Free Radical Scavenging activity of Multi-vitamin Plant (*Sauropus androgynus* L. Merr)

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Abstracts

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 which contains about 6-10% protein content. It is one of the most popular leaf vegetables in South Asia and Southeast Asia and is notable for high yields and palatability. In India it also known as Multivitamin Plant as it contains an excellent sources of provitamin A, Carotinoid, Vitamin B and C, protein and mineral. It has highly nutritive value and contains phytochemicals which can act as antioxidant. The antioxidant activity of Multi-solvent extracts of *Sauropus androgynus* obtained from ethanol, methanol and water were tested by measuring their ability to scavenge reactive hydroxyl radical through 2,2-diphenyl-1-picryl hydrazyl (DPPH) scavenging and Hydoxyl radical scavenging assay. In general, the ethanolic extracts were better free radical scavengers than the Mathanolic and aqueous extracts. Similar results were seen in the Hydroxyl assay. Our findings also showed a strong correlation of antioxidant activity with the total phenolic content. The findings indicated promising antioxidant activity of crude extracts of the above plant and needs further exploration for their effective use in both modern and traditional system of medicines.

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1. Introduction

Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infections and degenerative diseases. Literature shows that the antioxidant activity is high on herbal and vegetable plants. The presence of free radicals in the body causes cell and tissue damage. The most effective way to eliminate free radicals is with the help of antioxidant nutrients such as ascorbic acid (vitamin C), alpha-tocopherol (vitamin E) and beta-carotene (vitamin A) which can be found in vast amounts in fruits and vegetables (Rice-Evans *et al.*, 1997). Fruits and vegetables contain different antioxidant compounds, such as vitamin C, vitamin E andcarotenoids, whose activities have been established in recent years. Flavonoids, tannins and other phenolic constituents present in food of plant origin are also potential antioxidants (Salah *et al.*, 1995; Van Acker, Van den Vijgh, & Bast, 1996).

Recent reports suggest that cruciferous vegetables act as good source of natural antioxidants due to high levels of carotenoids, tocopherols, and ascorbic acid (Heinonen *et al.*, 1989) and convincing epidemiological evidence shows these compounds may help to protect the human body against damage by reactive oxygen and nitrogen species. Foremost are their antioxidative effects, manifested by the ability to scavenge free radicals (Soleas *et al.*, 1997) or to prevent oxidation of low-density lipoproteins (Rifci *et al.*, 1999). However, recent research indicates that in addition to carotenoids, tocopherols, and ascorbic acid most of the antioxidative effect in plants is mainly due to presence of phenolic compounds, which have not yet been characterized very well in vegetables (Deighton *et al.*, 2000). Phenolic compounds are secondary metabolites, which have been associated with flavour and colour characteristics of fruits and vegetables and are gaining considerable attention because of their potent antioxidant and health promoting properties (Kaur & Kapoor, 2001). Thus, growing evidence of their health benefits warrants their presence in varieties of fruits and vegetables and their quantification with special reference to cruciferous vegetables. In recent years, research in this area has focused on the detection of antioxidants in food, because there is evidence that they could play an important role in the prevention of several illnesses as well as in the

retardation of the aging process (Katalinic *et al*.,2004). Fruits and vegetables are reported to contain a wide variety of antioxidant components including phenolic compounds (Ames *et al*., 1993 and Ertan *et al*., 2001).

Studies carried out by the researchers have shown that low consumption of vegetables is associated with an increased risk of cancer. *Sauropus androgynus* of Euphorbiaceae family is a well known local vegetable which have been found to have antioxidant activity. Edible green leaves of *Souropus androgynus* is commonly known as 'katoo' in most parts of India locally it is also known as Multi-vitamin plant (Fig-1,2,&3). Katuk, *Sauropus androgynus*, a somewhat peculiar latin name. Common in Asia, Katuk is rarely found in the wild, occurring from India to Malaysia. The plant has small green leaves with yellow flowers that bloom occasionally. Lee, 1989 states that *Souropus androgynus* can be useful as a dye in food colouring a delicious hot weather green vegetable, widely considered to be one of the most prolific, nutritious and appetizing of all green-leaved vegetables. The leaves have about 6-10% protein content. The roots and leaves are sometimes used as medicine. The leaves and roots are used to relieve fever and treat urinary problems. The juice from its leaves is dropped into the ear as a remedy for earache (Frits Stoepman bNO, 2003). In addition, the young tips and leaves are a common nutritious vegetable for cooking. (Padmavathi and Rao, 1990; Kanchanapoom *et al*., 2003)

