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(RESEARCH ARTICLE)

GC-MS analysis of ethanol extract of leaves and aerial parts of *Andrographis stellulata* C. B. Clarke

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Abstract

The phytochemical profiling of ethanol extract of leaf and whole aerial parts of *Andrographis stellulata* was initially characterized and compared by Gas Chromatography-Mass Spectrometry (GC-MS). The results showed the ethanol crude extract of leaf contains bioactive compounds such as Diethyl Phthalate, Phytol, Octadecatrienoic acid, Octadecatrienoic acid, Flavone, 5-hydroxy-7,8-dimethoxy, Genkwanin, Nonacosane, Santonox, Desmethyltangeretin, Tetratriacontane, and α-Tocopherol compounds were determined. Also observed some of the similar bioactive compounds were identified such as Diethyl Phthalate, 2-Hexadecen-1-ol, Tetramethyl-, acetate, Phytol, Phthalic acid, di(2-propylpentyl) ester, Sitosterol, Flavone, 5-hydroxy-7,8-dimethoxy, Genkwanin, Nonacosane, Naphthalene, Octahydro-4,4- Dimethyl, 5-O-Desmethyltangeretin and α-Tocopherol were determined. Both of the extracts showed some bioactive compounds that were similarly presented. The unknown and known bioactive constituents must be studied in the future using pharmacological and biological properties.

Keywords: *Andrographis stellulata*; Ethanol Extract; GC-MS And A-Tocopherol; Bioactive constituents

1. Introduction

Natural resources are gaining popularity as an origin for establishing health nutritional supplements, tonics, pharmaceutical drugs, and numerous personal care products [1]. However, the scientific evidence about the biochemical components and biological properties of various environmental assets used in folk medicine is lacking, there is no written literature [2]. Plants have been identified as an important source of promising pharmacological biomolecules for treating various illnesses subsequently antiquity and frequently mentioned in traditional medicine orally by healers and ethnic peoples [3]. Ethnobotanically *Andrographis* species were used for the treatment of snake bites, bug bites, diabetes, viral fever, and diarrhoea [4], dysentery, fever, and malaria fever [5].*Andrographis paniculata* is the most predominantly used medicinal plant in Indian traditional medicinal systems like Ayurveda, Siddha, Unani, and Asian systems. *Andrographis* species is given in the form of tablets, decoction, kasayam, and infusion in the Indian system of medicine for the treatment of common flu, swine flu, chikungunya, malaria, diabetic, dengue, and other viral fevers [6, 7, 8].

The aerial part of the plant is used in the form of infusion, raw juice, powder, and decoction either alone or in combination with other medicinal plants [9]. The aerial parts of *Andrographis paniculata* plant extracts contain diterpenoids, diterpene glycosides, alkaloids, lactones, flavonoids and flavonoid glycosides. Whole plant leaves and root powders are also used as a folklore remedy for various diseases in Asia countries [10, 11]. Snake bite is a serious problem in tropical and subtropical countries like India. According to the World Health Organization (WHO), poisonous snakes are responsible for human mortalities annually at least 5 million counts [12].

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Andrographis stellulata, occurring in the southern Western Ghats of Nilgiris, among the different species of *Andrographis*, the present study provides the ethnobotanical data of rare, endemic/endangered species collected, and leaf extracts were orally administered with buffalo milk for treatment of poison bites and cold fever, jaundice [8]. Species of the genus *Andrographis* have been used in local folklore medicine for various diseases such as fever, malaria, diarrhoea, cough, and muscular pains also used for the expulsion of worms, in tribal and rural communities. Many pharmacological studies have been conducted to authenticate its use as a multipurpose medicinal agent [12, 13, 14].

2. Materials and Methods

2.1. Plant Material

The medicinal plant, *Andrographis stellulata* was collected from the surroundings of Manjoor, Nilgiris, and plant samples were washed thoroughly and allowed to dry under the shade. The identification and voucher specimens were deposited at the Department of Botany, LRG Government Arts College for Women, Tirupur, Tamilnadu. A voucher specimen (BSI/SRC/5/23/2024-25/Tech-308) was deposited in the Herbarium form to the Botanical Survey of India, Coimbatore, for authentication.

