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Research Article

NUTRITIONAL AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF *ANDROGRAPHIS LAWSONII* GAMBLE

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ABSTRACT

Objective: *Andrographis lawsonii* Gamble is a medicinal herb species, Endemic to India: Karnataka (Dakshina kannada or south Canara district), Kerala (Palakkad district), and Tamil Nadu (Coimbatore and Nilgiris districts). The *Andrographis* is a large genus of the family Acanthaceae. This study focused to check the preliminary phytochemical nutritive analysis, leaf stem and root and gas chromatography-mass spectrometry (GC-MS) analysis leaf methanol extract.

Methods: The plant samples were subjected to Soxhlet extraction for phytochemical analysis and further experimental studies. The test on phytochemical studies indicated the presence of alkaloids, saponins, glycosides, and flavonoids glycosides within the plant parts, respectively. The estimation of carbohydrate protein starch methanol leaves extracts to have high activity compared to others.

Results: Preliminary phytochemical and Nutritional analysis in *A. lawonii*. Nutritional analysis in carbohydrate protein and starch content was found to be high in leaf methanol extract. Highly medicinal compound analysis in this GC-MS Analysis 50 compound present in methanol leaf extract.

Conclusion: The leaf methanol extract of *A. lawsonii* has exhibited remarkable preliminary phytochemical analysis and nutritional analysis GC-MS analysis to the medicinal plant.

Keywords: *Andrographis lawsonii*, Preliminary analysis, Nutritional analysis, Gas chromatography-mass spectrometry.

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INTRODUCTION

The Acanthus family (Acanthaceae) is a dicotyledonous flowering plant taxon with almost 250 genera and 2500 species. The majority are tropical plants, shrubs, or twining vines, with a few epiphytes thrown in for good measure. Only a few species can be found in temperate climates. Indonesia, Malaysia, Africa, Brazil, and Central America are the main distribution hubs [1]. The Acanthaceae family is regarded to be one of the flavonoid-rich families [2].

In India, there are around 21 species of *Andrographis*. In mountainous settings, practically all of a species' plants flower in the same season. In tribal and rural cultures, species of the genus *Andrographis* have been utilized in folklore medicine for a variety of ailments such as fever, malaria, diarrhea, cough, muscle aches, and worm ejection.

Andrographis paniculata, a member of the Acanthaceae (Acanthus) family, has been used to treat upper gastrointestinal and upper respiratory infections, fever, herpes, and other chronic disorders for millennia. It has a diverse set of pharmacological properties. Andrographolide, a diterpene lactone, is the main therapeutic component of *A. paniculata.* Andrographolide has been linked to anti-cancer properties [3]. Anti-HIV [4], cardioprotective [5], and hepatoprotective [6] properties among others. The other active components include 14-deoxy-11,12-dihydroandrographolide (andrographolide D), homoandrographolide, andrographosterin, and stigasterol [7]. The antibacterial activity of *A. paniculata* had previously been studied and published [8]. The aqueous extract of *A. paniculata* was shown to have antibacterial activity [9]. In their study on *in vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method, found water extract of *A. paniculata* to posses potential antibacterial activity towards both gram-positive and gram-negative microorganisms [10]. *Andrographis lawsonii* is one of the highly used potential medicinal plants in the world.

METHODS

Collection of plant material

The leaves, stem, and root of *A. lawsonii* Gamble were collected in the month of October 5 from, Western Ghats, Tamil Nadu. The plant was identified taxonomically and authenticated through the BSI, the Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu (BSI/SRC/5/23/2020/Tech./15) and the voucher specimen has been deposited in Bharathiar University, Coimbatore. The freshly gathered plant materials were washed in the running water tap and wiped clean to take away the adhering dust. It is then dried separately at room temperature (30°C). The dried parts were then powdered by using the electric mixer and it is transferred into airtight containers. The powdered samples were then used for further studies.

Extraction of plant materials

Plant materials 50 g of the powdered were weighed, packed in the filter papers, and put into the thimble. A condenser and a round bottom flask were fitted above and below the thimble and were clamped firm into position. This assembly of apparatus is known as a Soxhlet extractor. The plant samples are separately extracted with organic solvents such as petroleum ether, chloroform, ethyl acetate, and methanol in the increasing order of their polarity. The Soxhlet extractor was then heated electrically on a heating mantle. Continuous extraction was carried out for a period of 10 h with about 22 refluxes. The samples were then removed and the solvents recovered. The extracts were poured into petriplates. Each time before extracting with the next solvent, the thimbles were air dried. The dried extract accumulated with every solvent turned into weighed.