The aim of the present investigation is to evaluate the aquatic, methanolic and ethanolic extracts of *Sauropus androgynus* L. Merr for *in vitro* antioxidant and free radical scavenging potential, vitamin C, total phenolics and nutritive values.

2. Material and Methods

2.1.1. Chemicals and Reagents

Folin-Ciocalteu reagent (Merck Pvt. Ltd, India), Sodium chloride (S.D. Fine Chem, India), Sodium carbonet (Merck Pvt. Ltd, India), HCL and H_2SO_4 (SD Fine, India), Anthron reagent and Catechol (Himedia Lab., India), 2, 2-Diphenyle-2-picryl hydrazyl (DPPH), 2-deoxyribose, and L-ascorbic acid, Sodium hydroxide, 2-thiobarbituric acid (TBA), gallic acid were purchased from Sigma, Hydrogen peroxide, Metaphosphoric acid, Potassium hydroxide, EDTA, Potassium hydroxide were purchased from Himedia Lab. and Ethanol, Methanol were obtained from SD-Fine Chem. Appropriate blanks were used for individual assays.

2.1.2 Plant Materials and Sample Preparation

The leaves of the species i.e *Souropus androgynus* of Meliaceae family were collected from the Medicinal Garden of B.J.B (A) College, Bhubaneswar, Orissa. The leaves were rinsed severally with clean tap water to make it dust and debris free. Then they were spread evenly and dried. Then the dried samples were ground in electric chopper to get fine powder form for further use.

2.2. Instrumentations

Collection of multi-solvent extract was done by Soxhlet apparatus (J.S.G.W) with varying temperatures according to the B.P. of the solvents. The samples were evaporated through the Rotary vacuum evaporator at 60-100^{0}C according to the B.P. of supplied solvents. Absorbance spectrophotometery was carried out using a UV-vis spectrophotometer (EI, model-1371).Wavelength scans and absorbance measurements were in 1ml quartz cells of 1cm path length.

2.3. Preparation of plant extracts

The dried and powdered leaves (50g) were extracted successively with multi-solvent extraction by using double distilled water, ethanol and methanol (each 400ml.) for 10-12 hrs. through Soxhlet apparatus. Then collected solutions were filtered through Whatman No-1 filer paper. The extracts were evaporated to dryness under reduced pressure at 90^{0}C by Rotary vacuum evaporator to obtain the respective extracts and stored in a freeze condition at -18^0C until used for further analysis.

2.4. Determination Nutritive Values

2.4.1. Determination of water content

10gms. of *Sauropus androgynus* leaves were cleaned and cut into small pieces and placed in an oven at 100 IC for 4 hrs. and then it was cooled in a desicator for 15 min and weighed. The weighed sample was dehydrated again at 100 IC for 1 hr. cooled and weighed in order to obtain a constant dried weight.

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The water content was obtained by a difference of weights.

