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Phytochemical Analysis and GC-MS profiling in the leaves of *Sauropus Androgynus* (l) MERR

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Abstract

In the present the phytochemical analysis of *Sauropus androgynus* was carried out. Phytochemical analysis of the leaves of this plant is reported for the first time. The leaves indicated the presence of proteins, resins, steroids, tannins, glycosides, reducing sugar, carbohydrates, saponins, sterols, terpenoids, acidic compounds, cardiac glycosides, catechol, phenols, alkaloids, flavonoids. In the GC-MS analysis the *Sauropus androgynus* extract result shows the presence of bioactive compounds which revealed a broad spectrum of many medicinal property and antioxidant activity were identified. The functional group present in these compounds was identified by IR spectral analysis. This study also helped to identify the formula and structure of biomolecules which can be used as drugs.

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Introduction

Traditional medicine is an important source of potentially useful compounds for the development of chemotherapeutic agents. [1]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties [2]. *Sauropus androgynus* L. Merr., also known as katuk, star gooseberry, or sweet leaf, is a shrub grown in some tropical regions as a leaf vegetable. It is most popular in South Asia and Southeast Asia. In India it also known as Multivitamin Plant as it contains an excellent source of vitamins A, B, C, carotenoid and also it has high nutritive value and contains phytochemicals which can act as antioxidant [3]. The

leafy vegetable *Sauropus androgynus* is commonly used as an effective medicinal herb in the treatment of diabetics, cancer, inflammation, microbial infection, cholesterol and allergy due to its antioxidant effect [4].

Materials and Methods

Collection of plant

Preparation of the extract: The fresh leaves of *Sauropus androgynus* were washed with tap water and shade dried at room temperature (28 ±2 ˚C). The dried leaves were powdered by electric blender. Ethanol was used for the extraction of 15g in the Soxhlet apparatus followed by the standard procedure [5]. The phytochemical analysis and GC-MS profiling of the plant extract was carried out.

Phytochemical analysis was performed using standard procedures[6]

Test for Carbohydrates by Molisch's test

Few drops of Molisch's reagent was added to each of the portion dissolved in distilled water, this was then followed by addition of 1 ml of conc. H_2SO_4 by the side of the test tube. The mixture was then allowed to stand for two minutes and then diluted with 5 ml of distilled water. Formation of a red or dull violet colour at the interphase of the two layers shows a positive test [7].

Tests for reducing sugar by Fehling's test

About 0.5 g each portion was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars [7].

Test for Protein by Xanthoprotein test

To 1 mL of extract, few drops of nitric acid was added by the sides of the test tube and observed for formation of yellow color [6].

Tests for sterols by Liebermann-buchard test

Two milliliter of acetic anhydride was added to 0.5 g of extract and 2 ml and 3 ml of sulphuric acid was added by the sides of the test tube and observed the colour change from violet or blue-green [6].

Test for resins

To 0.5g of each sample was added 5ml of boiling ethanol. This was filtered through Whatman No.1 filter paper and the filtrate diluted with 4ml of 1% aqueous HCl. The formation of a heavy resinous precipitate indicated the presence of resins [6].

Test for Tannins

About 0.5 g each portion was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins [8].

Test for Saponins

One gram of each portion was boiled with 5 ml of distilled water, filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins [7].

Test for Alkaloids by Mayer's test

Solvent free extract, 50mg is stirred with few ml of dilute hydrochloric acid and filtered. The filtrate is tested carefully with various alkaloid reagents as follows the Mayer's test of a few ml of filtrate, a drop or two of Mayer's reagent are added by the side of the test tube. A white or creamy precipitate indicates the test as positive [9].

Test for Flavonoids by Shinoda's test

About 0.5 of each portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids [8].

Test for Terpenoids by Salkowski Test

To 0.5g of the extract, 2mL of chloroform was added 3ml Conc. H_2SO_4 was carefully added to form a layer.

A reddish brown coloration of the interface indicates the presence of terpenoids. [9].

Test for Glycosides

For detection of glycosides, 50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2h on a water bath, filtered and the hydrolysate is subjected to the following tests.

Borntrager's test

To 2ml of filtered hydrolysate add 3ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates the presence of glycosides. [6,10].

Test for Phenols by Ferric chloride test

The 50mg extract is dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compounds [11].

Test for Steroids

To 0.5 g of extracts was added to 5 mL of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion [6,10]

Tests for acidic compounds

To the alcoholic extract sodium bicarbonate solution was added and observed for the production of effervescences [6,10]

Tests for cardiac glycosides by Keller Killiani's

Among 100 mg of extract was dissolved in 1 mL of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layer with 1mL of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of de-oxy sugar characteristics of cardenolides [6,10]

Tests for catechol [6,10].