Qualitative phytochemical analysis

The phytochemical screening of leaf, stem, and root of *A. lawsonii* was accomplished qualitatively to determine the presence of the following (Table 1) phytochemicals such as alkaloids, saponins, phenolic

compounds, tannins, flavonoids, cardiac glycosides, according to standard methods. Carbohydrates Molish's test [11], Protiens Biuret test [12], Amino acids Ninhydrin test [13], Alkaloids Hager's test [14], Saponins Frothing test [15], Phenolic compounds Ferric chloride test [16]. Flavanoids Alkaline reagent test [17]. Cardiac Glycosides Keller Killiani test [18].

MATERIALS AND METHODS

Nutritional analysis

Determination of total carbohydrates

The overall carbohydrates were envisioned as defined using [19] Glucose as a fashionable.

Estimation of starch

Estimate the amount of starch present in sample by perchloric acid method [20].

Determination of total proteins

The protein changed into predicted as defined by way of [21] the use of Bovine serum albumin as a fashionable.

Estimation of amino acids

Amino acids inside the samples were decided as per the manner of [22].

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using the instrument Thermo GC - Trace Ultra Ver: 5.0, Thermo MS DSQ II with Column: DB 35-MS Capillary Standard Non-Polar Column possessing Dimension: 30 Mts, ID: 0.25 mm, Film: 0.25 µm. The initial temperature of the instrument was set to 110°C and maintained for 2 min. At the end of this period, the oven temperature was raised up to 260°C, at the rate of an increase of 6°C/min, and maintained for 9 min. The temperature of injection port was ensured as 250°C and the flow rate of Helium as 1 ml/min. An injection volume of 1 µl of sample is considered in the analysis. The ionization voltage was maintained to be 70 eV and samples were injected in split mode in the ratio 10:1. Mass Spectral scan range was set at 45–450 (MHz). The chemical constituents were identified by GC-MS. The fragmentation patterns obtained from mass spectra were compared and analyzed with spectrometer database using the National Institute of Standards and Technology Mass Spectral database (NIST-MS). From the relative peak area of each component in the chromatogram the percentage of each component was calculated.

Identification of compounds

Using the database of NIST having more than 62,000 patterns the interpretation of mass spectrum of GC-MS was conducted. The spectrum components of known compounds were compared with unknown components stored in the NIST library. The name, molecular weight, and structure of the compounds were ascertained in above said way.

RESULTS AND DISCUSSION

Nutritional evaluation

The nutrient content in plants plays a vital role in maintaining metabolic and biological function. The primary metabolites which are typically present in the cells of many parts which directly involved in the growth. The group of pathways synthesizing the primary metabolites such as Carbohydrate, Protein, Starch, and Aminoacids are the key essential molecules for the physiological and biological cycles. Every plants depends energy from primary metabolites also essential for development, stress adaptation, and defence. The analysis for the nutrient contents is given in Table 2.

Estimation of total carbohydrate

Carbohydrates are the naturally occurring compounds normally found in all biological and organic matters it is one of the major constitutes found in plants. Carbohydrates are essential bio molecules produced during the photosynthesis. Carbohydrates are the building blocks to form cellulose (to develop cell wall)and also to derive energy from plants. The results of carbohydrate estimation from *A. lawsonii* from (Fig. 1) indicates that (43%) of leaf sample showed the high percentage of the carbohydrate, followed by (33%) and root yielded low percentage (24%).

Estimation of total starch

Starch is a polymer made by plants to store energy. Plants need energy to grow and function. During photosynthesis, they use light from the sun and convert them into the form of simple sugar and glucose. The

Table 1: Phytochemical screening of leaf, stem, and root of *Andrographis lawsonii*

Phytochemical constituents	Leaf	Stem	Root
Carbohydrates	+++	+++	++
Proteins	+++	++	+
Amino acids	++	++	++
starch	+	++	++
Alkaloids	+	++	++
Phenolic compounds	+++	+++	++
Flavonoids	++	+	+
Saponins	+	++	++
Tannins	++	++	++
Cardiac glycosides	++	++	+

(+): Presence of chemical compound, (-): Absence of chemical compound, (+)<(++)<(+++): Based on the intensity of characteristic color.

