Research Journal of
Phytochemistry

ISSN 1819-3471

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Phytochemistry

ISSN 1819-3471
DOI: 10.3923/rjphyto.2018.7.13

Research Article
Chemical Constituents of *Sauropus androgynus* and Evaluation of its Antioxidant Activity

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Abstract

Background and Objective: Antioxidants are involved in the prevention of cancer, aging and variety of diseases. The scientific community has begun to interest in the identification, recovery and enhanced performance of natural antioxidant principles from plant sources. The objective of this present study is also guide to the invention of new antioxidant sources from *Sauropus androgynus*. **Materials and Methods:** The antioxidant activity of plant material was assayed by DPPH radical scavenging assay and also the chemical compositions were investigated using GCMS-QP-2010 plus gas chromatography-mass spectrometry. Vitamins like ascorbic acid, riboflavin and thiamine were estimated according to the method of Koche. **Results:** Results showed that about 20 phyto active components are obtain from the leaves having vitamin C as major component while seed contains 10 components having fatty acids as major components. The leaf and seed extract showed significant DPPH scavenging activity (IC_{50} values are 49.62±2.52 and 52.47±2.21 μg mL^{-1}) compared with the values obtained for ascorbic acid standard (IC_{50} value 48.82±1.80). **Conclusion:** From the results obtained it was concluded that *Sauropus androgynus* contain various phytochemicals, justifying their use for various human ailments.

Key words: Phytochemicals, DPPH, antioxidant, GCMS, multi vitamin plant, vitamin C

Citation: Rajeswari Bose, Moorthy Saravana Kumar, Arumugam Manivel and Shanmugavadivelu Chandra Mohan, 2018. Chemical Constituents of *Sauropus androgynus* and evaluation of its antioxidant activity. Res. J. Phytochem., 12: 7-13.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Similar to pharmaceutical agents, functional foods and nutraceuticals also possess physiological and molecular targets that modulate clinical end-points associated with chronic diseases[1]. Edible plants possessing health promoting capacities are therefore being re-scrutinised to exploit their potentials as nutraceuticals, which are bioactive compounds that confer protection from chronic diseases[2]. Medicinal plants play an important role in preventive and curative treatments inchronic diseases such as cardiovascular diseases (CVD), diabetes and cancers. The preventive or curative role mainly due to the presence of phytochemicals is in medicinal plants. A diet rich in plant foods can provide over 25,000 phytochemicals that cannot be supplied by a refined oils, refined sugar and refined salt[1]. The protective role of phytochemicals may combine with antioxidant activity. Antioxidant phytochemicals possess strong free radical scavenging activity. Only traditional medicinal plants are the best sources of these phytochemicals. *S. androgynus* one of the most popular leafy-vegetables in India. The rich vitamin content of the leaves has led to its popular name, 'multivitamin plant'. Antioxidants present in plants were preventing cell and tissue damage as they act as scavenger. Micronutrient deficiencies have evolved as a serious global problem, especially in areas where the diet lacks variety[3]. A number of vitamins and minerals are considered essential for physical and mental development, immune system and various other metabolic processes. Recently, knowledge related to beneficial phytocomponents in medicinal plants has increased. Thus, screening on the active phytocompounds of plants has led to the invention of some new medicinal drugs. Hence the aim of the present investigation is to scrutinize the presence of vitamins, new chemical constituents of leaves and seeds of *Sauropus androgynus* by using GC-MS and evaluate the ethanolic extract of *Sauropus androgynus* for *in vitro* antioxidant potential.

MATERIALS AND METHODS

Plant material: The seeds and leaves and of *Sauropus androgynus* were collected from the local area of Thanjavur district, India, during the month of May-June, 2017. Accurately, 1.0 kg of shade-dried coarse powder of the plant material was extracted with 80% v/v aqueous ethanol by maceration at room temperature for 72 h. After the completion of each extraction, the extracts were filtered, concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50°C). The residues were stored in a vacuum desiccator for further use.

Table 1: Vitamin composition of leaves of *Sauropus androgynus*

Vitamin	Quantity (mg/100 g)
Vitamin C (Ascorbic acid)	298.32±0.34
Vitamin B2 (Riboflavin)	0.25±0.22
Vitamin B1 (Thiamine)	0.43±0.25

Values are Means±SD calculated as mg/100 g dry weight (DW) for *Sauropus androgynus* analyzed individually in triplicate

Preparation of extract: Different concentrations of leaf and seed extracts (20, 40, 60 and 80 μg mL^{-1}) were chosen for *in vitro* antioxidant activity. L-ascorbic acid (20, 40, 60 and 80 μg mL^{-1}) was used as the reference standard.

