

Full Length Research Paper

## Evaluation of Phyto-nutrients and Antioxidative Effects of *Sida acuta* Leaf Extract in Rats

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Phytochemical, micronutrient composition and anti-oxidative potential of ethanolic leaf extract of *Sida acuta* in albino wistar rats were investigated using standard analytical methods. The result (mg/100 for phytochemical composition were  $91.46 \pm 0.02$  tannin,  $1500.36 \pm 0.36$  alkaloid,  $530.27 \pm 0.03$  saponin,  $1163.86 \pm 0.1$  flavonoid,  $1454.50 \pm 0.85$  steroid,  $115.29 \pm 0.05$  terpenoids and  $851.62 \pm 0.01$  cardiac glycosides. The vitamin composition (mg/100 g) were  $0.36 \pm 0.01$  thiamin,  $0.19 \pm 0.02$  niacin,  $24.27 \pm 0.25$  ascorbic acid,  $1.85 \pm 0.32$  tocopherol,  $0.12 \pm 0.05$  riboflavin while mineral composition (mg/100 mg) was  $14428 \pm 0.02$ ,  $122.11 \pm 0.01$ ,  $325.12 \pm 0.02$  for calcium, magnesium and zinc, respectively. To determine the antioxidative potential, twenty-four adult wistar albino rats were divided into four groups of 6 rats each. Group 1 received feed and water (control) while group 2, 3 and 4 in addition to feed and water were treated with ethanol leaf extract of *S. acuta* at 20, 40 and 60 mg/kg body weight, respectively. After 14 days of treatment; the rats were sacrificed and plasma obtained for oxidative stress indices assay. The result showed a significant decrease ( $P < 0.05$ ) in mean values of plasma malondialdehyde concentration and a significant increase ( $P < 0.05$ ) in reduced glutathione concentration at 40 and 60 mg/kg body weight compared to the control group. Plasma catalase and superoxide dismutase activity were significantly increased ( $P < 0.05$ ) only in animals treated with 60 mg/kg body weight compared to the control group. The result showed that ethanolic leaf extract of *S. acuta* possesses an antioxidant property which, in a dose-dependent manner, reduces/ameliorates oxidative stress in rats.

**Key words:** *Sida acuta*, rats, micronutrients, phytochemical, oxidative stress.

### INTRODUCTION

Medicinal plants are local heritage of global importance and have continued to play essential role in health care with 80% of the world's inhabitants

relying on traditional medicinal plants for optimum health (Cragg and Newman, 2001). This has led to scientific validations to get evidences for their use in

traditional medicine. Africa has arguably the richest phytodiversities in the world which span approximately 216,634,000 hectares (Ijeh and Ejike, 2011) and their nutritional and medicinal potential and properties are empirically appreciated. The medicinal value of the plants useful for healing and cure of human diseases is attributed to presence of phytochemical constituents (Nostro et al., 2000). Among the phytodiversities, the genus *Sida* has been explored (Wake, 2012). Commonly referred as common wire weed, *Sida acuta* is a species of flowering plant which belongs to the family Malvaceae believed to have originated from Central America. The plant grows on roadside, waste areas, forests and fields. *S. acuta* is a perennial shrub which grows up to 1 m high and reproduced from their seeds. It is erect with branched woody stems having simple and alternate leaves. *S. acuta* has been used in the treatment of various ailments. These include headache, cough, ulcer, urinary infections, skin infections, liver disorder and malaria (Wake, 2012).

In southern part of Nigeria, *S. acuta* grows in every season of the year. It is a commonly used weed in rural communities mainly for the treatment of liver disorders and skin infections (Ekpo and Etim, 2009). Local/indigenous names include Udo (Igbo), Iyeye (Yoruba), Nsukerra (Efik) and Tsadar lamarudu (Hausa). It has also been used as anti-inflammatory and hypoglycaemic agent (Okwuosa et al., 2011). Rami et al. (2014) reported proximate, phytochemical and micronutrient composition of *S. acuta*. With increasing health challenges, the traditional use of herbal medicine has been brought to the fore. The use of *S. acuta* has been attributed to the presence of biological active compounds in the plant. Therefore, this study was aimed to investigate

phytochemical, micronutrient composition (some vitamins and minerals) and anti-oxidative potential of ethanolic leaf extract of *S. acuta* in wistar albino rats.

## MATERIALS AND METHODS

### Plant material

The fresh leaves of *Sida acuta* plant were harvested from roadsides and local farms in Umuahia, Abia State. The plant was authenticated by Dr. Omosu Garuba of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The leaves were washed and cleaned under running tap water and dried under shade at room temperature for three weeks. The dried leaves were ground to a fine powder with mechanical grinder and kept

in airtight containers under dry conditions. The dried ground leaves was used to determine phytochemical and micronutrient composition.

