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PHYTOCHEMICAL SCREENING, IN VITRO PHARMACOLOGICAL PROFILE OF *Sauropus androgynous* AND ITS CYTOTOXIC ACTIVITY AGAINST HUMAN OVARIAN CANCER CELLS PA1

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ABSTRACT

Sauropus androgynous is a shrubby plant having high potential of medicinal properties due to its extensive reservoir of nutraceutical compounds. The purpose of this cogitation was to investigate the phytochemistry of different leaf extracts which are methanol, chloroform and aqueous followed by an analysis of *in vitro* anti-inflammatory, anti-diabetic, antioxidant, antimicrobial and cytotoxicity effects against Human ovarian carcinoma cell line PA1. The phytochemical screening and *in vitro* bioactivities reveals the distribution of various metabolites in different extracts quantitatively by estimating their inhibitions. The IC50 value for Human ovarian cancer cell line was 60, 80 and 100µg/ml for methanol, aqueous and chloroform extracts respectively. This utilitarian study involving pharmacological profile would be useful in clinical and medical applications due to its therapeutic potential and this herb can be promoted to serve as a pharmacotherapy for prevalent diseases.

KEYWORDS: *Sauropus androgynous*, phytochemistry, anti-inflammatory, anti-diabetic, antioxidant, Cytotoxicity.

1. INTRODUCTION

Till date herbal medicine is a part of clinical treatment that have been used in medical field. At present the interest in pharmacognosy pulls the attention towards the medicinal plants and their properties to develop pharmacotherapies. The medicinal aspect of a plant relies on some

chemical compounds that exhibits diverse physiological actions on the human body that are commonly called phytochemical or phytopharmacological compounds.[1] The secondary metabolites from flora are always efficient and inspired models for drug development.[2] The most vital bioactive phytoconstituents of plants are alkaloids, tannins and flavonoids.[3] Medicine in the current trends exploits only the active ingredients of plants rather than concentrating on the whole plants. The phytochemicals can be isolated and purified from flora or can be manufactured artificially by pharmacological industries.[4]

Sauropus androgynous commonly known as star gooseberry is a Southeast Asian indigenous vegetable that belongs to the family Euphorbiaceae. This multivitamin perennial plant species grows well in humid and under high temperature conditions.[5] Morphologically the stem can reach up to 2.5 meters high and has dark green oval leaves 5-6 cm long. The plant can survive in acidic, heavy clay soils and moist soil. Nutritional content of the plant interprets they have precursors of vitamin molecules like A, B, C and K, fibre, proteins, carotenoid pigments, antioxidants, chlorophyll which serves as a blood-building block, cell rejuvenator and minerals like potassium, calcium, phosphorus, magnesium and iron, also increases production of steroid hormones, eicosanoids and also some aliphatic carbon chains are high. The usage of the leaves have plethoral benefits like in the form of tonic, decoction, febrifuge, antitussive and for soothing lungs. It is also suggested to use after prenatal stage to help the womb recover while in Indonesia, the leaves are made as an infusion, to enhance the flow of breast milk in lactation period of mother. In Taiwan, it is exploited as a slimming agent to counteract obesity. It is commonly used as an effective medicinal herb in the treatment of inflammation, microbial infection, cholesterol, diabetics, cancer and allergy.[6] At the same time, the over dosage of leaves may cause negative impacts while consuming it as raw and uncooked. The leaves contain a compound called papaverine, an alkaloid that drops the blood pressure and causes lung disease by making the blood vessels to open up.

In the present study, phytochemical analysis of methanol, chloroform and aqueous extracts of *Sauropus androgynous* leaves were carried out followed by the evaluation of anti-inflammation, anti-diabetic, antioxidant, antimicrobial and cytotoxicity effects collectively called as pharmacological profile. The entire research work of this plant presented in this paper is discussed with the literature and concluded.

2. MATERIALS AND METHODS

The fresh leaves of *Sauropus androgynous* were obtained from the moist areas of palakadu, kerala. The leaves were washed using running tap water 2-3 times to remove the impurities. They were shade dried and powdered using blender to a fine and coarse consistency. The powdered plant sample was used to carry solvent extraction.

2.1 Preparation of extract

The methanol, chloroform and aqueous extracts of the leaves were obtained using cold percolation method according to their decreasing polarity of solvents. The extracts were filtered using muslin cloth. The filtrate obtained from each solvent mixtures was dried and stored in four separate microfuge tubes representing the four extracts. The extracts were concentrated up to one-fourth of its original volume in each tube and are used to perform the following tests.

