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PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF *Sauropus androgynus* L. MERR.: A STUDY ON ITS POTENTIAL HEALTH BENEFITS AND ASSOCIATED RISKS.

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Abstract:

Sauropus androgynus L. Merr., a member of the Euphorbiaceae family, is a native vegetable of Southeast Asia known for its nutritional and medicinal benefits. Traditionally used in Malaysian medicine for urinary issues, fever reduction, and lactation enhancement, it is consumed in salads, curries, and stir-fries. Despite its health benefits, the plant has been linked to pulmonary dysfunction, with initial studies revealing drowsiness and constipation in the elderly due to high alkaloid content. Reports from Taiwan in 1995 highlighted severe respiratory issues, including irreversible respiratory failure, associated with excessive intake of *Sauropus androgynus*, necessitating lung transplantation in some cases.

This study aimed to determine the phytoconstituents and evaluate the antioxidant properties of *Sauropus androgynus* leaves. The extraction process involved maceration in 70% ethanol, yielding a 24.05% hydroalcoholic extract. Phytochemical screening revealed the presence of alkaloids, amino acids, carbohydrates, flavonoids, saponin glycosides, tannins, terpenoids, phenols, resins, phlobatannins, and vitamins B2 and C.

Antioxidant activity was assessed using H_2O_2 and DPPH scavenging assays. The extract showed significant free radical scavenging activity, with IC_{50} values of 357.1 µg/ml and 720.51 µg/ml for H_2O_2 and DPPH assays, respectively. These findings suggest that *Sauropus androgynus* has considerable antioxidant potential, which may contribute to its traditional medicinal uses and nutritional benefits.

(**Keywords**: *Sauropus androgynus*, Phytochemical screening, Antioxidant, DPPH, H_2O_2.)

Introduction:

Sauropus androgynus L. Merr. is a member of the Euphorbiaceae family. This native vegetable of Southeast Asia grows well in humid, warm climates and is widely grown for traditional medicinal uses[5].This plant is eaten raw in salads, curries, or stir fries, and is used in traditional Malaysian medicine to alleviate urinary issues, reduce fever[2], and boost the production of breast milk[18]. Because of its reputedly higher vitamin content and nourishment than other vegetables, it is regarded as a "multigreen" vegetable[12,17]. Despite being used as a food and medicine, *Sauropus androgynus* has been linked to pulmonary dysfunction in a number of investigations. Bender and Ismail carried out the initial investigation into the harmful consequences of *Sauropus androgynus*.[3]The study discovered that the elderly in Malaysia experienced drowsiness and constipation as a result of unnecessarily consuming this plant, proving that fresh *Sauropus androgynus* leaves contain 580 mg of the alkaloid "papaverine" per 100 grams, which is an excessive amount given that an antispasmodic medication only requires 200 mg of papaverine per day.[3,12] After *Sauropus androgynus* was originally launched as a weight-loss supplement in 1994, the first reports of issues resulting from excessive intake of the supplement appeared in Taiwan in 1995.[9,10,14] Breathing difficulties, irreversible respiratory failure, and fatalities after consuming *Sauropus androgynus* have all been documented. According to histopathological analyses, those individuals had constrictive bronchiolitis obliterans[7,15,19], for which there was no other treatment option but lung transplantation.[11,22,23,24] The current study is to determine the phytoconstituents and to determine free radical scavenging activity to investigate the potential Anti-oxidant bioactives from the leaves of *Sauropus androgynus.*

Materials and Methods:

Extraction: Fresh *Sauropus androgynus* leaves were gathered, cleaned, and then shade-dried. The plant was hand-pulverized to a coarse powder after being shade-dried. For 72 hours, 200 g of coarse powder were macerated in 70% ethanol. Using a rotary evaporator, the macerate was dried out after the extraction process. Furthermore, lyophillization was used to eliminate any leftover solvent. After that, the

dried extract was sealed in a tight-fitting container and weighed to determine its extractive value.