### Preparation of ethanolic leaf extract of *S. acuta*

Modified method of Abdulrahman et al. (2004) was used. Two hundred grams of grounded *S. acuta* leaves were macerated in 100ml absolute ethanol for 72 h properly covered and labeled. The extract was then filtered with sterile filter paper (Whatman No. 1). The filtrate was evaporated to dryness at 40°C in a vacuum using a rotary evaporator and stored at 50°C in a refrigerator until required for use. Approximate concentrations of the extract were made in 100 ml of 10% ethanol for the treatment of the animals.

### Determination of micronutrients

Micronutrient composition for vitamins (Thiamin, Riboflavin and Niacin) was carried out according to the method described by Okwu and Josiah (2006); ascorbic acid by the method described by Onwuka (2005) while tocopherol was determined by the method described by Umeh and Ogbuagu (2010). Mineral composition analysis (calcium, magnesium and zinc) was determined by the method described by Onwuka (2005). Quantitative determination of tannin, alkaloid, saponin, flavonoid, steroid, cardiac glycoside and terpenoid composition was carried out by the method described by Harborne (1973).

### Animals

Wistar albino rats of both sexes weighing daily between 150 to 180 g were used for the experiment. The rats were kept and acclimatized to dialing handling in the animal house of Imo State University, Owerri for 10 days. They were fed *ad-libitum* with normal commercial rat chow (Product of Pfizer Nig. Ltd) and water.

### Experimental design

Twenty-four rats randomly assigned into 4 groups of 6 rats each were used for this study.

#### Group 1

The rats in this group were fed with normal rat chow and had free access to water. They were given 1 ml of 10% ethanol daily for 14 days. They serve as control.

#### Group 2

The rats in this group were fed with normal rat chow and had

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**Abbreviations:** ROS, Reactive oxygen species; GSH, glutathione; MDA, malondialdehyde; DTNB, 5,5'-dithio-bis(2-nitrobenzoic acid) or Ellman's reagent.

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free access to water. They were orogastrically given 1 ml equivalent to 20 mg/kg body weight of *S. acuta* leaf extract daily for 14 days.

### Group 3

The rats in this group were fed with normal rat chow and had free access to water. They were orogastrically given 1ml equivalent to 40 mg/kg ethanol leaf extract of *S. acuta* daily for 14 days.

### Group 4

The rats in this group were fed with normal rat chow and had free access to water. They were orogastrically given 1 ml equivalent to 60 mg/kg ethanol leaf extract of *S. acuta* for 14 days.

### Analytical procedure

After 14 days of treatment, the animals were sacrificed under chloroform anaesthesia. Blood sample was collected by cardiac puncture from each rat using a sterile syringe into a heparinized tube. The plasma was separated by spinning at 1000rpm for 10 min with Wisperfuge model 1384 centrifuge (Samson, Holland) and collected with a pastuer pipette into clean test tubes. The plasma was used to estimate oxidative stress indices.

### Estimation of lipid peroxidation

Lipid peroxidation in plasma was estimated colorimetrically by measuring malondialdehyde as described by Wallin et al. (1993). Briefly, 0.1 ml of plasma was mixed with 0.9 ml of H<sub>2</sub>O, 0.5 ml of 25% trichloroacetic acid (TCA), 0.5 ml of 1% thiobarbituric acid (TBA) and 0.5 ml of 3% NaOH. The mixture was heated for 40 min in water bath and cooled in cold water. Then 0.1 ml of 20% sodium dodecylsulphate (SDS) was added and mixed properly. The absorbance was measured at 532 nm against a reference blank.

### Estimation of reduced glutathione (GSH)

Reduced glutathione (GSH) was estimated by the method of Ellman (1959). 1 ml of supernatant (0.5 ml of plasma precipitated by 2 ml of 5% TCA) was mixed with 0.5 ml of Ellman's reagent (0.0198% DTBN in 1% sodium citrate) and 3 ml of phosphate buffer (pH 8.0) was added. The fellow colour developed was read at 412 nm.

### Estimation of superoxide dismutase

Superoxide dismutase (SOD) activity was determined spectrophotometrically by the modified method of NADH-phenazinemetosulphate-nitrobluete-trazolium-formazon inhibition reaction at 560 nm (Kakkar et al., 1984). One unit of SOD is that which causes 50% inhibition of nitrobluetetrazolium reduction.

### Estimation of catalase

Catalase activity was estimated colorimetrically by the method described by Sinha (1972) using dichromate acetic acid reagent

**Table 1.** Phytochemical composition of the leaf of *Sida acuta*.

Phytochemical	Composition (mg/100 g)
Tannin	91.46 ± 0.02
Alkaloid	1500.26 ± 0.36
Saponin	530.27 ± 0.03
Flavonoid	1163.86 ± 0.10
Steroid	1454.50 ± 0.85
Terpenoid	115.29 ± 0.05
Cardiac glycoside	851.62 ± 0.01

Values are mean of triplicate determinations ± SD.