To 2 mL of test solution alcohol is added and erlich's reagent and few drops of conc.hydrochloric acid was added. The result was obtained.

Gas Chromatography – Mass Spectrum Analysis (GC-MS)

GC-MS was carried out at Indian Institute of Crop Processing Technology (IICPT) Thanjavur. This was carried out to study the phytochemical components present in the extract. 20 g of the powdered leaves were soaked in 95% ethanol for 12 h. The extracts were then filtered through Whatmann filter paper No. 41 along with 2 g sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant material was used. 2µl of these solutions was employed for GC/MS analysis.

GC analysis

GC-MS analysis was carried out on a GC clarus 500 Perlin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrophotometer (GC – MS) instrument employing the following conditions: column Elite – 1 fused silica capillary column (30 x 0.25 mm ID x 1 EM df, composed of 100% Dimethyl polysiloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1 injector temperature 250˚C; ion-source temperature 280˚C. The oven temperature was programmed from 110˚C (isothermal for 2 min). With an increase of 10 C/min, to 200 C then 5 C/min to 280˚C, ending with a 9 min isothermal at 280˚C. Mass spectra were taken at 70 eV; a scan interval of 0.5 and fragments from 40 to 550 Da.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute

Standard and Technology (NIST) having more than 62,000 patterns. 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 phytochemical constituent of *Sauropus androgynus* was qualitatively analyzed and the results are presented in Table:1. In the GC-MS analysis the mass spectra of identified compounds from ethanolic leaf extract of *Sauropus androgynus* were matched with those found in the NIST/NBS spectral database are given in Table:2 and the chromatographic peak are represented in Figure1. The medicinal properties of the analyzed phytochemical constituents were listed in Table:3. The identification compound based on comparison of their mass spectra with those of NIST and Wiley Libraries [12]. Further analysis was done with Infrared spectroscopy to identify the functional group present in the above listed compounds such as alcohol, phenol, alkane and sulphate groups are present in these compounds.

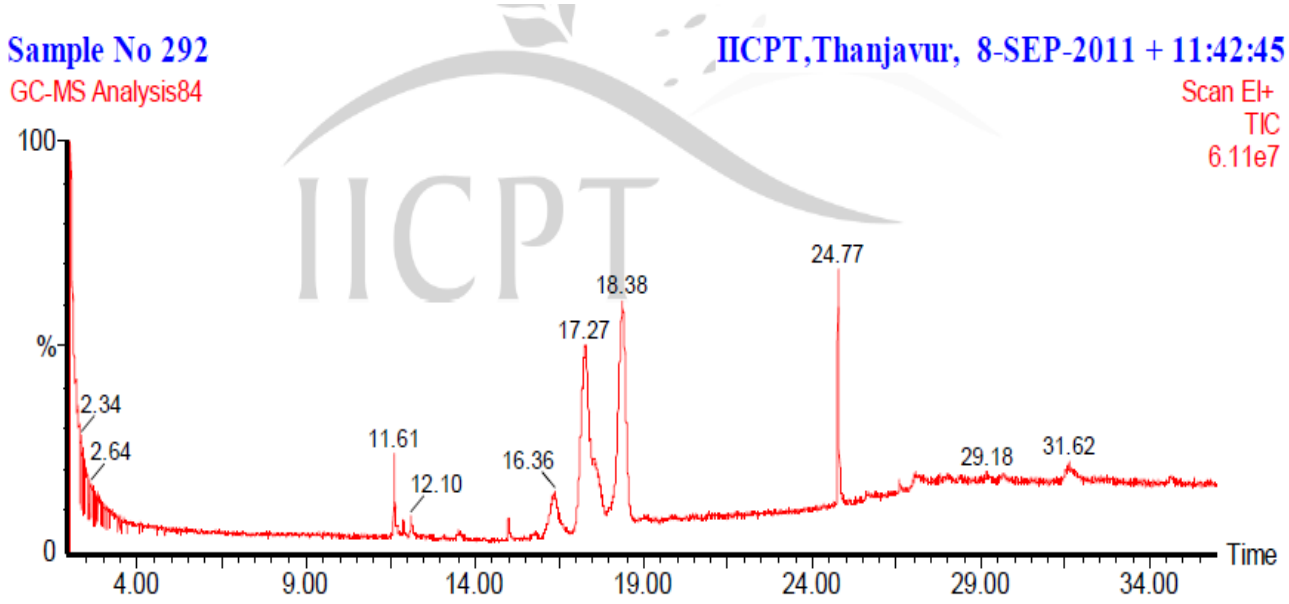

Fig1: Chromatogram obtained from the GC-MS with the extract of *Sauropus androgynus*.

