



## Evaluation of nutritional and cytotoxic effect of *Sauropus androgynus* (L.) Merr

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### Abstract

The present investigation deals with the nutritional quality of *Sauropus androgynus* and it is compared with *Amaranthus tricolor* and *Amaranthus dubis*, two commonly used and market available leafy vegetable. To assess the edible value, estimation of nutritional factors, antinutritional factors, vitamins and minerals were conducted. To analyse the photosynthetic efficiency, pigment estimation also conducted. Cytotoxicity and DNA damage assay was conducted to clarify some doubts among common people that the intake of *S. androgynus* can cause some toxic effect to our body. This study shows that the *S. androgynus* have more nutritional value than *A.tricolor* and *A.dubis* and cannot cause any DNA damage. Hence this plant can be recommended for further crop improvement programmes.

**Keywords:** *Sauropus androgynus*, *Amaranthus tricolor*, *Amaranthus dubis*, cytotoxicity, DNA damage assay, MTT assay

### Introduction

Leafy vegetables have always gained interest among most of the communities of the world due to its ease of availability, cultivation and nourishing potentials. Edible leaves which are cultivated as well as from the wild are available from perennial trees, aquatic plants and terrestrial annuals and are consumed by the people especially in rural areas (Hamada,2002) [11]. These vegetables are a source of economy and ensure the supply of nutrients to the rural people (Ibukun, 2008) [14]. Most of the leafy vegetables have high medicinal property also. In general, most of the edible green leafy vegetables appear to be underutilized due to its limited usage by the people in a specific region or race which eventually lead to its diminished use in the future (Leung,1968) [16].

*Sauropus androgynus* (L.) Merr. belongs to the family Euphorbiaceae, is a shrub grown as a leafy vegetable in Southeast Asia extensively cultivated for traditional medicine. (Bunawan, 2015) [6]. It is commonly known as katuk, star gooseberry or sweet leaf (Santoso, 2005) [26]. This plant was introduced to India from Malasia in the year 1950s (Eganathan and panda, 2011) [7]. It is used as a supplementary food item, rich in vitamins and minerals that are essential for the growth and development of our body (Agarwal, 2014) [1]. Apart from being a multivitamin plant because of its high carotenoid, vitamin B and C, protein, lipid, carbohydrate and mineral content *Sauropus androgynus* is also known for its high yield and palatability. It is also known as multivitamin leafy vegetable and multi minerals packed green leafy vegetables. (Price 1992) [23]. Tender shoot and young leaves are used in culinary purpose because they are vastly nutritious in nature (Eganathan and panda, 2011) [7]. Although it has many nutritional benefits, there is a growing concern among the general public that eating this plant can cause health problems.

Several research works were conducted on *S. androgynus*, to explore its phytochemical constituents (Selvi and Basker, 2012) [27]. Those works revealed the presence of active phytochemical components, owing to its therapeutic potential. *S. androgynus*' effectiveness as a conventional

herbal remedy, a breast milk inducer, a wound healer, and an antioxidant (Fikri and Purnama,2020) [10]. Many conventional treatments of *S. androgynus* extract include sauroposide and certain secondary metabolites such as alkaloids, flavonoids, phenols, terpenoids, glycosides, and certain vitamins such as carotenoids and tocopherol. At the same time, researchers like Hock Eng Khoo H, Azlan A and Ismaila (2015) [9], reported that antinutrients and heavy metals detected from *S. androgynus* leaves possessed adverse health effects, like heavy metal toxicity and induced lung injury. Bronchiolitis obliterans and obstructive ventilatory impairment of the patients were also reported to be caused by ingestion of uncooked or fresh *Sauropus androgynus* leaves (Bunawan, 2015) [6]. Hence, in general there is a belief that consumption of *S. androgynus* cause serious health issues in human beings. An attempt has been made in this piece of work to efface such a misconception by comparing the nutritional parameters of *S. androgynus* with *A. tricolor* and *A.dubis*, the two widely used leafy vegetables. Furthermore, the cytotoxicity and DNA damage assay were also conducted to authenticate the fact that, consumption of *S. androgynus* can cause little or in fact no damage to the DNA. This is the first attempt, where, a comparative study on the nutritional parameters of *S. androgynus* have been done with locally available green leafy vegetables.

There is space for further research into the edible leaves of indigenous and underutilised plants' potential for use as food and medicine, as well as incorporation into traditional agri-food systems.