Estimation of vitamins: Vitamins like ascorbic acid, riboflavin and thiamine were estimated according to the method by Koche[4] (Table 1).

GC-MS analysis: Gas chromatography mass spectrum analysis was carried out using GCMS- QP 2010 PLUS SHIMADZU JAPAN with injector temperature of 270°C, carrier gas Helium (5.5 lb/in2) and carrier gas pressure of 11.6 kpa. RestekRtx- 5MS capillary column used, the column length was 30.0 m with a diameter of 0.32 mm and the flow rate of 41 mL min^{-1}. The mass spectrum was also equipped with a computer fed mass spectra data bank.

DPPH radical-scavenging activity: DPPH radical-scavenging activity was determined by the method of Shimada *et al.*[5].

Procedure: Briefly, a 2 mL aliquot of DPPH ethanol solution (25 μg mL^{-1}) was added to 0.5 mL sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. Then the values of absorbance are placed within the formula given below to obtain the scavenging activity of *Sauropus androgynus* phytocomponents:

$$\text{Redical scavenging activity (\%)} = 100 - \left(\frac{A_C - A_S}{A_C}\right) \times 100$$

Where:

A_C = Absorbance of the control

A_S = Absorbance of the sample

Statistical analysis: The results were presented as Mean±SD. Data were statistically analyzed using student t-test. For the calculation of IC_{50}, linear regression analysis was done using Graph Pad prism statistical software.

RESULTS AND DISCUSSION

Chemical composition by GC-MS analysis: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST Library. In the GC-MS analysis, 20 bioactive phytochemical compounds were identified in the ethanol extract of leaves (Fig. 1 and Table 2) and 10 bioactive compounds were identified in the ethanol extract of seeds (Fig. 2 and Table 3). Biological activity of some identified components listed in Table 4.

Twenty compounds were identified in the GCMS analysis of leaves of *Sauropus androgynus*. The Chromatogram (Fig. 1) showed 20 prominent peaks. The largest peak (RT 19.564) is due to the presence of l-(+)-Ascorbic acid 2,6-Dihexadecanoate. The dominant component (peak height 27.82) of this plant L-(+)-Ascorbic acid 2,6-Dihexadecanoate has been reported to have antioxidant, anti-inflammatory and anti-nociceptive properties[6,7]. The1-(+)-Ascorbic acid 2,6-Dihexadecanoate which is vitamin C fatty acid conjugate, indicating the presence of vitamin C. The n-Hexadecanoic acid-palmitic acid found in seed extract (RT 19.611), possesses an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant and antiandrogenic activities[8]. *cis*-Vaccenic acid, is an

Table 2: Compounds identified in the leaves of *Sauropus androgynus*

Peak#	R. Time	Area (%)	Height (%)	Name
1	8.471	0.40	0.53	Cyclopentasiloxane, decamethyl
2	11.488	0.25	0.26	Cyclohexasiloxane, dodecamethyl-
3	13.758	0.30	0.14	1,2-Benzenedicarboxylic acid, 2-Ethoxy-2-oxoethyl methyl ester
4	15.557	1.98	1.94	2,4-Imidazolidinedione, 1-[{(5-nitro-2-furanyl)methylene}amino]-
5	17.381	0.32	0.26	9-Octadecenoic acid (Z)-
6	17.721	0.53	0.59	Heptadecanoic acid, ethyl ester
7	18.029	0.25	0.28	2,6,8-Trimethyl-bicyclo [4.2.0]oct-2-ene-1,8-diol
8	18.230	2.65	4.27	2,6,10-Trimethyl,14-ethylene-14-pentadecane
9	18.333	0.33	0.27	2-Pentadecanone, 6,10,14-trimethyl-
10	18.502	0.64	0.94	7-Octadecyne, 2-methyl-
11	18.710	1.33	2.04	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
12	19.393	0.43	0.35	cis-Vaccenic acid
13	19.564	27.81	27.82	l-(+)-Ascorbic acid 2,6-Dihexadecanoate
14	19.866	13.76	17.85	Hexadecanoic acid, ethyl ester
15	21.174	3.27	4.12	Phytol, acetate
16	21.435	4.29	3.76	9,12-Octadecadienoic acid (Z,Z)-
17	21.513	12.90	7.47	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
18	21.706	11.28	9.40	Ethyl (9Z,12Z)-9,12-Octadecadienoate
19	21.806	15.93	16.32	Ethyl 9,12,15-Octadecatrienoate
20	22.010	1.35	1.39	Octadecanoic acid, ethyl ester
		100	100	