2.2. Preparation of Extract

The collected plant leaves and aerial parts were washed with water. Washed leaves and aerial parts were dried at room temperature for 10-15 days in a shaded place and ground into a coarse powder. The required quantity of the leaf and aerial parts of *Andrographis stellulata* was weighed and transferred to a flask treated with ethanol until the coarse powder was fully immersed, incubated overnight then filtered through a Whatman filter paper. The coarse powder was subjected to successive extraction with ethanol. The crude extracts were collected for further usage in phytochemical and pharmacological studies. The collected extracts were mixed with diethyl ether to separate non-polar compounds using a separating funnel three to five times. The alcohol layer and Diethyl ether layer were segregated using a separating funnel then collected in separate beakers and subjected to evaporation. The dried substance obtained is used for further analysis. The active compounds were identified by the the Gas- Chromatography-Mass Spectrometer technique.

2.3. Gas Chromatography – Mass spectrum analysis

The GC-MS analysis was done at the South Indian Textiles Research Association (SITRA), Coimbatore, Tamilnadu, India. The GC-MS analysis of volatile compounds from crude extracts of *Andrographsstellulata* by using a Shimadzu-GCMS-QP-2010plus (Auto system XL) Gas chromatograph equipped and coupled to a mass detector SHIMADZU Rts-5MS-5.1 spectrometer, 30m × 0.32 mm× 0.50µm, of capillary column. The injector temperature of the instrument was set to an initial temperature of 270 ℃ and maintained at this temperature. The oven temperature was maintained at 45 degrees centigrade. The oven temperature was raised to 150-degree centigrade (0 min hold) and the oven temperature was again raised from 150 ℃ to 250℃ at the rate of 5 ℃/min, and maintained for 10 min at the end. The total sample running time is 30 minutes. Injection port temperature was ensured as 270 ℃ and Helium flow rate as 5.5lb/in2. The pressure was 11.6Kpa. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). The NIST library is used to identify the compounds present in the sample extract [1]. The mass spectrum of individual compounds is matched with the mass spectrum of compounds in the sample chromatogram shown as peaks and identifies the nature of compounds.

2.4. Identification of bioactive compounds

Interpretation on mass spectrum GC-MS was conducted using the database of the National Institute of Standard and Technology (NIST) having more than 1,000,000 patterns. The mass spectrum of the unknown volatile components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, chemical formula, and structure of the bioactive compounds of the test materials were ascertained.

2.5. Data Analysis

GC-MS data are analyzed using online software to identify chemical components, Molecular formula, retention time (RT), Chemical group and peak area. The detected volatile compounds are further analyzed for biosynthesis pathways, properties, and bioactive properties using PubChem, KEGG, ChEBI, PlantCyc, Aldric and SpectraBase websites.

3. Results

The results obtained from the bioactive compounds present in the ethanol extract of leaf and whole plant crude extracts of *Andrographis stellulata*first time were determined and identified by GC-MS analysis. The GC-MS analysis of the leaf crude extract of *Andrographis stellulata* revealed the presence of seventeen compounds that could contribute to the medicinal quality of the plant. The bioactive principles with their retention time (RT), molecular weight (MW), molecular formula (MF), and peak area in percentage are presented in Table 1 and Figure 1. It was found that main phytoconstituents such as Diethyl Phthalate (1.97%), Phytol (2.4%), Phthalic acid, di(2-propylpentyl) ester (1.52%), Phthalic acid, di(2-propylpentyl) ester (1.78%), Genkwanin (3.52%), Nonacosane (1.62), Santonox (2.93%), 5-O-Desmethyltangeretin (1.35%), Tetratriacontane (2.29%) and α- Tocopherol (3.79%). The aerial parts of the crude extract contained volatile compounds such as Diethyl Phthalate (1.89%), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate (4.46%), Phytol (1.95%), Phthalic acid, di(2-propylpentyl) ester (1.47%), Sitosterol (6.32%), Flavone, 5-hydroxy-7,8-dimethoxy (4.13%), Genkwanin (2.10%), Nonacosane (10.6), Naphthalene, octahydro dimethyl (8.02%), 5-O-Desmethyltangeretin (3.35%) and α- Tocopherol (18.28%).

Table 1 Volatile compounds identified in GCMS analysis of ethanol crude extract of *Andrographis stellulata* leaves