2.4.2. Determination of protein content

Protein content was determined by the Kjeldahl method (Kjeltec system, Tecator), in which 0.5 g of dried sample, Kjeltabs (K_2SO_4+$CuSO_4$) as a catalyst and 10 ml conc. H_2SO_4 were added into a digestion tube. Blanks containing all these chemicals were simultaneously processed. The tubes were placed in the heated digestion block at 420 IC for 45 min, cooled at room temperature and diluted by adding 50 ml of distilled water. Then the tube was placed in a distillation unit and 50 ml of 40 % NaOH solution added. The tube was heated by vapor from boiling water for 5 min. Ammonium sulphate was degraded to ammonia. Ammonia was condensed together with water vapor into 25 ml of 4 % boric acid solution in a conical flask. Borate in a solution was titrated with 0.1 M HCl. The volume of 0.1 M HCl used for titration was directly proportional to the nitrogen content of the sample. The percentage nitrogen content was calculated by the equation below:

% N = 1.401 x (volume of HCl used for the sample-volume of HCl used for the blank) x M of HCl weight of sample

The percentage of protein was obtained by multiplying percent nitrogen by 6.25.

2.4.3. Determination of lipid content

Lipid content of this plant was determined by the direct extraction method. Three grams of dried sample was extracted by 50 ml of petroleum ether at 60^0C for 60 min in a soxtec system HT (series 1043 Extractor unit, Tecator). The lipid value was obtained by mean of weighing.

2.4.4. Determination of Total Carbohydrate content

The total carbohydrate content was determined by Anthrone method (Sadasivam and Manickam, 2008). In this method glucose was taken as standard solution. The values were read through spectrophotometer at 630nm and calculation was done by multiplying the value by a factor 0.9.

2.4.5. Determination of Vitamin C content

The vitamin C content was determined by the titrimetric method according to the method of AOAC, 1990. Eighteen grams of sample was cleaned and cut into small pieces, ground in a mortar and extracted with 250 ml of metaphosphoric acid-acetic acid solution. The extract was filtered through a filter cloth. The filtrate was used to determine vitamin C content by adding 10 ml of distilled water to 20 ml of sample solution and titrating with 2,6-dichloroindophenol (DICP) solution. Ascorbic acid was used as a standard.

2.5. Phenolic Estimation

The total phenolic content of plant extracts were determined by using Folin-Ciocalteu Spectrophotometric method according to the method described (Kim *et al.,* 2007). Reading samples on a UV-vis Spectrophotometer at 650nm. Results were expressed as catechol equivalents (µg/mg).

2.6. Antioxidant activity

The antioxidant activity of the *Sauropus androgynus* leaves on the basis of the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method described in (Brand-Williams, *et al.,* 1995) with slight modification. The following concentrations of extracts were prepared 40µg/mL, 80µg/mL, 120µg/mL, 160 µg/mL and 200µg/mL. All the solutions were prepared with methanol. 5 ml of each prepared concentration was mixed with 0.5mL of 1mM DPPH solution in methanol. Experiment was done in triplicate. The test tubes were incubated for 30 min. at room temperature and the absorbance measured at 517nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid was used as a standard and the same concentrations were prepared as the test solutions. The different in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

$$\text{Scavenging effect (\%)} = (1 - As/Ac) \times 100$$

As is the absorbance of the sample at t =0 min.

Ac is the absorbance of the control at t=30 min.

2.7. OH^- radical scavenging activity

2-Deoxyribose is oxidized by OH^- radicals that are formed by Fenton reaction and degraded to malondialdehyde (Chung et al., 1997) which can be detected by reacting with Thiobarbituric acid (TBA). A reaction mixture composed of 0.1 ml of 10 mM $FeSO_4.7H_2O$; 0.1 ml of 10 mM EDTA, 0.2 ml of 10 mM 2-deoxyribose and 0.02 ml of sample (Multi-vitamin crude extract) in 1.38 ml of 0.1 mM phosphate buffer (pH 7.4) was made. The reaction was started by adding 0.2 ml of 10 mM H_2O_2 and incubating at 37 IC for 1 hr. After incubation, 1 ml each of 2.8 % Trichloroacetic acid (TCA) solution and 1 ml of 1 % Thiobarbituric acid (TBA) solution were added to the reaction mixture, which was then boiled for 10 min, cooled in ice and its absorbance recorded at 532 nm.

