenyl) and it may be acts as an Anticancer, Antimicrobial, Antioxidant, Anti inflammatory, Antiinvasive activities, Antimitotic activity and Anti-TB activity. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Lupeol and Squalene are the terpenes and they may be employed as an Antidiabetic activity, Analgesic, Anti-inflammatory, Ulcerogenic, Antioxidant, Antibacterial, Anthelmintic activity, Anticancer activity and *In vitro* cytoprotective activity, Vitamin K is suggested to be a vitamin compound and it may be acts as Anticoagulation therapy,Antioxidant activity. n-Hexadecanoic acid (Palmitic acid) and 9,12- Octadecadienoic acid (Z,Z)- (Linoeic acid) have the pharmacological activities like Analgesic, Anti-inflammatory, Ulcerogenic,Antihyperglycemic, Antihyperlipidemic and Antioxidant activity. Stigmasterol is the unsaturated pant sterols and it may be acts as an Immunomodulatory activity, Antiproliferative, Apoptotic, Antimutagenic, Antiulcerogenic, Antitumor activities.The active principle, area of the peak, Concentration (%) and Retention Time (RT) are presented in Table 1. The activity of phyto components identified in *Andrographis alata* and structure of the compoundsare tabulated in Table 2. The chromatogram obtained by ethanol fraction of *Andrographis alata* whole plant extract was shown in Fig. 1.

CONCLUSION

Phytochemical investigation and GC-MS analysis of ethanolic extract of whole plant of *Andrographis alata* showed the presence of carbohydrates, steroids, alkaloids, phenols, flavonoids, fatty acids, vitamins, palmitic acid, lioleic acid, and esters. The presence of various bioactive compounds confirms the application of *Andrographis alata* for various ailments by traditional practitioners.The isolation of individual phytochemical constituents may proceed to find a novel drug. It can be concluded that in future *in vitro* and *in vivo* studies on biological systems can find a new way for drugs that can be employed in clinical trials.

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