

ANTIOXIDANT SURVEY IN CERTAIN FOOD PLANTS

¹ MAHUYA DE GHOSH* & ² RAMAKRISHNA TM.**

INDIAN ACADEMY DEGREE COLLEGE, CENTRE FOR RESEARCH & PG STUDIES,
HENNUR CROSS, HENNUR MAIN ROAD,
BANGALORE – 560 043, INDIA
EMAIL: DEMAHUYA@YAHOO.COM

ABSTRACT

In vitro antioxidant activity of methanolic extracts of leaves of *Sauropus* and *Alternanthera* were screened for their free radical scavenging properties using ascorbic acid as standard antioxidant. The free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical and reducing power. The reducing ability of a compound generally depends on the presence of reductants which are antioxidative potentially, breaking the free radical chain, by donating a hydrogen atom. The DPPH and reducing power was increased with the increasing concentration. Both the plants are potential source of antioxidants of natural origin of which, *Alternanthera* has exhibited more antioxidant property.

KEYWORDS: Antioxidants assay in certain vegetable greens

INTRODUCTION:

The free radicals are generated in living systems as a part of the normal metabolic process and the free radical chain reactions are usually produced in the body. In addition, chemical mobilization of fat, starch under various conditions such as lactation, exercise, fever, infection and even fasting can result in increased radical activity and damage. The antioxidants are radical scavengers which protect the human body against free radicals. The reactive oxygen species, including superoxide, hydroxyl radical and hydrogen peroxide are generated in specific organelles in the cell under normal physiological conditions (Hargguchi 2001)¹. Excessive production of these ROS, beyond antioxidant defence capacity of the body can cause oxidative stress (Aruoma 1996)². The reactive oxygen species (ROS) and free radicals mediated reactions are involved in various pathological conditions

such as anaemia, asthma, arthritis, inflammation, neurodegeneration against ageing process and perhaps dementia (Polterait 1997)³. A general consensus has been reached during the last few years that diet has a major role in the development of chronic diseases, such as, cancer, coronary heart disease, diabetics type2, hypertension and cataract.

Studies have also shown that consumption of food and beverages rich in phenolic content is correlated with reduced incidence of heart diseases, anaemia, asthma, arthritis, inflammation, neurodegeneration⁴⁻¹² (American Institute for cancer Research 1997, US dept. of Agriculture 1995, American Heart Association 1996, American cancer society 1996, World Health organization 1990, Willett 1998 1999, Jacob's *et al* 1998, US dept. of Health and Human services 1996)

Recently, there has been considerable interest in finding natural antioxidants from plant sources to replace synthetic ones. Natural antioxidant substances are considered to be safe since they occur in plant foods and are desirable than their synthetic counter parts. The scientific reports and experimental studies have shown that plants contain a large variety of phytochemicals that have antioxidant property (Pratt 1992)¹³ The most common plant phenolic antioxidants include flavonoid compounds, cinnamic acid derivatives, coumarins, tocopherols and poly functional organic acids (Hertog *et al* 1993)¹⁴. Studies on antioxidants have been explored in various plants and plant products. In this antioxidant survey two common plants like *Sauropus androgynous* and *Alternanthera pungens* were selected based on their use as edible greens. There is scanty information on the antioxidant property of these plants and hence the present work was taken up to fill the gap.

Methods and Materials

Sauropus androgynous (Linn.) Merr. is commonly known as chekkurmensis. Taxonomically classified under the family Euphorbiaceae. It is a leafy perennial undershrub. The plant is cultivated in gardens and also in parks for the beautiful foliage. It is not authentically known when this was introduced into India. The leaves and the top portion of 15 cms stem tips of plants have a pleasant taste. Normally, the leaves are eaten as raw and sometimes along with other popular leafy greens brought from the market is cooked together to prepare soup. The plant is being used in Taiwan and other South East Asian countries as regular leafy vegetable. The plant has alternate leaves, with short petioles and small stipules. The flowers appear in the axils of leaves. Each flower has 6-perianth lobes in 2- whorls. Female

flowers have bigger perianth lobes. Male flowers have 3-stamens. Fruit is a berry. Presently this plant is placed in a family Phyllanthaceae (Euphorbiaceae).