2.2 Phytochemical qualitative analysis

The secondary metabolites can be classified into alkaloids, flavanoids, phenylpropanoids, quinones, terpenoids, steroids, tannins and proteins based on their structures.[7] The leaf extracts were subjected to phytochemical qualitative analysis for the identification of presence of secondary metabolites by using the standard methods.[8,9]

2.3 Anti-inflammatory activity

Inflammation is reflex immunogenic mechanism pertaining to the participation of vasoactive, chemotactic and proliferative factors in response to an injury or infection.[10] The *in vitro* anti-inflammatory effect of the extracts was done using inhibition of albumin denturation method.[11] Different concentrations of extracts were taken in separate test tubes along with control. 1 ml of egg albumin, 3 ml of PBS were added and kept for incubation at 37^0c for 15 min. Then, the test tubes were heated at 70^0c for 10 min. The absorbance was measured in the spectrophotometer at 660 nm. The percentage inhibition of protein denaturation was calculated using

% inhibition= [{Abs control-Abs sample}/Abs control]*100

2.4 Anti-diabetic activity

About 0.5 ml of α-amylase solution was added to the different concentrations of the extracts taken in separate test tubes along with control and incubated at 37^0c for 5 min. 1ml of starch (initiating agent) was added in all test tubes and incubated again at 37^0c for 3 min. 1 ml of

DNS reagent (stopping reagent) was added to the test tubes, followed by boiling the mixture at 100^0c for 5 min and brought back to bearable temperature. The absorbance was measured at 540 nm in the spectrophotometer. The percentage inhibition was calculated using

% inhibition= [{Abs control-Abs sample}/Abs control]*100

2.5 Anti-oxidant activity

Among various *in vitro* and in vivo methods, DPPH method was found to be used mostly for the *in vitro* tests.[12] The antioxidant activity of the extracts was assayed by DPPH free radical scavenging test. Different concentrations of the extracts were added to 100 µl of 0.2 mM DPPH in methanol solution in separate test tubes along with control. The mixture was incubated at 25^0c for 5 min. The absorbance is measured at 520 nm. The free radical scavenging activity was calculated by the percentage inhibition

% inhibition= [{Abs control-Abs sample}/Abs control]*100

2.5 Cytotoxicity assay

In vitro cytotoxicity assay was performed using methanol, aqueous and chloroform extracts of *Sauropus androgynous* leaves against Human ovarian cancer cell line PA1. Cancer cell line was grown in T-flask containing DMEM medium supplemented with 100U/ml Penicillin, 10% (v/v) heat in activated fetal bovine serum and 100µg/ml streptomycin maintained in CO2 incubator at 37^0c providing 5% CO2- 95% air humidified incubator. Exponential growth of the cells was counted, viability of the cells estimated using tryphan blue assay. About 100µg/ml of cell suspension was added in 96-well microtiter plates along with different concentrations of test extracts 20, 40, 60, 80,100µg/ml. Cytotoxicity was determined by performing 3-(4,5-dimethyl- thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay and subsequently incubated for three days. [13] The IC_{50} value is measured by the concentration of each extract causing 50% growth inhibition of cancer cells.

2.6 Anti-bacterial activity

The antibacterial activity of the extracts was evaluated by using standard well diffusion agar technique against the virulent species pertained to both gram positive and gram negative bacteria.[14] These test organisms were *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, E.coli* respectively. Nutrient agar medium were kept solidified in five petri dishes, while each organism was swabbed over the medium

in separate plates. About five wells were diffused using T-rod in each plate such that each plate holds 40 µl of each extract and 30 µl of gentamicin as control. The plates were incubated for one day to observe the results.

2.7 Anti-fungal activity

The antifungal activity was performed in the same manner by using potato dextrose agar medium and poured in two petri dishes. The test organisms were *Candida albicans* and *Aspergillus niger* respectively. About five wells were diffused using T- rod in each plate such that each plate holds 40 µl of each extract and 30 µl of gentamicin as control. The plates were incubated for two days to observe the results.

3. RESULTS AND DISCUSSIONS

3.1 Phytochemical analysis

The phytochemical analysis of the leaf extracts are presented in Table 1, the presence of the secondary metabolites tested has confirmed the plant has superior nutrition and vitamins.[15,16] It is shown that presence of proteins and steroids in methanol extract, terpenoids in aqueous extract and steroids in chloroform extract are higher.

Table 1: Phytochemical qualitative analysis of *Sauropus androgynous* leaves

S. No	Name of the test	Phytochemical analysis		
		aqueous	Methanol	chloroform
1.	Test for alkaloids	_	++	+
2.	Test for carbohydrates	–	–	–
3.	Test for glycosides	_	_	_
4.	Test for proteins	_	+++	_
5.	Test for phenol	_	_	_
6.	Test for flavanoids	+	+	_
7.	Test for terpenoids	+++	++	_
8.	Test for steroids	_	+++	+++
9.	Test for saponin	++	_	+
10.	Test for tannin	+	+	_
11.	Test for quinones	+	_	_
12.	Test for coumarin	_	_	_
13.	Test for phytosterols	_	++	++

"+"= positive, "-"= negative

3.2 Anti-inflammatory activity

The percentage inhibition by the four extracts are tabulated and presented in Table 2. The extracts have inhibited the heat induced albumin denaturation. It is shown that chloroform

leaf extract is more potent against inflammation. This may be due to the presence of high amount of phytosterols, steroids and alkaloids responsible for inhibition of inflammation which is found lower in other extracts. The compounds in leaves of Sauropus androgynous are immunomodulating agents that can either stimulate or suppress the immune system to prevent chronic inflammations.[17]

Table 2: Anti-inflammatory activity of the *Sauropus androgynous* leaves.