(5% potassium dichromate and glacial acetic acid mixed in 1:3 ratio). The intensity was measured at 620 nm and the amount of H<sub>2</sub>O<sub>2</sub> hydrolyzed was calculated for catalase activity.

### Statistical analysis

Statistical analysis was carried out using one-way analysis of variance of the SPSS version 21.0. This was followed by student's t-test of significance. Values at P<0.05 were considered statistically significant.

## RESULTS

Preliminary evaluation of the phytochemical, some vitamins and minerals composition and the effect of ethanolic leaf extract of *S. acuta* on oxidative stress indices were investigated in this study. Table 1 shows the phytochemical composition of *Sida acuta*. The result shows that the composition (mg/100 g) of tannin, alkaloid, saponin and flavonoid were 91.46 ± 0.02, 1500.26 ± 0.36, 530.37 ± 0.03, 1163.86 ± 0.10, respectively. The composition (mg/100 g) of steroids, terpenoid and cardiac glycosides were 1454.50 ± 0.85, 115.29 ± 0.05 and 851.62 ± 0.01 respectively. The composition (mg/100 g) of thiamin, niacin, ascorbic acid, tocopherol and riboflavin are shown in Table 2. Thiamin, niacin and ascorbic acid were 6.36 ± 0.01, 0.19 ± 0.40 and 23.27 ± 0.25, respectively, while tocopherol and riboflavin were 1.85 ± 0.32 and 0.12 ± 0.05 respectively. The composition (mg/100 mg) of calcium, magnesium and zinc were 114.28 ± 0.02, 122.11 ± 0.01 and 325.12 ± 0.02, respectively, as shown in Table 3. The effect of ethanolic leaf extract of *S. acuta* on lipid peroxidation and antioxidant activity are shown in Table 4. The result showed a significant decrease (P < 0.05) in malonaldehyde in the rats treated with 40 and 60 mg/kg ethanolic leaf extract of *S. acuta* compared with the control. Also, reduced glutathione (GSH) showed a significant increase (P < 0.05) in rats treated with 40 and 60 mg/kg ethanolic leaf extract of *S. acuta* when compared with the control while catalase and superoxide dismutase activity

**Table 2.** Composition of some vitamins in the leaf of *Sida acuta*

Vitamin	Composition (mg/100 g)
Thiamin	0.36 ± 0.01
Niacin	0.19 ± 0.02
Ascorbic acid	24.27 ± 0.25
Tocopherol	1.85 ± 0.32
Riboflavin	0.12 ± 0.5

Values are mean of triplicate determinations ± SD.

**Table 3.** Composition of some Minerals in the Leaves of *Sida acuta*.

Mineral	Composition (mg/100 mg)
Calcium	144.28 ± 0.02
Magnesium	122.11 ± 0.01
Zinc	325.12 ± 0.02

Values are mean of triplicate determinations ± SD.

**Table 4.** Effect of ethanolic leaf extract of *Sida acuta* on plasma malondialdehyde and antioxidants.

Group	Treatment	MDA (IU/L)	GSH(IU/L)	CAT(IU/L)	SOD(IU/L)
1	1 ml of 10% ethanol	4.31 ± 0.18	12.06 ± 0.92	0.48 ± 0.01	0.76 ± 0.09
2	1 ml 20 mg/kg <i>Sida acuta</i>	3.15 ± 0.45	13.37 ± 1.20	1.54 ± 0.01	1.25 ± 0.45
3	1 ml 40 mg/kg <i>Sida acuta</i>	1.83 ± 0.24*	17.29 ± 0.29*	1.95 ± 0.03	1.87 ± 0.01
4	1 ml 60 mg/kg <i>Sida acuta</i>	0.34 ± 0.40*	21.98 ± 0.69*	5.50 ± 0.54*	3.14 ± 0.07*

Values are mean of three determinations ±SD. (n = 6) \*Significantly different from control (P < 0.05).

showed a significant increase (P < 0.05) only in rats treated with 60 mg/kg ethanolic leaf extract of *S. acuta* when compared to the control group.