### Materials and Methods

#### Estimation of nutritional and anti- nutritional factors Analysis of nutritional factors

For the nutritional analysis the factors such as moisture content (Huie,2002) [13], reducing sugar (Thayumanavan and Sadasivum,1990) [29], total carbohydrates (Hedge and Hofreiter,1962) [12], crude protein (Bradford method, Jones,1989) [15], total lipid(Blight and Dare,1959) [5], ascorbic acid (Sadasivum and Manickam,2008) [25],

reducing sugar (Miller, 1959) [18] amin acid (Moore and Stein,1948) [19], carotenoids (Thimmaiah, 1999) [30] and chlorophyll (Arnon,1949) [4] in *S.androgynus* were estimated. The data thus obtained were compared with that of nutritional factors of *A. tricolor* and *A.dubis*, which are widely used as leafy vegetable in Kerala.

#### Analysis of antinutritional factors

Anti- nutrients like oxalate (AOAC, 1990) [2], saponins (AOAC,1984) [3] and total phenolics(Malik and Singh, 1980) [17] were estimated and compared with *A.tricolour* and *A.dubis*.

#### Mineral analysis

Mineral analysis were done in the dried powdered leaves of *S.androgynus*, *A. tricolour* and *A.caudatus*. Micro elements like Ca, Mg, K, Na, N and macro elements like Fe, Mn, Zn, Cu and B were analysed using GCM method. (GC model – 7890 A, MS model – 5975 C).

#### Invitro antiproliferative effect determination by MTT assay

MTT assay was done to find out the cytotoxicity of plant extract in Hela cell line, initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium Himedia. The cell line was cultured in 25 cm<sup>2</sup> tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO<sub>2</sub> incubator (Galaxy<sup>®</sup> 170 Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

#### Cells seeding in 96 well plate:

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x10<sup>4</sup> cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator.

#### Preparation of plant extracts and compound stock

1 mg of each plant extract or compound was added to 1ml of DMEM and dissolved completely by cyclomixer. After that the extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

#### Ant proliferative effect Evaluation

After 24 hours the growth medium was removed, freshly prepared samples in 5% DMEM were five times serially diluted by two-fold dilution (6.25µg, 12.5µg, 25µg, 50µg, 100µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator.

#### Ant proliferative effect by Direct Microscopic observation

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Labomed TCM-400 with MICAPS™ HD camera) and microscopic observation were recorded as

images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

#### Ant proliferative effect by MTT Method

Fifteen mg of MTT (Himedia, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 3 0µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilisation Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilise the formazan crystals. The absorbance values were measured by using micro plate reader at a wavelength of 570 nm (Laura B. Talarico *et al.*, 2004) [28].

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of Viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control group}}$$

#### DNA cleavage study

The DNA cleavage activity of sample was studied using gel electrophoresis method (Zhang and Lippard, 2003)[35] and the respective photograph was given below. The DNA was treated with 50 µg of the respective compound in a reaction volume of 15 µl and incubated at 37 °C for 2 h. After incubation the treated as well as control DNA were subjected to agarose gel electrophoresis in 1% gel in 0.5 % TAE buffer for 1 hour.