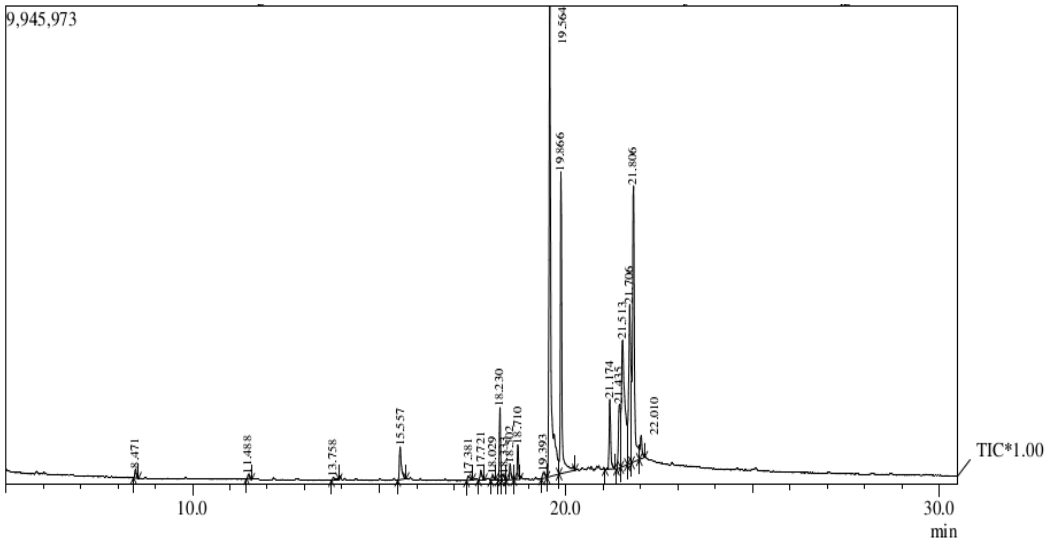

Fig. 1: GCMS chromatogram of leaves of *Sauropus androgynus*

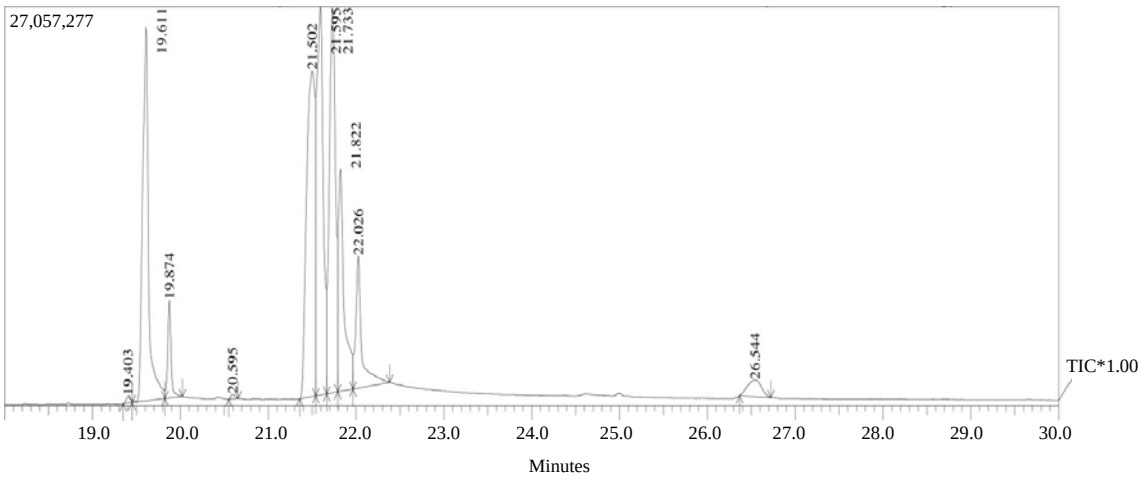

Fig. 2: GCMS chromatogram of seeds of *Sauropus androgynus*

Table 3: Compounds identified in the seeds of *Sauropus androgynus*

Peak#	R. Time	Area (%)	Height (%)	Name
1	19.403	0.23	0.36	cis-9-Hexadecenoic acid
2	19.611	17.74	19.11	Hexadecenoic acid
3	19.874	2.60	5.03	Tetradecanoic acid, ethyl ester
4	20.595	0.14	0.23	l-(+)-Ascorbic acid 2,6-Dihexadecanoate
5	21.502	22.53	16.70	9,12-Octadecadienoic acid (Z,Z)-
6	21.595	19.61	19.87	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
7	21.733	18.98	19.69	9-Octadecenoic acid (Z)-
8	21.822	10.04	11.38	Ethyl 9,12,15-Octadecatrienoate
9	22.026	6.29	6.77	Octadecanoic acid, ethyl ester
10	26.544	1.84	0.85	Squalene
		100	100	

Table 4: Biological activity of some identified compounds

Name of the identified compounds	Biological activity**
9,12-Octadecadienoic acid (Z,Z)- (linoleic acid)	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic and antiarthritic[19]
Tetradecanoic acid (myristic acid)	Anti oxidant, cancer preventive, hypercholesterolemic, nematicide, lubricant, cosmetic, lubricant and antibacterial[20]
1-(+)-Ascorbic acid 2,6-Dihexadecanoate (Vitamin C derivative)	Anti oxidant, anti scorbutic, anti inflammatory, anti nociceptive, anti mutagenic and wound healing property.[6,7]
Heptadecanoic acid (margaric acid)	Antioxidant, anti fungal, surfactant[20]
9-Octadecenoic acid (Z) (Oleic acid)	5-α reductase inhibitor, allergenic, α-reductase inhibitor, anti inflammatory, anti androgenic, cancer preventive, anemiagenic, anti alopecic, anti leukotriene-D4, choleretic, dermatitigenic, hypocholesterolemic, insectifuge, perfumery, propecic and flavour[19]
Octadecanoic acid (stearic acid)	5-α reductase inhibitor, hypo cholesterolemic, suppository, cosmetic, lubricant, surfactant and softening agent, perfumery, propecic and flavor[19]
Cis-11,14-Eicosadienoic acid, methyl ester	Anti inflammatory, anti oxidant, anti arthritic and anti coronary
Hexadecanoic acid (Palmitic acid)	Anti oxidant, hypocholesterolemic, nematicide, pesticide, lubricant, anti androgenic, flavour and hemolytic-5-α reductase inhibitor[8]
Hexadecanoic acid, ethyl ester	Antioxidant[19]