The yield of extracts were calculated using following formula:-

Percentage yield = a/b× 100

Where a= weight of hydroalcoholic extract

b= weight of *S. androgynus*

Phytochemical screening:

Test for alkaloids:

a. *Dragendorff's test:*

Extract gives reddish brown precipitate with Dragendorff's reagent (potassium bismuth iodide solution)

b. *Mayer's test:*

Extracts gives cream colour precipitate with Mayer's reagent (potassium mercuric iodide solution)

c. *Hager's test:*

Extracts gives yellow precipitate with Hager's reagent. (saturated solution of picric acid)

1. Test for amino acids:

a. *Million's test:*

To 2 ml of extract add 2ml of Million's reagent, observed for white precipitate of amino acids.

b. *Ninhydrine test:*

To the 2ml of extract added Ninhydrine solution, boiled and observed for violet colour.

2. Test for carbohydrates:

a. *Molisch's test:*

To the 3ml of extract few drops of alcoholic a-napthol added, then added few drops of concentrated sulfuric acid through sides of test tubes and observed for purple to violet colour ring at the junction.

b. *Test for reducing sugars:*

a. 0.5gm of extract was dissolved in water then filterd, filtrate heated with 5ml of fehling solution and B and observed for formation of red precipitate of cuprous oxide.

3. Test for flavonoids:

a. *Shinoda test:*

To the extract added few magnesium turnings and concentrated hydrochloric acid dropwise, and observed for pink scarlet, crimson red or green to blue colour appears after few minutes.

b. *Alkaline hydrochloride test:*

To the extract solution added few drops of sodium hydroxide soution and observed for formation of intense yellow colour which turns to colourless on addition of few drops of dilute acid.

4. Test for glycosides:

Chemical test for specific glycosides

I. Anthraquinone glycosides

a. *Borntrager's test:*

Bolied the 2ml of extract with 1ml of sulphuric acid in a test tube for 5 minutes. Filtered while hot, cooled the filtrate and shaked with equal volume of dichloromethane. Seperated the lower layer of dichloromethane and shake with half of its volume of dilute ammonia, observed for rose pink to red colour produced in the ammonical layer.

II. Cardiac glycosides

b. *Keller-killiani test (test for deoxy sugars):*

Extract the test solution with chloroform and evaporated to dryness. Added 0.4ml of glacial acetic acid containing trace amount of ferric chloride. Then transferred to the test tube, added 0.5ml of concentrated sulphuric acid by the side of the test tube, observed for blue colour at acetic acid layer.

c. *Baljet's test:*

Treated the extract solution with oicric acid, observed for formation of orange colour.

III. Saponin glycoside

a. *Froth formation test:*

Placed 2ml of extract solution in water in a test tube, shake well and kept for formation of stable froth (foam).

5. Test for tannins

Extract was dissolved in 10ml of distilled water and filtered. 1% aqueous iron chloride ($FeCl_3$) solution was added to the filtrate. The appearance of intense blue or black colour indicate presence of tannins in the test sample.

6. Test for terpenoids (Salkowski test)

3ml of extract was mixed in 2ml of chloroform followed by addition of 3ml of concentrated (H_2SO_4). A layer of reddish brown colouration was formed at the interface thus indicate presence of terpenoids.

7. Test for phenols

To 2ml of extract, 2ml of distilled water followed by few drops of 10% aqueous ferric chloride were added. Formation of blue or green colour indictaes presence of phenols.

8. Test for sterols

0.5 gm of extract was added to 5ml of distilled water in a test tubes. 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.

9. Test for resins

About 0.5gm each portion was added 5ml of boiling ethanol. This was filtered through whatman No.1 filter paper and filtrate diluted with 4ml of 1% aqueous HCl. The formation of a heavy resinous precipitate indiactes presence of resins.

10. Test for phlobatannins

2ml HCl (1%) was added to 2ml of extract and heated. Formation of red precipitate indicates presence of phlobatannins.

11. Test for vitamin B2

1mg of extract dissolved in 100ml of water. Solution is pale yellow green colour by transmitted light and intense yellow green colour fluorescence by reflected light that disappear on addition of mineral acids or alkali.