Discussion

The GC/MS analysis showed that at least 8-9 compounds were present in ethanolic extract of *Sauropus androgynus*. The fragmentation pattern of the major compound is 2(1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl) retention time is 17.27 and peak area percentage is 41.17. The next highest found compound is Azulene retention time is 18.38 with 36.20 peak area percent. These compounds have good pharmacological activity viz., anticancer, antitumor and antibiosis [13,14]. The compounds Pyrene hexadecahydro and Squalene with peak area percentage 9.07 and 8.06 respectively have shown to improve human immunity [15]. Phytol was also detected 0.88% relative amount with 15.00 retention time; this compound is known to possess an antimicrobial, antioxidant activity [16]. Futhermore the alcoholic compound 1, 14 Tetradecanediol identified compounds reported to have antimicrobial property were also found [17].

Table: 1 Phytochemical Constituents analysis of *Sauropus androgynus*

S. No	Name of the Test	Phytochemical analysis of *Sauropus androgynus*
1.	Test for Carbohydrates Molisch's test	+
2.	Tests for reducing sugar Fehling's test for free reducing sugar	+
3.	Test for Protein Xanthoprotein test	++
4.	Tests for sterols Liebermann-buchard test	+
5.	Test for resins	+
6.	Test for Tannins	++
7.	Test for Saponins	++
8.	Test for Alkaloids Mayer's test	++
9.	Test for Flavonoids Shinoda's test	++
10.	Test for Terpenoids Salkowski Test	+
11.	Test for Glycosides Borntrager's test	++
12.	Test for Phenols Ferric chloride test	++
13.	Test for Steroids	-
14.	Tests for acidic compounds	+
15.	Tests for cardiac glycosides Keller Killiani's	+
16.	Tests for catechol	-

Table: 2 GC-MS analysis of *Sauropus androgynus*

RT	Name	Peak area %
11.61	1,14-Tetradecanediol	2.82
11.89	1-Octadecyne	0.48
12.10	1-Hexadecyne	0.75
13.51	Decanoic acid, ethyl ester	0.57
15.0	Phytol	0.88
16.36	Pyrene, hexadecahydro-	9.07
17.27	2(1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	41.17
18.36	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1-methylethenyl)-	36.20
24.77	Squalene	8.06

Table: 3 Medicinal properties of *Sauropus androgynus*

RT	*Name of the compound*	*Biological activity*
11.61	1,14-Tetradecanediol	Antimicrobial
11.89	1-Octadecyne	Anti-inflammatory agent, Anti-bacterial agent, Fragrance
12.10	1-Hexadecyne	Antibacterial
13.51	Decanoic acid, ethyl ester	Flavour, Nematocide
15.0	Phytol	Cancer prevention, Auto immune response
16.36	Pyrene, hexadecahydro	*Anti-bacterial agent, Fragrance*
17.27	2(1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	*Anti-inflammatory*
18.36	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1-methylethenyl)-	*Antiallergic, Antihistaminic, Anti-inflammatory, Antipyretic, Antiseptic, Antispasmodic, Antiulcer*
24.77	Squalene	*Antibacterial, Antioxidant, Antitumour, Anticancer prevention, Chemopreventive, Immunostimulant, Lipooxygenase inhibitor,*

Conclusion

Efforts in this regard have focused on plants because of their use historically and the fact that a good portion of the world's population rely on plants for the treatment of infections and non infectious diseases [18]. The leaves of *Sauropus androgynus* leaves has a high level of provitamin A carotenoids, especially in freshly picked leaves, as well as high levels of vitamins B and C, protein and minerals. Nutrient content of the leaves is usually higher in more mature leaves. [19]. *Sauropus androgynus* ethanolic plant extracts showed anti-inflammatory effects on nitric oxide inhibitory activity and antioxidant activity [20].

The present investigation may be used to authenticate the scientific reason of free radical-scavenging with use of plant in the treatment or prevention of the onset of deadly disorders like arthritis, breast cancer, atherosclerosis, etc. And also it is a right step in the direction of searching for novel and more effective Gas chromatography and mass spectroscopy analysis which showed the existence of various compounds with variable chemical structures. At end point it is conclude that the in vivo studies on biological systems can open up new way for natural antioxidants that can also be employed for clinical trials which may generate successful results in future.

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