S.No	RT	Compound Name	Formula	Peak Area %	Compound Nature	Biological Activity
1	3.92	Isobutane	C4H10	0.25	Ether compound	No activity reported
2	4.89	Acetic acid, 2-fluoroethyl ester	C4H7FO2	0.42	Carboxylic compound	Food acidity regulator, antimicrobial drug, food preservation
3	7.00	Disiloxane, 1,3-diethoxy, tetramethyl	C8H22O3Si2	0.34	Ether compound	No activity reported
4	12.66	Dodecane	C12H26	0.47	n-alkane hydrocarbon	No activity reported
5	21.40	Diethyl Phthalate	C12H14O4	1.97	Ether compound	No activity reported
6	24.31	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate,	C22H42O2	0.73	Diterpenoids	Flavor and fragrance agents
7	25.69	n-Hexadecanoic acid	C16H32O2	0.79	Fatty acid	No activity reported
8	27.44	Phytol	C20H40O	2.4	diterpenoid	cytotoxic, antimicrobial, antioxidant, antiinflammatory
9	27.80	Octadecatrienoic acid	C18H30O2	1.3	Carboxylic compound	Food additives
10	31.59	Phthalic acid, di(2-propylpentyl) ester	C24H38O4	1.52	Ether compound	No activity reported
11	33.11	Flavone, 5-hydroxy-7,8-dimethoxy-	C17H14O5	1.78	Carbonyl group	Antifungal activity, oxidative damage, inflammation, allergies, and tumors
12	33.76	Genkwanin	C16H12O5	3.32	Flavonoid	Antioxidant, chronic disease
13	34.36	Nonacosane	C29H60	1.62	Hydrocarbon	Antibacterial
14	35.90	Santonox	C22H30O2S	2.93	Phenol group	Antioxidant

15	36.26	5-O-Desmethyltangeretin	C19H18O7	1.35	Methoxy group	No activity reported
16	36.61	Tetratriacontane	C34H70	2.29	Alkane group	Memory-enhancing effect
17	37.25	α- Tocopherol	C29H50O2	3.79	Phenolic compounds	Strong antioxidant activities

Table 2 Volatile compounds identified in GCMS analysis of ethanol crude extract of *Andrographis stellulata* aerial parts

S.No	**RT**	**Compound Name**	**Formula**	**Peak Area %**	**Compound Nature**	****Activity**
1	3.59	2-Chloroethyl methyl ether	C3H7ClO	0.40	Ether compound	No activity reported
2	4.87	Ethanol, 2-(trimethylsilyl)-	C5H14OSi	0.41	Alcoholic compound	No activity reported
3	12.60	Methyl salicylate	C8H8O3	0.66	Ether compound	No activity reported
4	21.40	Diethyl Phthalate	C12H14O4	1.89	Ether compound	No activity reported
5	24.31	2-Hexadecen-1-ol, etramethyl-, acetate	C22H42O2	4.46	Fatty acid	No activity reported
6	27.44	Phytol	C20H40O	1.95	diterpenoid	cytotoxic, antimicrobial, antioxidant, anti-inflammatory
7	31.59	Phthalic acid, di(2-propylpentyl) ester	C24H38O4	1.47	Ether compound	No activity reported
8	32.61	Sitosterol	C29H50O	6.32	Steroids	Pain relief, inflammatory
9	33.13	Flavone, 5-hydroxy-7,8-dimethoxy-	C17H14O5	4.13	Carbonyl group	Antifungal activity, oxidative damage, inflammation, allergies, and tumors
10	33.76	Genkwanin	C16H12O5	2.10	Flavonoid	Antioxidant, chronic disease
11	34.36	Nonacosane	C29H60	10.6	Hydrocarbon	Antibacterial
12	35.97	Naphthalene, octa hydro dimethyl-	C18H22N4O4	8.02	Methyl group	Metabolic pathways
13	36.31	5-O-Desmethyltangeretin	C19H18O7	3.35	Methoxy group	No activity reported
14	37.24	α- Tocopherol	C29H50O2	18.28	Phenolic compounds	Strong antioxidant activities

4. Discussion

The usage of medicinal plants for pharmacological purposes relies on their phytochemical composition which exhibits some interesting and specific biological activities. Different phytochemicals identified in the present study have been found to possess a wide range of biological activities. It has long been reported that Acanthaceae families contained several phytochemicals such as terpenoids, flavonoids, phenypropanoids and sesquiterpenes which exhibited antitumor activities [8, 15, 16]. In this study, the bioactive compounds of *Andrographis stellulata* have been analyzed

and identified using the Gas Chromatography-Mass Spectrometer (GCMS) technique. The chemical compounds of the whole plant extracts of *Andrographis stellulata* were investigated using Shimadzu GCMS. Analysis was carried out of *Andrographis stellulata* whole plant extraction to check the presence of bioactive compounds using ethanol extraction. The important bioactive compounds determined from leaf extract showed Phytol (2.4%), Genkwanin (3.52) and α-Tocopherol (3.79%). Similarly present in the aerial part of the *Andrographis stellulata* extract showed Phytol (1.95%), Sitosterol (6.32%), Genkwanin (2.10%), Nonacosane (10.6) and α- Tocopherol (18.28%). The existence of the major compounds were Bis (2-ethylhexyl) phthalate and 9,12-Octadecadienoic acid (Z, Z)-2,3-dihydroxy Propyl ester [9]. GC-MS analysis revealed the presence of fifty-three phytoconstituents in methanol leaf extract, out of them fifty-two was identified. The presence of many bioactive compounds proved the therapeutic importance of this *Tephrosia* species; hence it can be considered a valuable medicinal plant for developing novel drugs to cure several diseases [17]. The various biomedical potentials of *Andrographis paniculata* leaf methanol extract showed more phytochemicals in the GC-MS analysis. Accordingly, the methanol alone showed Nonacosane, Phthalic acid, di(2-propylpentyl) ester, Flavone, 5-hydroxy-7,8-dimethoxy and considerable bactericidal and antioxidant activity [18].