#### Result and Discussion

##### Estimation of nutritional and anti-nutritional characters

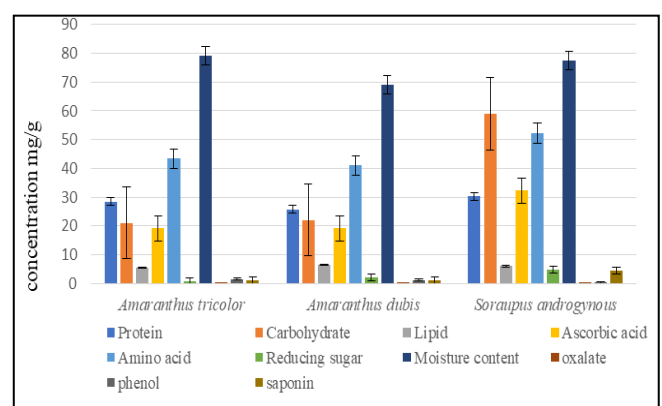


Fig 1

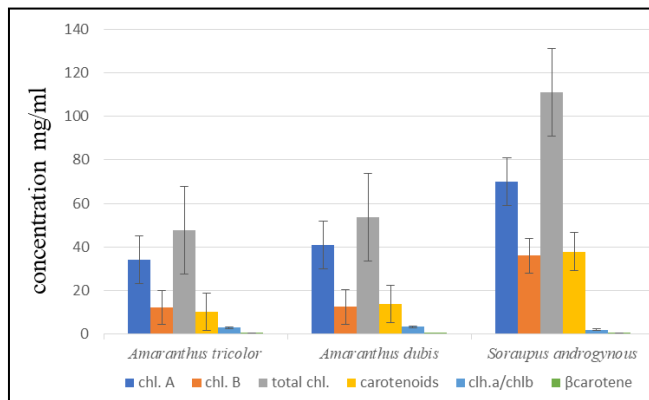
Nutritional and anti- nutritional characters of *Sauropus androgynus* were estimated and it is compared with *Amaranthus tricolor* and *Amaranthus dubis*. *Sauropus androgynus* have high nutrient content than *Amaranthus tricolor* and *Amaranthus dubis*. *Sauropus androgynus* contain meagre amount of saponin, but it can be removed through cooking. Moisture content of the *S. androgynus* is approximately same as to the *A. tricolor* and *A.dubis*. High

moisture content revealed its freshness and health benefits.(Ellis,1986)<sup>[8]</sup>

*Sauropus androgynus* is a common leafy vegetable used by indigenous people. According to Rehman,2014 <sup>[24]</sup> these plants are also used against various diseases based on their traditional knowledge.

Green leafy vegetables are high in nutrients that are important for human health and happiness (WJD, 1959) <sup>[33]</sup>. Amino acids, vitamins, essential fatty acids, minerals, protein, starch, and dietary fibre are among these nutrients. It has major socioeconomic advantages as well (Padmavati, 1990) <sup>[22]</sup>. According to Natesh *et al* (2017) <sup>[20]</sup> green leafy vegetables are grown and harvested by women farmers in the tropics and subtropics to supplement household income. Traditional green leafy vegetables are commonly considered to be the cheapest food source for supplementing vitamins and micronutrients to prevent nutrient deficiencies. vegetables, which are available all year, play an important role as a food source in rural areas. In various cultural and traditional settings, it's also used as an herbal and medicinal plant for a variety of ailments. Prior to consumption anti-nutrient levels are reduced by thermal processing of leafy vegetables, such as boiling, frying, and blanching (Padmavati, 1990) <sup>[22]</sup>.

**Pigment estimation**



**Fig 2**

Chlorophyll is a valuable nutrient. It is essential to human life. To confirm the nutritional values of this photosynthetic pigment, chlorophyll content were estimated in *Sauropus androgynus*, an underutilised edible green, and it was compared to two commercially available leafy vegetables, *A.tricolor* and *A.dubis*. Chlorophyll a, b, total chlorophyll and carotenoids are high in *Sauropus androgynus*. It reveals its photosynthetic efficiency than *Amaranthus tricolor* and *Amaranthus dubis*. Photosynthesis, growth, and development of plants are all influenced by plant pigments. (Wee Sim Choo, 2019) <sup>[34]</sup>. Freshness and health effectiveness of leafy vegetables are determined by the amount of chlorophyll in green leaves. These pigment-rich foods not only battle with cancer cells, but also have beneficial effects on a wide range of human diseases and have other health benefits. (Upadhyay,2018) <sup>[31]</sup>. Total chlorophyll content was high in *Sauropus androgynus*. It reveals its photosynthetic efficiency. According to Shankar *et al.*, (2013) <sup>[32]</sup> consumption of edible greens, instead of

purchasing chlorophyll supplements, chlorophyll liquid solutions, or chlorophyll tablets for dietary purposes, will be more beneficial.

**Mineral analysis**

Minerals are required by living organisms for their normal growth and development and it can prevent the occurrence of deficiency diseases. Mineral analysis helps to identify the macro and micro elements present in the sample. Macro elements are natural elements that the body requires in greater quantities and are thus more essential than other minerals. Trace elements are needed in trace quantities to keep the body safe. Trace elements are needed in trace quantities to keep the body safe (Neravathu, 2021) <sup>[21]</sup>

**Table 1:** Macroelements in *S.androgynus*, *A.tricolor* and *A.dubis*

Sl no.	Elements	Constituents		
		<i>S.androgynus</i>	<i>A.tricolor</i>	<i>A.dubis</i>
1	Calcium (Ca) (%)	0.91	0.77	0.72
2	Magnesium (Mg) (%)	0.48	1.48	1.68
3	Potassium (K) (%)	2.3	4.18	5.25
4	Sodium (Na) (mg/ kg)	61.54	703.55	756.18
5	Nitrogen (N) (%)	4.47	3.99	4.37

In humans, microelements are involved in the synthesis of enzymes, hormones, and other substances, as well as the growth, development, and functioning of the immune and reproductive systems.