3. Results and Discussion

Sauropus androgynus L. Merr. is highly nutritious. Its protein content is higher than other leafy vegetables. Fresh leaves are an excellent source of provitamin A, Carotinoid, Vitamin B and C, protein and mineral. Mature leaves have more nutrient than young leaves. Table-1 shows the nutritive values of *Sauropus androgynus*. The plant exhibited Water 82.45%, Protein 5.24%, Lipid 0.13% and Carbohydrate 4.86% and Ascorbic acid 85.65%.

There are variations in the yields of crude extracts obtained from 3 different solvents i.e. Ethanol, Methanol and Water. The yield of extracts using Soxhlet apparatus were 3.83gm, 4.54gm and 5.16gm respectively. The variation in yield may be due to the polarity of the solvents used in the extraction process (Table-2). It is reported that phenols are responsible for the variation in the antioxidant activity of the plant (Cai *et al*., 2004). They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals. (Pokorney, 2001;Pitchaon *et al*., 2007). Total phenolic content of the plant extracts were determined using the Folin-Ciocalteu's Phenol reagent. The exact mechanism of the reaction is complex but it is essentially a redox reaction occurring between antioxidants with phosphotungstic and phosphomolybdic acids. Since the reaction is based on redox, the assay would not be specific to just phenolics but to any other substance that could be oxidised by the Folin reagent. This was not surprising as numerous workers has reported the poor specificity of this assay (Singleton *et al.,* 1999; Escarpa and González, 2001). The ethanolic fraction of multivitamin plant exhibited the highest concentration of total phenolic content i.e. 230µg/ml followed by Methanolic extract with 158µg/ml and aqeous extract with 120µg/ml respectively.

The antioxidant activity of Multi-solvent extracts of Multi-vitamin plant was measured by two different spectrophotometric methods, DPPH assay and OH^- radical assay. In DPPH assay, DPPH is a stable free radical with purple color. The antioxidants scavenge DPPH radical by donating hydrogen atoms leading to a non-radical with yellow color (Bondent *et al*., 1997). Fig-1 shows the DPPH radical scavenging activity of *Sauropus androgynus* L. Merr. obtained from 3different solvents i.e. Water, Methanol and Water. The results revealed that the ethanolic fractions of *Sauropus androgynus* in both DPPH and OH- radical methods exhibited the highest free radical scavenging activity i.e. 62.90±0.03% and 54.36±0.03% respectively followed by methanolic extracts with i.e. 53.67±0.04% and 44.67±0.04% and aqueous extracts with 48.48±0.03% and 36.55±0.08% respectively (Table-3). It was observed that the ethanolic extracts show the highest scavenging activity followed by the aqueous followed by methanol (Fig-4&5). Methanol and ethanol has been proven as effective solvent to extract phenolic compounds (Siddhuraju and Becher, 2003). In the present study, the values of ethanolic and aqueous extracts were higher than that of methanolic ones. Among solvents used in this study ethanol has proved to be highly effective in extracting phenolic components. Ethanol as solvent is preferred for the extraction of antioxidant compounds mainly because of its lower toxicity (Karadeniz *et al*., 2005).

The antioxidant capacity is also expressed as 50 % inhibitory concentration (IC_{50}) in Table-2. 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 means a higher antioxidant capacity of the sample.

In Hydroxyl radical assay, OH^- radicals degrade 2-deoxyribose to malodialdehyde. The oxidized products from the reaction from complexes with TBA and show a pink color. The antioxidants of crude extract decreased formation of oxidized product of 2-deoxyribose leading to less formation of thiobarbituric acid reactive substances (TBARS). Here too ethanolic fraction of *Sauropus androgynus* L. Merr. exhibited highest IC_{50} value of 0.016 mg/ml (Table-2).

Table-1. The nutritive values of *Sauropus androgynus* L. Merr.