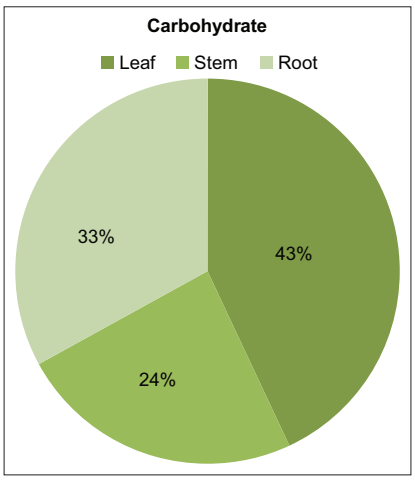

Fig. 1: Percentage of carbohydrate

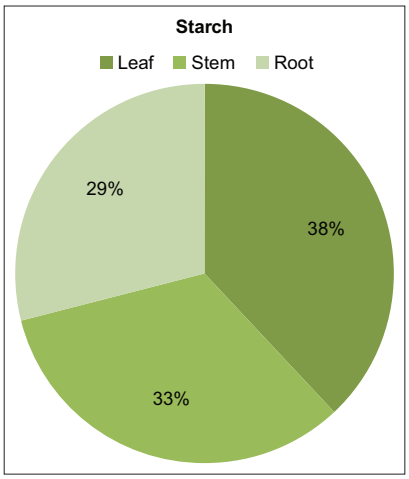

Fig. 2: Percentage of starch

simple sugars and glucose are stored in the form of starches and utilized them when they are required in *A. lawsonii* (Fig. 2) shows the highest percentage of starch in leaf (38%) stem (33%) root (29%).

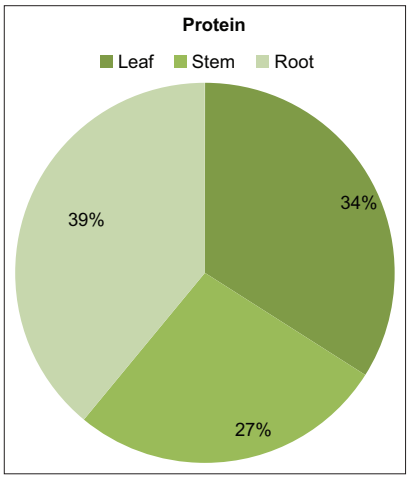

Fig. 3: Percentage of protein

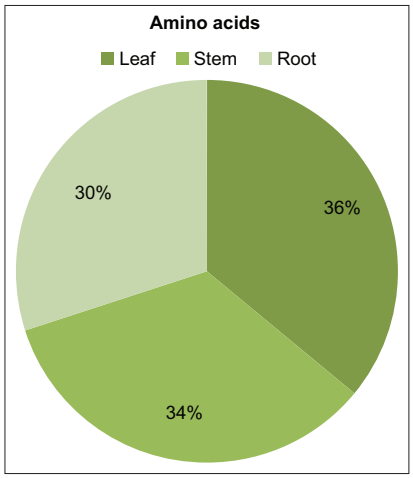

Fig. 4: Percentage of amino acids

Estimation of total protein

Proteins are the nitrogenous compounds which composed of the long chain and short-chain monomers in amino acids which are very much essential to life. Proteins are important in plants because highly evolved and diverse class and molecules also key compound to synthesis hormones enzymes and membrane channels and pumps. Proteins also function the immune system and can be used in the production of energy. In plants, proteins are the vital element that plays a key role as macromolecules which promote enzymes being the biological catalyst. The results of the protein estimation in *A. lawsonii* (Fig. 3) show that leaf (34%) stem (27%) root (39%).

Estimation of total amino acids

Amino acids are the important organic compounds containing amine and carboxyl groups. Along with side chains they contain key elements such as carbon, hydrogen, oxygen, and nitrogen for the biological process. Some other elements are also found in certain amino acids. In the fundamental ingredient in the process of protein synthesis. There are about 20 important amino acids involved in each life function. Many studies proved either directly or indirectly they involve in the physiological activities of the plant. From (Fig. 4) amino acids estimation in *A. lawsonii* leaf higher percentage (36%) stem (34%) and root (30%).