The exploration of volatile bioactive compounds analyzed by GC-MS of *Andrographis stellulata* leaf and aerial parts. The important phytochemical constituents can be summarized as follows: The ethanol extract of leaves identified 17 compounds and aerial parts determined 14 compounds. Both extracts showed the presence of a few similar compounds such as Phytol, Flavone, Dodecane, Genkwanin, Tetratriacontane, Nonacosane, α and tocopherol. Further exploration of leaf and aerial parts of *Andrographis stellulata* containing secondary metabolites is recommended to investigate other bioactivities such as anti-diabetic, anti-oxidant, anti-cancer properties, and pharmacological industries to develop into potential herbal drugs for future usage.

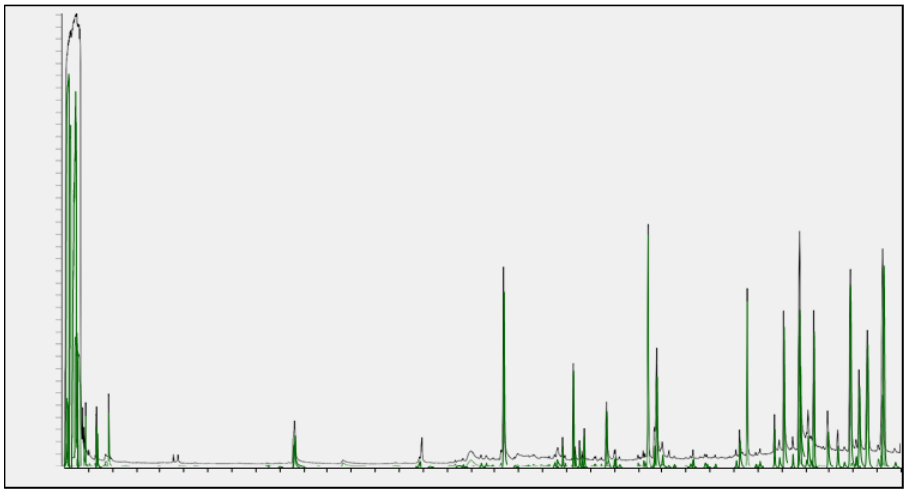

Figure 1 The chromatogram of the mass spectrum of leaf of methanol crude extract of *Andrographis stellulata*

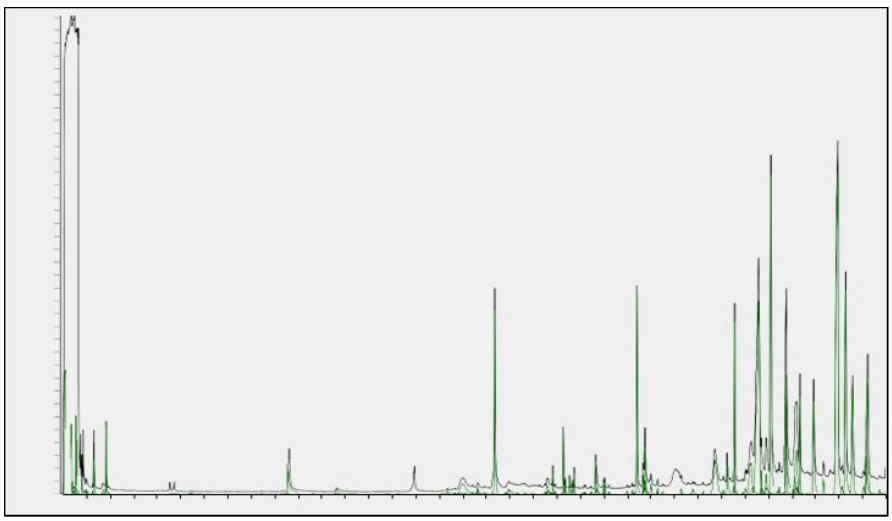

Figure 2 The chromatogram of the mass spectrum of aerial parts of methanol crude extract of *Andrographis stellulata*

Compliance with ethical standards

Statement of Conflict of Interest

No conflict of interest to be disclosed.