Altrananthera pungens Kunth. This has been taxonomically classified under the family Amaranthaceae, is commonly known as creeping chaffweed. It is a noxious perennial weed growing on the ground in the form of dense mat, around the houses, roadsides and in waste places etc. Stems prostrate, rooting at nodes. Leaves ovate to obovate, glabrous. Flowers in sessile, pubescent spikes, bracts tipped with a spine, bracteoles acute, abaxial sepals are tipped with spine. This is not being used as regular leafy food, but used as mixed leafy vegetable from ages, my grandmother (second author) used to pluck along with other wild leafy greens during food gathering. Our observations have confirmed that, during the months of june, july, august, the spines are not produced in the younger parts. This is an attempt to present it as a future food plant.

The plants were collected locally and leaves were dried in shade for 4-5 days. The dried leaves were powdered using a mixer grinder. The dried plant materials were soaked in methanol (100gms of plant material in 1000ml of methanol) for 5-7 days, by stirring it at every 18 hours using a sterilized glass rod. The final plant extracts were filtered seperately by using whatman No.1 filter paper. The filtrate obtained was concentrated under rotary vacuum evaporator at 40° celsius for further use. The stock solution of crude extracts 5mg/ml was prepared by dissovng a known amount of dry extract in 98% methanol. From the stock solution working solutions of 50,100, 150, 200, 250µg/ml were prepared separately using methanol (AR grade).

PRELIMINARY SURVEY OF ANTIOXIDANTS:

The methanolic extract was tested by applying general chemical test for alkaloids. The controls used in this test are Green tea, Cardamom. The reagents such as Murexide and Dragendroff's were used to estimate alkaloid content. The table represents the presence and absence of alkaloids.

Sl. No	Reagents	Controls		Plant extracts	
		Green tea	Cardamom	Alternanthera Pungens	Sauropus androgynous
1.	Murexide	+	-	+	-
2.	Dragendroffs	-	+	-	-

Table - 1

ANTIOXIDANT ASSAY:

The antioxidant activity of plant extracts were determined by different methods such as the DPPH free radical scavenging method and reducing power method.

ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT:

The antioxidant activity of plant extracts and the standard was assessed on the basis of the radical scavenging effects of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) a free radical activity by modified method (Braca *et al* 2002)¹⁵ The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 micro grams/ml solution. DPPH was freshly prepared in methanol and this solution was mixed with some ml of the plant extract solution and standard solution separately. These solutions were kept in dark for 30 minutes and optical density was measured at 517nm in UV Visible Spectrophotometer. Methanol is used as an blank. The optical density was recorded and the percentage

inhibition of DPPH was calculated using formula:

Percentage inhibition of DPPH activity

$$= A - B / A \times 100.$$

Where

A=Optical density of the control,
B= Optical density of the plant extract.

REDUCING POWER METHOD:

This was carried out as described by (Lu, Y and Y. Foo. 2000)¹⁶ 1ml of plant extract was mixed with phosphate buffer & potassium ferricyanide were mixed and incubated at 50 degree C for 10 min. Then, to that mixture trichloro acetic acid was added and centrifuged at 3000 rpm for 10min, finally to the supernatant distilled water & ferric chloride was added and absorbance was measured at 700nm in UV visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer used as a blank. The measured absorbance of the reaction mixture indicates stronger reducing power.

RESULTS AND DISCUSSIONS:

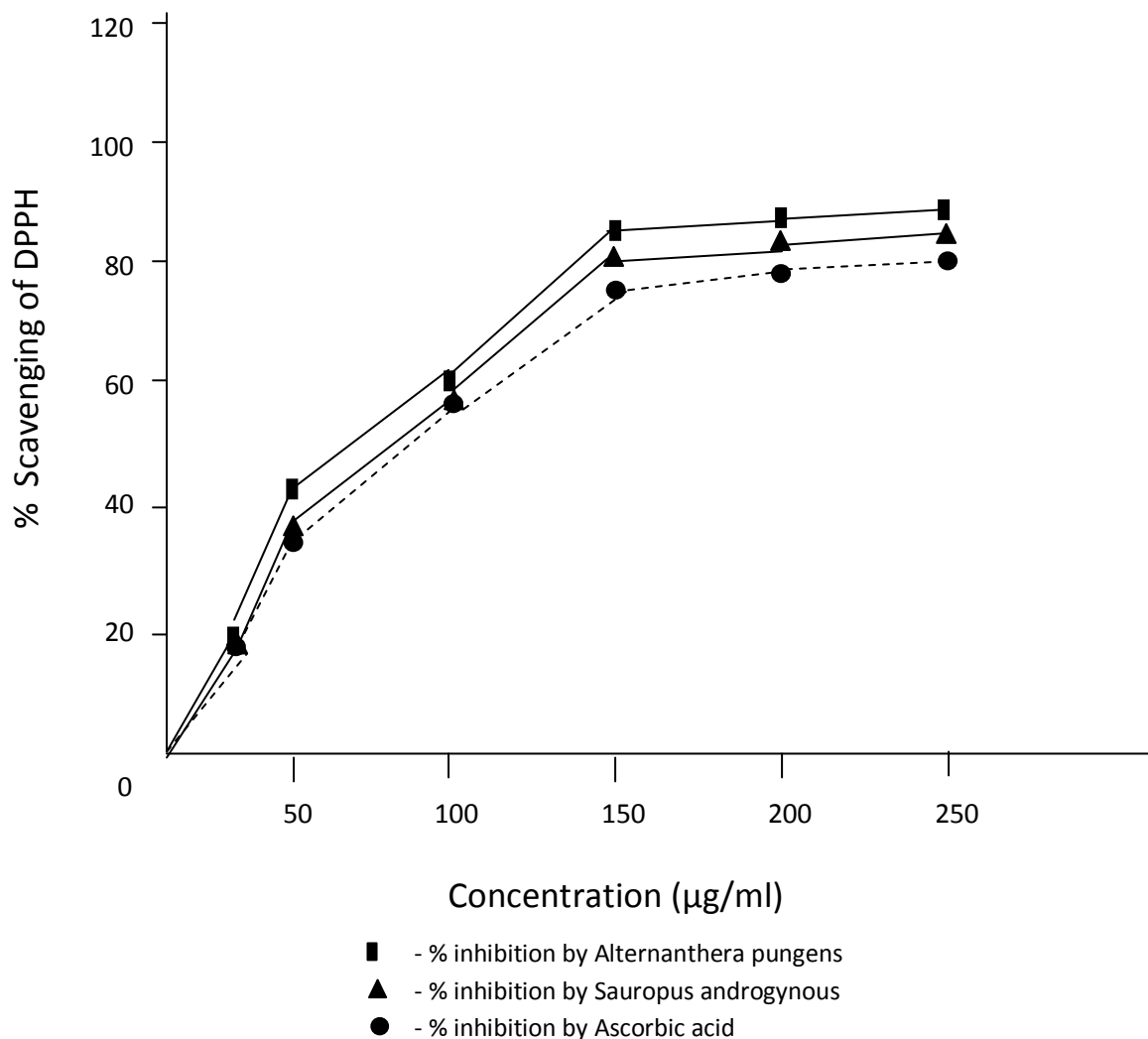
a) DPPH RADICAL SCAVENGING ACTIVITY:

DPPH radical scavenging activity of leaves of *Sauropus androgynous* and *Altrananthera pungens* species reveals that they have got profound antioxidant activity. The DPPH antioxidant assay is based on the ability of DPPH a stable free radical to decolourize the presence of antioxidants. The DPPH radical contains odd electrons, which is responsible for the absorbance at 517nm and also for visible deep purple colour. When DPPH accepts an electron donated by antioxidant compound, the DPPH is decolourized which can be quantitatively measured from the changes in absorbance. The

antioxidant activity was compared for both plant extracts and standards as

shown in fig- 1

Fig – 1: DPPH radical scavenging activity of methanolic leaves extracts of *Alternanthera pungens*, *Sauropus androgynous* measured at 517 nm as compared to standard Ascorbic acid. Values are the average of triplicate experiments and represented as mean $\pm$ standard deviation.