## DISCUSSION

The leaves of plants provide both nutritional and medicinal benefits principally due to their nutrient composition and secondary bioactive metabolites which are known to possess antioxidant, anti-bacterial, anti-inflammatory, anti-sickling, hypoglycaemic and immunomodulatory properties (Owolabi et al., 2011, Egba et al. (2012). Alkaloids, tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides were found in amounts of medicinal value. Alkaloids saponins and flavonoids can be used in the management of cold, fever, headache and diabetes mellitus (Nwanjo et al., 2007, Ujowundu et al., 2010). Flavonoids are known for their antioxidant activity and can help protect the body against Reactive Oxygen Species (ROS) which are involved either in the

initiation or progression of carcinogenesis and other degenerative diseases. Reactive oxygen species cause oxidative damage by peroxidation and oxidation of cellular lipids, proteins and deoxyribonucleic acids (Poli et al., 1990). Tannins possess antibacterial, antiviral and also potent against degenerative diseases. It has been reported that tannin plays a major role as anti-diarrhoea and anti-haemorrhagic agent (Asquith and Butler, 1986). Steroids are important in pharmaceutical and play important role in the functions of sex hormones (Okwu, 2001). Saponin has been shown to have hypocholesterolaemic, hypotensive and cough depressant activities (Mensah et al., 2013). Tijjani et al. (2012) has also reported saponins as active agents against fungal infections. Glycosides are potent signal transducers acting on several intracellular targets thus modulating the activities of enzymes and hormones (Neer, 1995). Terpenes has been reported to have both antimalarial and hypoglycaemic effects (Kuzuyama and Seto, 2003; Tijjani et al., 2012).

Vitamins are important for optimum growth and

development since they participate in metabolic activity as coenzymes. Inadequate intake and utilization lead to characteristic diseases and subsequently death (Bakare et al., 210). Thiamin, niacin and riboflavin (water soluble vitamins) are energy releasing vitamins and their appreciable composition in the leaf of *S. acuta* are important in preventing pellagra, beri-beri and dermatitis which are characteristic deficiency diseases of niacin, thiamin and riboflavin respectively (Chaney, 2011). Antioxidant vitamins (ascorbic acid and tocopherol) are shown to be present in the leaf of *S. acuta*. Ascorbic acid and tocopherol are potent antioxidants which scavenge reactive oxygen species thereby ameliorating lipid peroxidation with its attendant deleterious effects (Nwankpa et al., 2012). The appreciable content of ascorbic acid and tocopherol in the leaf is suggestive of their role in protecting cellular components from oxidative damage. This corroborates the significant decrease in malondialdehyde (Table 4).

Mineral (calcium, magnesium, zinc) content of the leaf of *S. acuta* is in appreciable amount. Calcium plays a major role in sustenance and maintenance of strong bone and is important in muscle contraction and blood clotting. Magnesium is known to depress blood pressure and in conjunction with calcium sustains strong bones and prevents circulatory diseases (Alinnor and Oze, 2011). Zinc is a major component of superoxide dismutase which is an enzymic antioxidant. Zinc plays a major role in insulin secretion, wound healing, synthesis of mononucleotides and stimulate growth and development (Chatterjea and Shinde, 2012). The appreciable content of zinc in the leaf of *Sida acuta* (Table 3) suggests its possible role in curbing oxidative stress in the animals.

Malondialdehyde concentration depicts the level of cellular lipid peroxidation in cells. In this study, there was a significant decrease in malondialdehyde (MDA) in rats treated with 40 and 60mg/kg when compared to the control. This suggests the role of *Sida acuta* leaf extract in reducing lipid peroxidation hence reduction of reactive oxygen species (ROS) which cause oxidative damage. Similar report by Nwanjo et al. (2007) and Nwankpa et al. (2012) have shown the anti-lipid peroxidation effect of *Phyllanthus niruri* and *Chromolaena odorata* in diabetic rats and *Salmonella typhi* infested rats. The result of this study showed a significant increase ( $P < 0.05$ ) in reduced glutathione in rats treated with 40 and 60 mg/kg of *S. acuta* leaf extract when compared to the control while catalase and superoxide dismutase activity showed significant increase ( $P < 0.05$ ) in rats treated with 60mg/kg leaf extract of *Sida acuta* when compared to the control group. The results obtained in this study agrees with the report of Ogulanna and Ogulanna (2008), Ashok et al. (2012) and Omotosho et al. (2013) on antioxidant potential of *Newbouldia leavis*, *Gingo biloba* and

*Chrysophyllum* fruit extracts, respectively. Glutathione, catalase and superoxide dismutase are natural antioxidants (non enzymic and enzymic) which are known to play a critical role in ameliorating oxidative stress and preventing oxidative tissue damage (Nwankpa et al., 2012). This possibly may be linked to their role in scavenging free radicals species and reducing them to non-toxic products.

## Conclusion

Phytochemical and micronutrient composition of *S. acuta* leaf are authenticated in this study. Lipid peroxidation was reduced while anti-peroxidative indices (CAT, SOD and GSH) in rats treated with leaf extract of *S. acuta* were increased. Therefore, this study has shown the anti-oxidative property of the leaf extract of *S. acuta* which explains the use in traditional medicine.

## Conflict of Interests

The authors have not declared any conflict of interests.

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