Biological activity of the gc-ms phytocompounds

Characterization of secondary metabolites from medicinal plants provides an extensive resource for the isolation and identification of novel therapeutic agents for Nutrition analysis. The major non-volatile compounds can be identified (Fig. 5) by GC-MS analysis. The result shows (Table 2) crude methanolic extract revealed the high peak intensity compound predominant presence of major compounds such as Quinic acid (13.976) Cycloheptasiloxane, tetradecamethyl- (4.020) Pentadecanoic acid, 3-oxo-, methyl ester (4.880) were also identified.

CONCLUSION

The analytical characterization of methanol extract of *A. lawsonii* leaf witness the presence of active metabolites. GC–MS reveals that the plant and its extract has valued agents which fulfill the pharmaceutical need. From the present study, nutritional quantification of *A.lawsonii* leaf, stem and root rich in carbohydrate starch protein aminoacids. Further, the fractions containing active compounds should be isolated from the extract and has to be examined through *in-vivo* experiments. This will confirm their mechanism of action as novel therapeutic agent

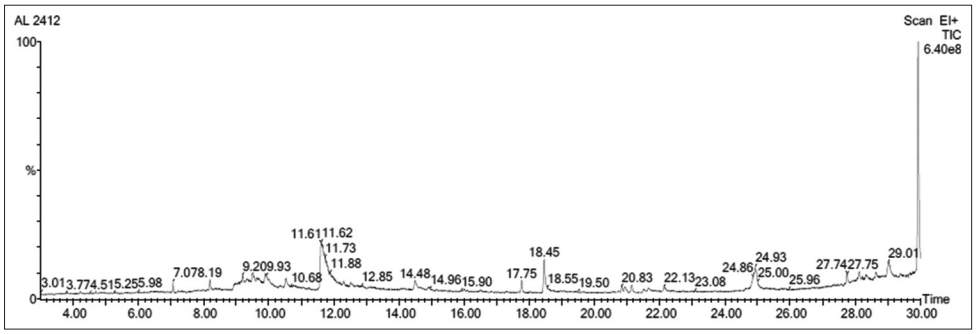

Fig. 5: Gas chromatography-mass spectrometry chromatogram analysis

Table 2: Determination of carbohydrates, starch, protein, and amino acids contents from the plant sample

Plant parts	Carbohydrate Mg GuE/mg	Starch GuE/mg	Protein BSAE/mg	Amino acids LE/mg
leaf	19.22±3.55^{c}	25.01±5.40^{b}	5.48±1.54^{a}	4.06±0.89^{b}
stem	32.30±1.25^{b}	21.79±0.83^{c}	7.79±3.56^{a}	3.66±0.45^{c}
root	36.42±5.17^{a}	28.87±4.01^{a}	6.78±1.58^{a}	4.31±0.59^{a}

Values are mean of three independent analyses±standard deviation (n=3). Mean values followed by different superscript letters a>b > c indicate the significant statistical difference in each column (GuE: Glucose equivalents, BSAE: Bovine serum albumin equivalents and LE: Leucine equivalents).