Sl.No	Nutritive Tests	Nutritive Values (%)
1	Water	82.45
2	Protein	5.24
3	Lipid	0.13
4	Carbohydrate	4.86
5	Ascorbic acid	85.65

Table-2

Crude extracts, phenol contents & IC_{50} Value in *Sauropus androgynus* L. Merr. Leaves

Solvent used	Crude Extracts (gm)	Phenol content (µg/ml)	IC_{50} Value in OH^- (mg/ml)	IC_{50} Value in DPPH (mg/ml)
Water	3.83	120	---------	----------
Methanol	4.54	158	----------	0.014
Ethanol	5.16	230	0.012	0.016

Table-3

Antioxidant activities of *Sauropus androgynus* in different solvents

Concentration of extracts (µg/ml)	Antioxidant activity (%)					
	Water		Methanol		Ethanol	
	DPPH	OH^-	DPPH	OH^-	DPPH	OH^-
40	28.24±0.07	21.56±0.073	34.41±0.04	27.41±0.04	42.30±0.01	35.50±0.01
80	30.18±0.10	24.11±0.05	37.11±0.06	31.11±0.06	43.46±0.03	38.41±0.03
120	33.48±0.08	28.78±0.02	43.46±0.06	34.46±0.06	47.69±0.06	44.34±0.06
160	35.47±0.02	31.66±0.09	49.23±0.10	39.23±0.10	52.11±0.13	48.08±0.13
200	48.48±0.03	36.55±0.08	53.67±0.04	44.67±0.04	62.90±0.03	54.36±0.03

Fig-1. Flowers of Multi-vitamin Plant

Fig-2 Multi-vitamin Fruits

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Fig-3. Leaves of Multi-vitamin Plant