12. Test for vitamin C

Dilute 1ml of 2%w/v solution of extract with 5ml of water and 1 drop of freshly prepared 5% w/v solution of sodium nitropruside, 2ml dil. NaOH and 0.6ml of concentrated HCl was added dropwise. Yellow colour turning blue indicates presence of vitamin.[6,8,16]

In vitro studies:

H_2O_2 antioxidant assay

40mM H_2O_2 reagent was prepared by adding 1.32ml of H_2O_2 in 100ml of 50mM phosphate buffer (PH 7.4). Ascorbic acid was taken as standard. The concentrations of extracts and standard studied were 100,200,300,400 & 500 μg/ml. To this 0.6ml of H_2O_2 reagent and make up phosphate buffer upto 10 ml and the absorbance values were read using in UV-visible spectrophotometer (Labindia 3000+) at 230nm. The blank solution used was 50mM phosphate buffer and the % of peroxide free radicals scavenged was calculated by the formula.[1,13,21]

$$\% \text{ scavenged} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

DPPH *a*ssay:

In this assay, 1ml of different concentrations (100-500 μg/ml) of extracts was added to the reference solution in test tubes. The tubes were incubated in dark for 1hr at room temperature. After that, the absorbance of reaction mixture was measured in UV visible spectrophotometer (Labindia 3000+) at 517nm. Ascorbic acid was used as standard. Methanol replacing the extract/ascorbic acid served as control (i.e., 1ml of methanol + 3ml of DPPH radical solution). Inhibition of DPPH radicals (%) was calculated and IC50 value was determined.[4,20]

$$\% \text{ scavenged} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Results and Discussion:

Extraction: The yield of hydroalcoholic extract was 24.05% (crystals with brown color)

Phytochemical screening:

Table no.1: Phytoconstituent analysis

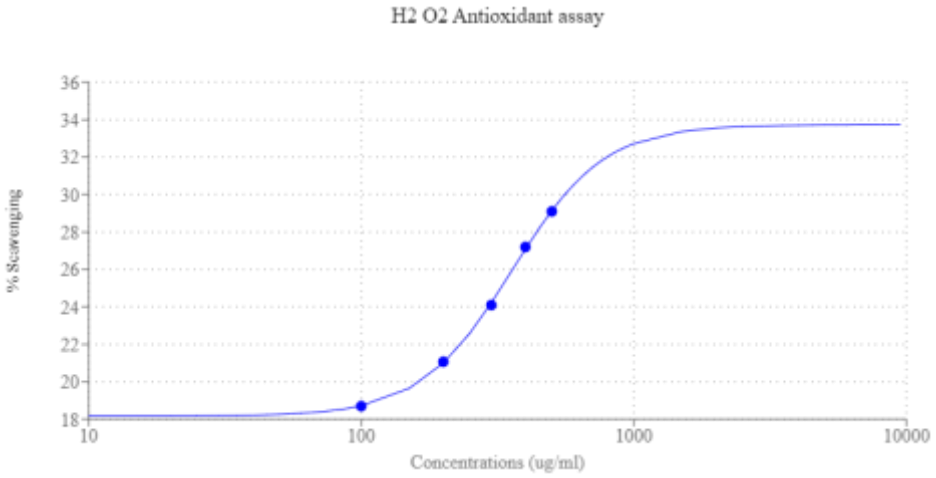
Sr. No.	Chemical test	Present (+) or Absent (-)
1.	Test for alkaloids	
	Dragendorff's test	+
b)	Mayer's test	+
c)	Hager's test	-
2.	Test for amino acids	
a)	Million's test	+
b)	Ninhydrine test	+
3.	Test for carbohydrates	
a)	Molisch's test	+
b)	Test for reducing sugars	+
4.	Test for flavonoids	
a)	Shinoda test	+
b)	Alkaline hydrochloride test	+
5.	Test for glycosides	
I.	Anthraquinone glycosides	
a)	Borntragger's test	-
II.	Cardiac glycosides	-
a)	Keller-killiani test	-
b)	Baljet's test	-
III.	Saponin glycoside	
a)	Froth formation test	+
6.	Test for tannins	+
7.	Test for terpenoids (Salkowski test)	+
8.	Test for Phenols	+