3,7,11,15-Tetramethyl-2- hexadecen-1-ol (Phytol)	Anti microbial, anti cancer, anti-inflammatory and hepatoprotective[21]
Squalene	anti bacterial, anti oxidant, anti tumor, cancer preventive, immunostimulant and chemo preventive[11-14]
Phytol, acetate	Antimicrobial, anti-inflammatory anticancer and diuretic[21]
9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (Linolenic acid)	Antiinflammatory, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistamic, anticoronary, antieczemic, antiacne[19]

omega-7 fatty acid, is found in leaves extract. Its IUPAC name is (Z)-11-Octadecenoic acid and its lipid shorthand name is 18:1*cis*-11.

A well known antibiotic 2,4-Imidazolidinedione, 1-[{(5-nitro-2-furanyl)methylene}amino] available in leaves extract, its general name Nitrofurantoin, is an antibiotic that fights bacteria in the body. Nitrofurantoin is used to treaturinary tract infections. Nitrofurantoin, sold under the trade name Macrobid among others, is an antibiotic used to treat bladder infections. Nitrofurantoin is sometimes described as a urinary antiseptic, owing to its value in clearing infections of the urinary tract caused by Gram-negative pathogens such as *Pseudomonas aeruginosa, Serratia marcescens* and *Proteus mirabilis*[9].

Decamethylcyclopentasiloxane(D_5) found in leaves extract (RT 8.471), is an organosilicon compound with the formula $[(CH_3)_2SiO]_5$. It is becoming more common in hair conditioners as it makes the hair easier to brush without breakage. In Canada, among the volume used in consumer products approximately 70% were for antiperspirants and 20% for hair care products[10].

Squalene ($C_{30}H_{50}$) available in seed extract at RT 26.544. Squalene has several beneficial properties. It is a natural antioxidant, serves in skin hydration[11] and has been used as emollient in adjuvant for vaccines[12]. As a compound of olive oil, it also has a preventive effect on breast cancer, possesses tumor-protective and cardio-protective properties[13,14] and decreases the serum cholesterol level[15]. It has been reported that squalene emulsions given simultaneously with anti-cancer drugs provide favorable effects either directly or indirectly by enhancing efficacy of anti-cancer drugs[16-18].