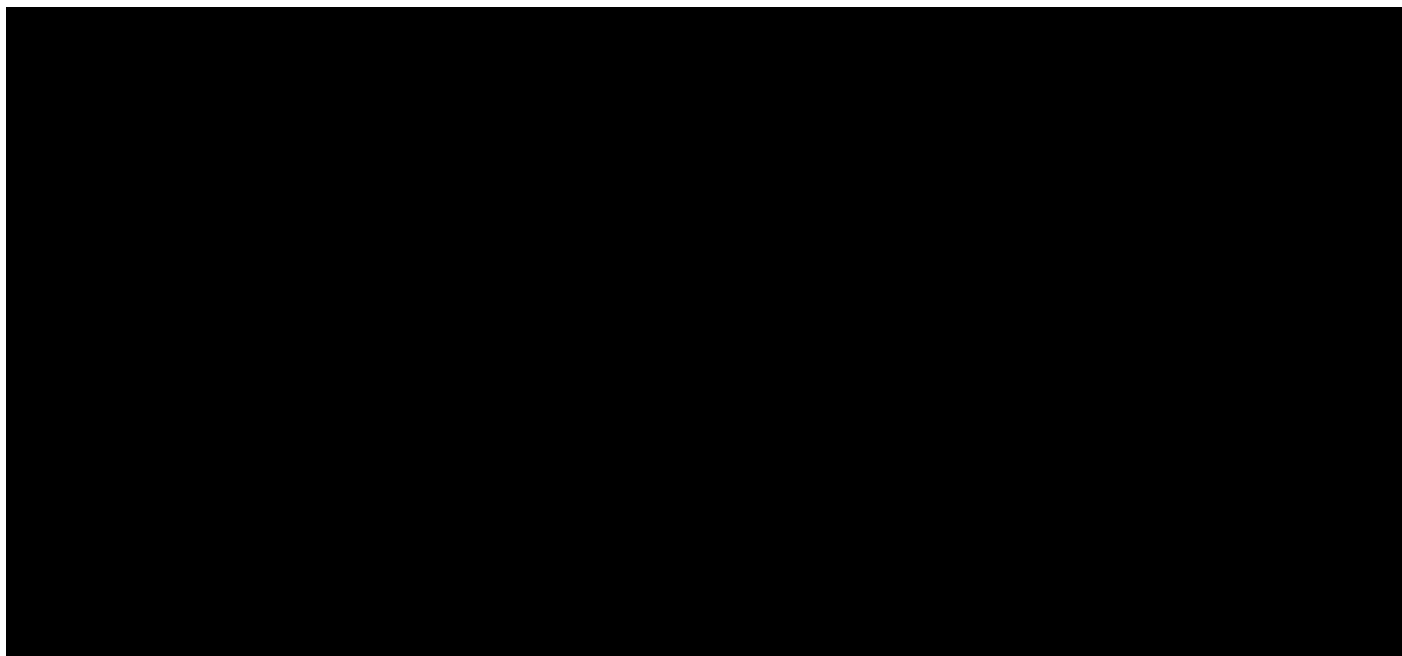

Fig-4. DPPH Free Radical Scavenging Activity of *Sauropus androgynus* L. Merr.

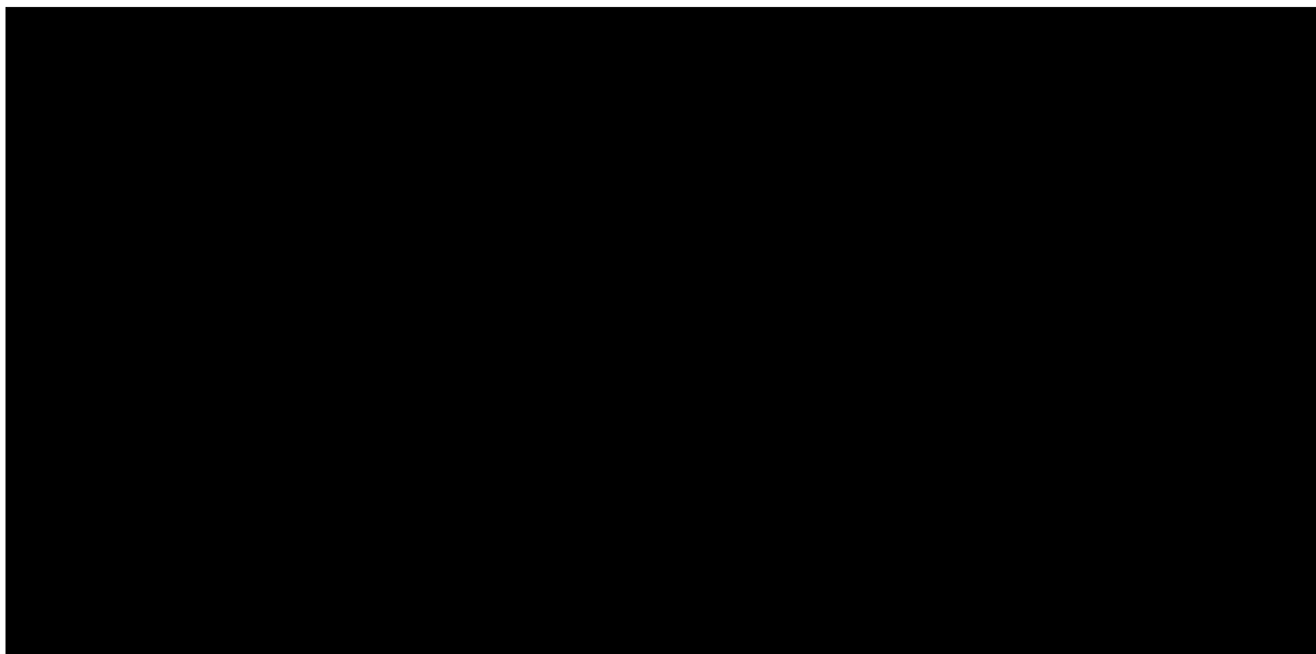

Fig-5. OH^- Free Radical Scavenging Activity of *Sauropus androgynus* L. Merr.

4. Conclusion

The results of the present study demonstrate higher IC_{50} values and strong antioxidant activity of an uncommon leafy vegetables plant with nutritional properties and medicinal value. This multivitamin green leafy vegetable plant can be explored for its vitamin content, phytochemicals which can act as low cost antioxidant.

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