References

[1] Hussein, J.H., Imad Hadi, H, and Mohammed Yahya, H., (2017). Using Gas Chromatography-Mass Spectrometry (GC-MS) technique for analysis of bioactive compounds of methanolic leaves extract of Lepidium Sativum. Research Journal of Pharmacy and Technology, 10(11); 3981-3989.

[2] Aboyewa, J.A., Sibuyi Nicole, R.S, and Meyer, Mervin., (2021). Green synthesis of metallic nanoparticles using some selected medicinal plants from Southern Africa and their biological applications. Plants, 10, 1929. https://doi.org/10.3390/plants10091929.

[3] Alagu Lakshmi, S., (2021). Ethnomedicines of Indian origin for combating COVID-19 infection by hampering the viral replication: using structure-based drug discovery approach. J. Biomol. Struct. Dyn. 39 (13): 4594-4609.

[4] Biswajit, S, and Goswami, B.C., (2018). Evaluation of Hypoglycemic effect of ethanol extract of Musa paradisiaca unripe fruit pulp on normal and alloxan-induced diabetic mice. Research Journal Pharmacy and Technology, 11(3):1048-1052

[5] Jarukamjorn, K, and Nemoto, N., (2008). Pharmacological aspects of Andrographis paniculata on health and its major diterpenoid constituent Andrographolide, Journal of Health Science, 54(4): 370–381.

[6] Alagesaboopathi, C., (2000). Andrographis SPP.: A Source of Bitter Compounds for Medicinal Use. Anc Sci Life, 19(3-4):164-168.

[7] Samydurai, P., Rajendran, A., Sarvalingam, A, and Rajasekar, C., (2017). Ethnobotanical knowledge of threatened plant species Andrographis in Nilgiris biosphere reserve, Tamil Nadu, India. International Journal of Herbal Medicine, 5(6): 103-107.

[8] Karuppusamy, and Janakiraman, N., (2024) Andrographolide and its Analogs: Botanical Sources, Phytochemistry, Pharmacology and Biotechnology, (15):52-66. DOI: 10.2174/9789815256567124010006.

[9] Prabha, N, and Rahmath Bushra, J., (2019). Gas Chromatography Mass Spectrometry Analysis of Andrographis paniculata. Asian J. Research Chem. 12(1): 01-06. doi: 10.5958/0974-4150.2019.00001.4

[10] Akbar, S., (2011). Andrographis paniculata: A review of pharmacological activities and clinical effects. Alternative Medicine Review, 16(1): 66–77.

[11] Kabir, M.H., Hasan, N, and Rahman M.M., et al., (2014). A survey of medicinal plants used by the Deb barma clan of the Tripura tribe of Moulvibazar district, Bangladesh. Journal of Ethnobiology and Ethnomedicine, 10(1): 19.

[12] Chippaux, J.P., (1998). Snake-bites: Approval of the global situation. Bull WHO, 76(5) : 524.

[13] Abhishek, N, and T Shri Krishna, T., (2010). Alok Lehri Biological activities of Kalmegh (Andrographis paniculata Nees.) and its active principles-A review. Journal of Natural Product and Resources, 1: 125-135.

[14] Alagesaboopathi, C., (2013). Ethnomedicinal plants used for the treatment of snake bites by Malayali tribal and rural people in Salem district, Tamilnadu, India. International Journal of Bioscience, 2:42-53.

[15] Sreevani, P., (2013). Phytochemical and pharmacological activities of Andrographis Paniculata Nees.: A Review. International of Journal Science and Research, 1887-1890

[16] Thangavel, M.S., Umavathi, Y., Thangam, A., Thamaraiselvi, K, and Ramamurthy, M., (2015). GC-MS analysis and larvicidal activity of Andrographis paniculata (Burm.F) Wall.Ex Nees against the dengue vector Aedes aegypti (L) (Diptera: Culicidae). Int. J. Curr. Microbiol. App. Sciences, 4(7): 392-403.

[17] Vandana, S., (2025). Phytochemical Screening and GC-MS Profiling of methanol leaf Extract of Tephrosia wallichii Graham of Indian Thar Desert. Research Journal of Pharmacy and Techn, 18(1):159-4.

[18] Chau, T.P., Samdani, M.S., Kuriakose, L.L, and Sindhu R., (2024). Assessment of multi-biomedical efficiency of Andrographis paniculata shoot extracts through in-vitro analysis and major compound identification, Environmental Res, 242: 1-10.