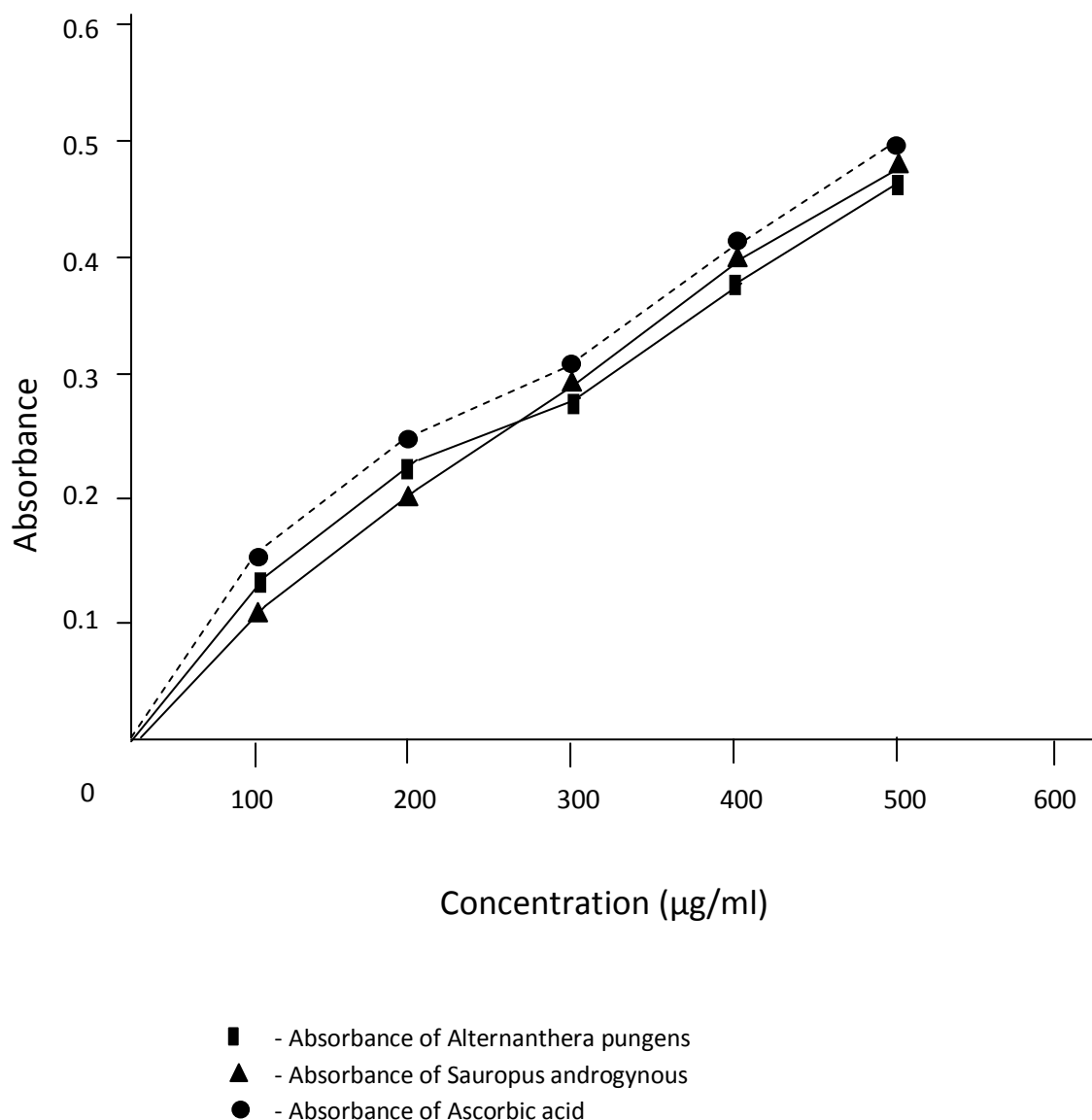


b) REDUCING POWER:

Different extracts of *Sauropus* and *Alternanthera* exhibited reducing power of methanolic extracts and ascorbic acid at concentrations between 100-500 mg/ml. The

reducing power of *Sauropus* and *Alternanthera* was very potent and the power of the extract was increased with the increase in concentration of the sample. (Fig:-2)

Fig:2: Reducing power of methanolic leaves extracts of *Alternanthera pungens*, *Sauropus androgynous* measured at 700 nm as compared to standard Ascorbic acid. Values are the average of triplicate experiments and represented as mean $\pm$ standard deviation.



CONCLUSIONS:

These plants are not popularly used as food plants. Further, investigations on isolation and identification of antioxidant components in the plants may lead to potential plant resources as food plants for the future. Since several decades, may be for centuries *Alternanthera* has been used along with other green vegetable plants. However, this is being used rarely as food plant, and not recognised as a potential food plant. The

results of antioxidant property has shown that *Alternanthera* and *Sauropus* are the good source of antioxidants. The detailed account of nutrients available in these plants would certainly give added value to the plants.

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