9.	Test for resins	+
10.	Test for steroids	-
11.	Test for phlobatannins	+
12.	Test for vitamin B2	+
13.	Test for vitamin C	+

H_2O_2 antioxidant assay:

Table no.2: H_2O_2 Scavenging activity

S.no	Concentration (µg/ml)	Hydrogen peroxide	Phosphate buffer (7.4) Volume upto 10ml	Absorbance	% Scavenging
1.	100	0.6ml	9.3 ml	0.212	18.7%
2.	200	0.6ml	9.2 ml	0.206	21.07%
3.	300	0.6ml	9.1 ml	0.198	24.1%
4.	400	0.6ml	9 ml	0.190	27.2%
5.	500	0.6ml	8.9 ml	0.185	29.1%
6.	control	0.6ml	9.3ml	0.261	-

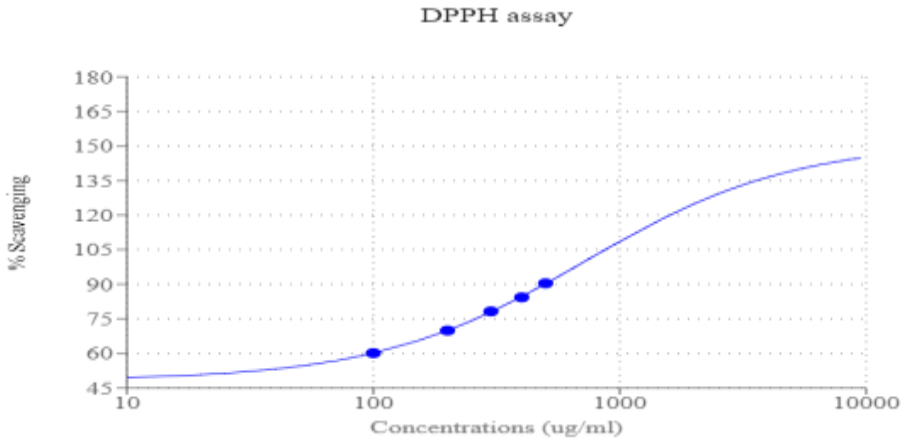


Graph no.1: H_2O_2 scavenging activity of *Sauropus androgynus* Hydroalcoholic extract. IC_{50} was found to be 357.1 (µg/ml)

DPPH assay:

Table no.3: DPPH scavenging activity

S.no	Concentration (µg/ml)	DPPH	Absorbance	% Scavenging
1.	100	3ml	0.359	60.1%
2.	200	3ml	0.271	69.9%
3.	300	3ml	0.196	78.2%
4.	400	3ml	0.141	84.3%
5.	500	3ml	0.086	90.4%
6.	control	3ml	0.902	-



Graph no.2: DPPH scavenging activity of *Sauropus androgynus* Hydroalcoholic extract. IC_{50} was found to be 720.51.

Conclusion:

The current study investigated the phytoconstituents and antioxidant properties of the hydroalcoholic extract of *Sauropus androgynus* leaves. The findings indicated that the extract had a yield of 24.05% and contained a range of bioactive compounds including alkaloids, amino acids, carbohydrates, flavonoids, saponin glycosides, tannins, terpenoids, phenols, resins, phlobatannins, and vitamins B2 and C. These constituents contribute to the plant's traditional medicinal uses, especially its reputed nutritional benefits.

The antioxidant potential of the *Sauropus androgynus* extract was evaluated through H_2O_2 and DPPH scavenging assays. The results demonstrated notable free radical scavenging activity, with IC_{50} values of 357.1 µg/ml and 720.51 µg/ml for the H_2O_2 and DPPH assays, respectively. These findings suggest that *Sauropus androgynus*

possesses significant antioxidant properties, which may be beneficial in counteracting oxidative stress.

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