Table 3: GC-MS compound

S. No	Rt	Rt. area	Compound	M. formula	M. weight
1	3.324	0.513	Digitoxin	$C_{41}H_{64}O_{13}$	764.9
2	3.669	1.183	2-Hexadecanol	$C_{16}H_{34}O$	242.44
3	3.844	0.859	1-Methoxy-3-hydroxymethyloctane	$C_{10}H_{22}O_2$	174.28
4	4.219	0.896	4-Hydroxy-4-methylhex-5-enoic acid, tert.-butyl ester	$C_{11}H_{20}O_3$	200.27
5	4.409	0.773	Tetraacetyl-d-xylonic nitrile	$C_{14}H_{17}NO_9$	343.29
6	4.689	0.735	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	370.77
7	4.934	1.286	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	484.7
8	5.684	0.605	4-Benzoyloxy-1-morpholinocyclohexene	$C_{25}H_{44}N_2O_5S$	484.7
9	5.945	1.242	2,6,6-Trimethylundeca-1,3-dien-9-yn-5-one	$C_{14}H_{20}O$	204.31
10	6.180	0.869	Benzenepentanenitrile, ë-oxo-à-phenyl-	–	–
11	6.420	0.620	2-Methyl-2,3-divinyloxirane		
12	6.550	1.605	12-Dimethylamino-10-oxododecanoic acid	$C_{14}H_{27}NO_3$	257.37
13	6.900	0.501	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322.5
14	7.065	0.716	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	444.92
15	7.215	0.765	Phenol, 3-methyl-5-(1-methylethyl)-, methylcarbamate	$C_{12}H_{17}NO_2$	207.27
16	7.385	0.516	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl (ester)	–	–
17	7.830	1.556	3,5-Methano-2H-cyclopenta[b] furan-2-one, 3,3a, 4,5,6,6a-hexahydro4,4-dimethoxy-, (3R,3a-trans, 5-cis, 6a-trans)-	–	–
18	8.000	0.654	2-Hydroxy-6-methyl-3-cyclohexen-1-carboxylic acid	$C_{16}H_{29}NO_4$	299.41
19	8.190	1.218	2-Propenoic acid, 3-phenyl-, methyl ester	$C_{10}H_{10}O_2$	162.18
20	8.371	0.853	2-Propenoic acid, 3-phenyl-	$C_9H_8O_2$	148.16
21	8.491	0.532	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	$C_{18}H_{30}O_2$	278.4
22	8.661	1.511	Tetraacetyl-d-xylonic nitrile	$C_{14}H_{17}NO_9$	343.29
23	9.196	4.020	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	519.07
24	9.331	2.338	1-Dodecanol, 3,7,11-trimethyl-	$C_{15}H_{32}O$	228.41
25	9.511	2.134	Bicyclo[5.3.0]dec-1 (7)-ene-2,5-dione	$C_{11}H_{16}O$	164.24
26	9.646	2.536	D-Allose	$C_6H_{12}O_6$	180.16g
27	9.891	4.880	Pentadecanoic acid, 3-oxo-, methyl ester	–	–
28	10.341	1.344	Methyl 10-oxohexadecanoate	$C_{17}H_{32}O_3$	284.4
29	10.531	1.979	Dodecanoic acid	$C_{12}H_{24}O_2$	200.32
30	10.766	2.301	d-Glycero-d-ido-heptose	$C_7H_{14}O_7$	210.18
31	11.062	0.943	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312.5
32	11.232	1.167	Propanedioic acid, 2-(2-oxiran-2-yl) ethyl-, diethyl ester	$C_{22}H_{40}O_4$	368.5
33	11.617	13.976	Quinic acid	$C_7H_{12}O_6$	192.17
34	12.302	1.669	4,4,5,8-Tetramethylchroman-2-ol	$C_{15}H_{22}O_2$	234.33
35	12.522	1.365	7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3-hydroxy-1-butenyl)-1,5,5-trimethyl	$C_{13}H_{22}O_3$	226.31
36	12.732	1.215	Desulphosinigrin	$C_6H_{12}O_5S$	196.22
37	12.877	1.149	4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0 (1,4)]dec-2-en-7-ol	$C_{13}H_{20}O_2$	208.3
38	13.137	0.887	Strychane, 1-acetyl-20à-hydroxy-16-methylene-	–	–
39	13.177	0.653	Octadecanedioic acid	$C_{18}H_{34}O_4$	314.5
40	13.303	0.525	cis-5-Dodecenoic acid, dimethyl (3,3,3-trifluoropropyl) silyl ester	$C_{17}H_{31}F_3O_2Si$	352.5
41	13.528	1.073	Glycyl-D-asparagine	$C_6H_{11}N_3O_4$	189.17
42	13.918	0.464	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326.4
43	14.163	1.291	tert-Hexadecanethiol	$C_{48}H_{99}AuS_3$	969.5
44	14.483	2.205	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37
45	14.918	0.912	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$	222.28
46	17.764	0.505	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5
47	18.455	2.123	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
48	24.932	3.060	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	$C_{30}H_{52}O$	428.7
49	28.133	0.556	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390.6
50	29.034	1.615	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.7

against cancer activity. This research article also emphasizes varied pharmacological properties of *A. lawsonii*.

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AUTHORS' CONTRIBUTIONS

Sathya E has performed data collection, drafting of manuscript, and graphical representation of statistical data, Sekar T performed the study design and editing of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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