Macro and micro elements in *S. androgynus* compared with *A.tricolor* and *A.dubis*. It revealed that calcium, nitrogen, iron and manganese are high in *S.androgynus*. Boron is high in *A.tricolor* and other elements are more in *A.dubis*.

**MTT Assay**

**Table 1**

	Response 1 (OD)	Response 2 (OD)	Response 3 (OD)	Average
Control	0.819	0.86	0.871	0.85
Conc. of samples (µg/ml)	OD values of sample			
6.25	0.79	0.781	0.806	0.792333
12.5	0.73	0.69	0.596	0.672
25	0.56	0.551	0.57	0.560333
50	0.319	0.302	0.351	0.324
100	0.254	0.221	0.2	0.225

**Table 2**

Concentration µg/ml	Percentage of viability	IC50
6.25	92.94	29.325
12.5	78.82	
25	65.88	
50	37.64	
100	27.05	

At low concentrations above 65% of the cells are viable. But when the concentration of the drug increases, the percentage of viability decreases. 50 % of cells can be inhibited by 29.325 µg/ml concentration of drug.

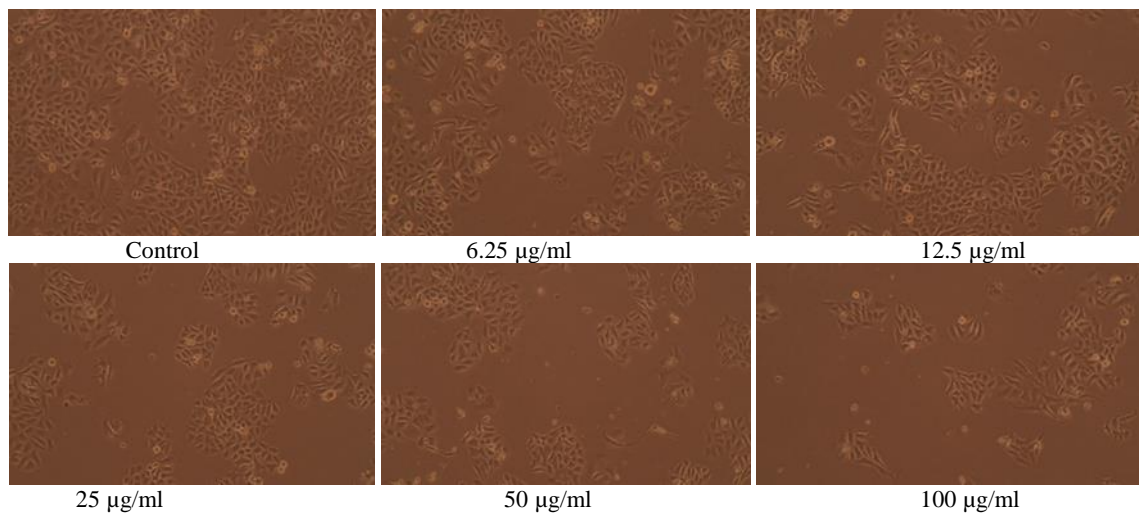


Fig 3

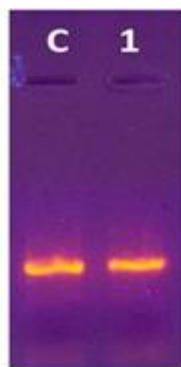


Fig 4

**C:** Control- Untreated DNA (*E coli* Genomic DNA)

**1:** 50 µg MS + *E coli* Genomic DNA

The plant extract did not show any DNA damaging effect and can be concluded that it will not cause any DNA toxicity. So, the consumption of this plant as vegetable did not cause any damage to our DNA.

### Conclusion

Local vegetables are semi-cultivated or wild species that are important in both rural and urban areas around the world. The leaves or aerial parts of these plants were ingrained in people's eating habits and are highly recommended as a supplement to exotic varieties due to their high nutritional value. Green leafy vegetables play a vital role in a balanced diet. Many of the underutilised wild plants are nutrient-dense. The nutritional value of inexpensive, easily available, and lesser-known vegetable plant for a balanced diet is revealed here. The findings of this study showed that the selected plant materials contain adequate amounts of various nutritional factors. *Sauropus androgynus* have high nutrient content and low anti nutrient content. MTT assay shows that percentage of cell viability may decrease when concentration increases. Above 65% of the cells are viable at low concentration. DNA cleavage study shows that there is no genome toxicity. So, this leafy vegetable can be recommended for further cultivation and crop improvement programmes.

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