Concentration of extract µg/ml	Anti-inflammatory activity in %		
	methanol	chloroform	aqueous
25	42	66.8	30.7
50	64.1	72.5	50
75	70.5	87.3	64.5
100	80.4	90.4	82.6

3.3 Anti-diabetic activity

The anti-diabetic activity of the leaf extracts are represented as percentage inhibitions and presented in Table 3. Among the four, chloroform extract exhibited more activity against diabetics due to the presence of mild amount of polyphenol content in the leaves when compared to aqueous extract which has the least potential. Some articles report that leaves of *Sauropus androgynous* have compounds that are potent α-glucosidase inhibitor with anti-diabetic activity and they possess compounds responsible for low glycaemic index.[18,19]

Table 3: Anti-diabetic activity of the *Sauropus androgynous* leaves.

Concentration of extract µg/ml	Anti-diabetic activity in %		
	methanol	chloroform	aqueous
25	62	70	8.3
50	75	75	25
75	76.4	82	31
100	78	85	32

3.4 Anti-oxidant activity

The anti-oxidant activity of the leaf extracts are calculated and presented in Table 4. The violet colour of the DPPH turns to yellow due to the biological reduction of DPPH by the compounds present in the extracts. The change in colour of the solution was measured with respect to absorbance to yield the percentage of inhibition. Antioxidants are substances that prevent free radical induced tissue damage by preventing the formation of free radicals, scavenging them or by promoting their decomposition.[20] The methanol and aqueous extracts

is more potent as a result of immense flavanoids, phenolics and alkaloids responsible for scavenging activity when compared to the latter. Phenolic compounds are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the initiation step.[21]

Table 4: Anti-oxidant activity of the *Sauropus androgynous* leaves.

Concentration of extract µg/ml	Anti-oxidant activity in%		
	methanol	chloroform	aqueous
10	32	5.4	28
20	42	10	31
30	50	13.3	43
40	56	30.8	53
50	65	36	63

3.5 Cytotoxicity assay

Cytotoxicity assay using different extracts of *Sauropus androgynous* leaves against the Human ovarian cancer cell line PA1 were estimated and cell viability is represented graphically in Fig1. Methanol, aqueous and chloroform extracts had shown the following percentage inhibition of proliferating cancer cells determined to be the IC50 values at the concentrations 60, 80 and 100µg/ml. Morphology of the cells attached to scaffold were visualized at the respective IC50 values shown in Fig 2.

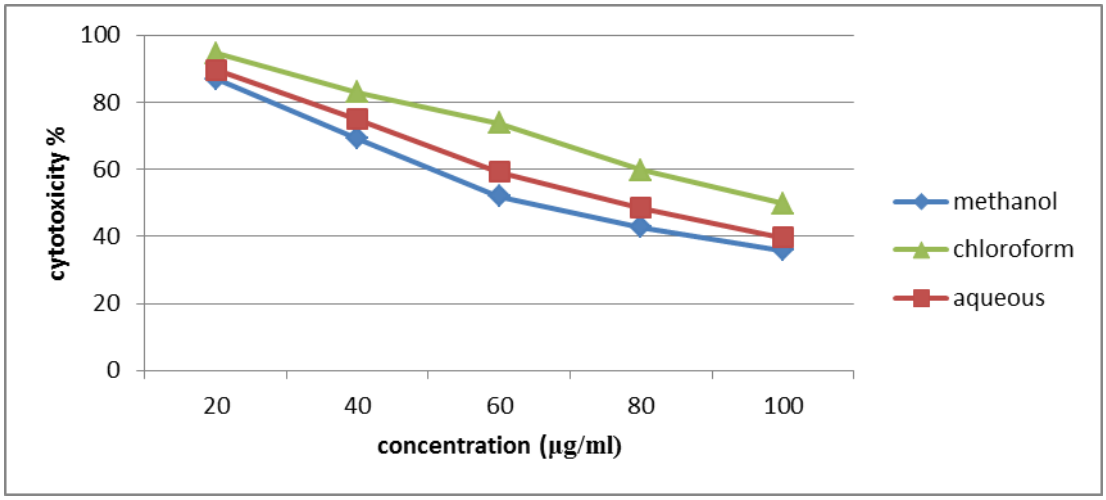

Fig 1: Cytotoxicity assay using *Sauropus androgynous* leaves against Human ovarian cancer cell line PA1.