10 components identified in seeds extract in which the major compound were fatty acid such as 9,12-Octadecadienoic acid (Z,Z)-, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-, 9-Octadecenoic acid (Z)- and Hexadecenoic acid. Samad *et al.*[19] also conducted GCMS analysis in the leaves extract of *S. androgynus* and identified major components were 9,12,15-Octadecatrienoic acid (Z,Z,Z)-, hexadecanoic acid.

Antioxidant activity: The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[22]. Extracts of plants are allowed to react with the stable radical, DPPH, in methanol solution. The reduction capability of DPPH radicals is determined by the decrease in its absorbance at 517 nm, induced by an anti-oxidant (AH) after 30 min as follows:

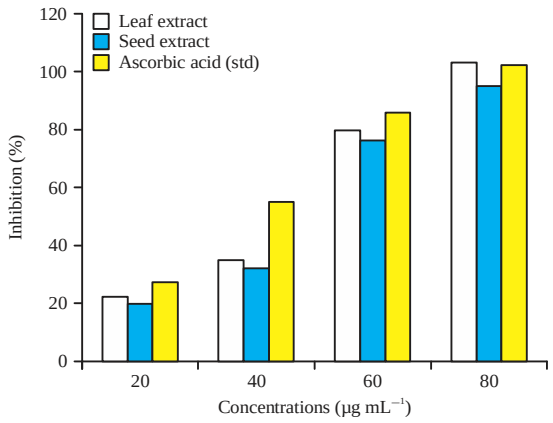

Fig. 3: DPPH Radical scavenging activity of leaf and seed extracts of *Sauropus androgynus* at different concentrations

$$DPPH+AH \rightarrow DPPH-H+A^{\bullet}$$

The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure the antioxidant activity[23]. The IC_{50} value was obtained from a graph plotted between sample concentration and the absorbance of the DPPH radical. A lower IC_{50} value corresponds with a higher antioxidant power. The leaf and seed extracts showed significant DPPH scavenging activity (IC_{50} values are 49.62±2.52 and 52.47±2.21 µg mL^{-1}) compared with the values obtained for ascorbic acid standard (IC_{50} value 48.82±1.80). DPPH Radical scavenging activity of leaf and seed extracts are shown in Fig. 3.

Previous study revealed that *S. androgynus* leaves possess a high total phenolic content and antioxidant activities. Based on the electron transfer reaction assays, *S. androgynus* leaves contained higher antioxidant activities than many other leafy vegetables of Indonesian origin[24]. A previous study on antioxidant activities on aqueous extracts of 25 tropical plants showed that *S. androgynus* has a high polyphenol content, cupric ion chelating activities, free radical scavenging and reducing ferric ion antioxidant properties[25]. The leaves have containing bioactive antioxidants, such as carotenoids, flavonoids and other phytochemicals. These phytochemicals can reduce oxidative stress, thus reduce the risk of some chronic diseases[24].

CONCLUSION

It was concluded that *Sauropus androgynus* extracts constitute a wide range of bioactive phytochemicals with high therapeutic values. The identified phytochemicals have several activities such as antioxidant, analgesic and anti-inflammatory,

anti biotic, anti cancer, anti nociceptive, anti arthritic and hypoglycemic. The results revealed that *Sauropus androgynus* leaves were excellent source of vitamin C derivatives. Due to good antioxidant phytochemicals and vitamin composition, *Sauropus androgynus* would be an important plant in pharmaceutical formulations and play an important role in improving the human health by participating in the antioxidant defense system against free radical generation.

SIGNIFICANT STATEMENT

The present study conducted to evaluate the antioxidant activity and components of *Sauropus androgynus* seeds and leaves and found that about 20 phyto active components are obtain from the leaves having vitamin C as major component while seed contain 10 components having fatty acids as major components which confirmed its strong antioxidant activity whereas, antibiotic component nitrofurantoin also obtained from it. The study result helps the researchers in determining its strong antioxidant activity *in vivo* and also other active components of plant in future. Thus best theory on it may be arrived at.

ACKNOWLEDGMENT

The authors would like to acknowledge Periyar TBI, Periyar Maniammai University, Thanjavur for GCMS analysis.

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