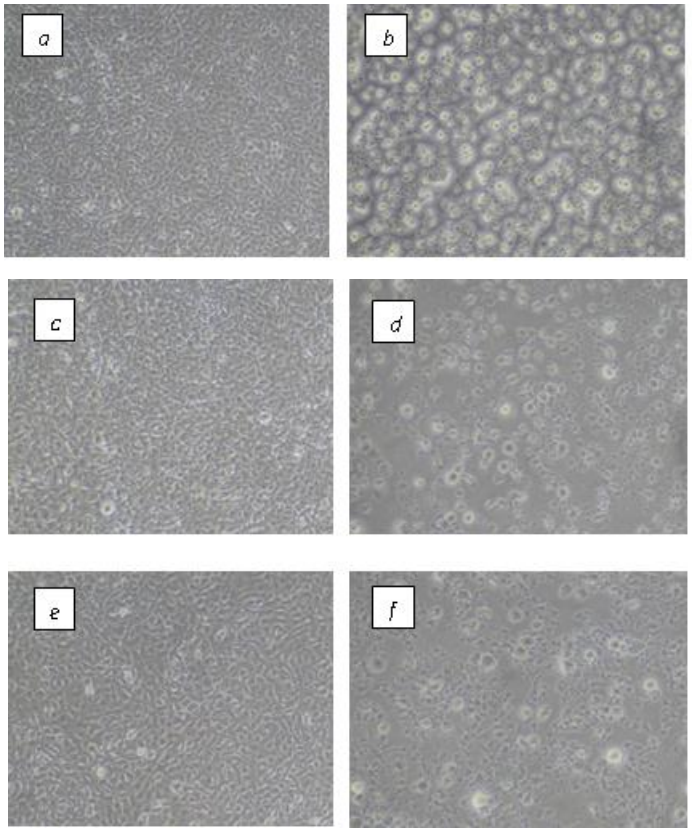

Fig 2: Morphology of the Human ovarian cancer cell line PA1 during inhibition by S. androgynous leaf extracts: Effect of methanol extract on PA1 cells at 24 hrs. (a) control, (b) treated (60 µg/ml). Effect of aqueous extract on PA1 cells at 24 hrs. (c) control, (d) treated (80 µg/ml). Effect of chloroform extract on PA1 cells at 24 hrs. (e) control, (f) treated (100 µg/ml).

3.6 Antibacterial activity

The antibacterial activity of the extracts against four common pathogenic bacterial species was evaluated by measuring the inhibition zones in cm after the period of incubation given in Table 5. Terpenoids, polyphenols, saponins are some of the antibacterial agents that can inhibit any common pathogenic organisms. The effects of plant secondary metabolites on deleterious human and microorganisms indicate their perspectives of antimicrobial uses.

Table 5: Antibacterial activity of the *Sauropus androgynous* leaves.

Organism	Zone of inhibition (in cm)			
	Control	methanol	Chloroform	Aqueous
Klebsiella pneumoniae	1.6	1.3	0.6	0.7
Pseudomonas aeruginosa	0.9	0.7	0.6	0.6
Staphylococcus aureus	0.8	0.5	0.3	0.3
Escherichia coli	0.7	0.4	0.3	0.5
Bacillus subtilis	0.7	0.4	0.2	0.2

3.7 Antifungal activity

The antifugal activity of the extracts against two common virulent fungal species Candida albicans and Aspergillus niger are presented in Table 6 and their zones were also measured. The activity is vital for many applications due to the presence of alkaloids, saponins, tannins, polyphenols, flavanoids, anthocyanins and terpenoids in the extracts varying in quantity.[22]

Table 6: Antifungal activity of the *Sauropus androgynous.*

Organisms	Zone of inhibition (in cm)			
	Control	methanol	Chloroform	Aqueous
Candida albicans	1.9	0.9	0	1.5
Aspergillus niger	1.5	0.5	0	1

CONCLUSION

The phytopharmacological evidence reveals that the medicinal significance and benefits of this plant has outnumbered. The phytochemical screening of the mentioned leaf extracts has surplus of secondary metabolites enhancing their protection and serves for beneficial of mankind as a pharmacotherapy. One step forward, the ethanobotanical studies with respect to these metabolites has proved their crucial role in bioactivities of anti-inflammation, anti-diabetic, anti-oxidant as well as cytotoxicity. The antibacterial and antifungal activity depicts the therapeutic essential of the leaves acting as an antibiotic against any disease causing virulent pathogens. Thus, this piece of research work supports the rationale that *Sauropus androgynous* would produce a remarkable successful alternate replacing other chemotherapeutic drugs in the futuristic research and development in pharmacology.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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