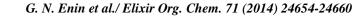
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Chemical assessement of the proximate, minerals, and anti-nutrients composition of *Sida acuta* leaves

G. N. Enin, B. S. Antia and F. G. Enin

Department of Chemistry, Faculty of Science, University of Uyo, Uyo, Nigeria.

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ABSTRACT

The leaves of *Sida acuta* "Broom Weed" (Family: Malvaceae) was analysed for its proximate, anti-nutrients and mineral elements composition using standard methods. The proximate analysis showed the results to be (%) moisture 54.82, ash 7.94, crude protein 17.85, crude fat 2.70, carbohydrate 6.21, crude fibre 5.30, and caloric value 360.54 Kcal. The concentration of anti-nutrients (mg/100g) was observed to be oxalate 140.8, hydrocyanic acid 98.3, phytate 210.1 and tannin 603.7. The mineral element contents (mg/100g dry weight) were Na (81.90), K (117.40), Ca (44.32), P (1.15), Fe (1.01), Zn (1.07) and Mg (14.40). The study showed that the high concentration of anti-nutrients in *Sida acuta Burn.f.* obtained from this locality might cause health hazard if consumed in large quantity over a long period of time.

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Introduction

Sida acuta known commonly as Broom weed is a species of flowering plant in the mallow family, Malvaceae. The most favourable habitat is in pastures, roadsides and waste grounds (Peirce, 2000). Sida acuta burm F. (Malvaceae) traditionally known as 'akananwanidipekisoro' among the Ibibio's of the south Eastern Nigeria is a small erect branched perennial shrub with woody tap root and hairy branches. Its hairy branches measure up to 1 m high and are reproduced from their seeds (Akobundu and Agyakwa, 1987). The stem is woody, rounded and slender, and is fibrous and hairy especially when young. The plant as well as other species of Sida has medicinal properties; the roots of S. acuta are astringent, cooling tonic and antipyretic, it is useful in nervous and urinary disorder and diseases of the blood and bile. The juice from the leaves is considered as an antihelmintic for intestinal worms (Peirce, 2000). It is believed in Nicaragua, that the decoction of the entire plant if taken orally can remedy asthma, fever, aches and pains, ulcer and venereal diseases (Caceres, et al, 1991; Coee and Anderson, 1996). Sida acuta is regarded as herbal plant by Botanists and Pharmacists because of its discovered bioactive 1. constituents in herbal medicine and chemotherapy (Trease and Evens, 1990). The antimicrobial screening of the plant using the disk diffusion assay showed that the methanolic extract of the plant had a significant activity on Stephylococcus aureus, Escherichia coli, Bacillus subtilis and Mycobacterium phlei. However, the extract was not active on Salmonella thyphimurium, Pseudomonas aeruginosa and Candida albican (Anani et al., 2000; Rajakaruna et al., 2002). The plant has also been screened for its cancer chemo-preventive properties and the result reported showed that flavonoids, compounds of tannins, steroids and triterpenoids present in S. acuta may be involve in this action as flavonoids have been reported to posses significant antiulcer activity in various experimental models of gastric and duodenal ulceration (Mandal et al., 2004). In another study, Otero et al., (2000) reported that the ethanolic extract of the plant had a moderate activity against the lethal effect of Bothrops alrox venom.

Phytochemical screening on S. acuta revealed that the plant contains alkaloids and steroidal compounds, with the potential to induce quinine reductase and to inhibit 7,12-dimethylbenz-(a)anthacene induced preneoplastic lesions in mouse mammary organ (Cao and Qi, 993; Dinan et al., 2001; Jang, et al., 2003). The alkaloid isolated from S. acuta, were reported to be of great interest in pharmacological studies. Ekpo and Etim, (2009) reported the presence of flavonoids in trace amount. Karou et al., (2007) attributed the great potentials of S. acuta to the presence of different bioactive components. Further research revealed that the juice from leaves of S. acuta is antihermitic for intestinal worms (Sofowora, 1982). The invitro activities and antioxidant properties of S. acuta has been reported (Ekor et al., 2010; Karou et al., 2005; Messina et al., 1994; Karou et al., (2003). However the report on the proximate composition, mineral elements and anti-nutrients analysis of this medicinal plant to ascertain its safety or otherwise to human, and its usefulness as an herbal drug and/or food is scanty. This study has been designed to determine the proximate composition, nutritional and anti-nutritional component of S. acuta.

Materials and method

Analytical procedure:

Fresh leaves of *Sida acuta* were Obtained from Obong-Itam in Itu Local Government Area in Akwa Ibom State of Nigeria and were identified by a taxonomist, Dr. (Mrs.) Bassey of Botany Department, University of Uyo, Nigeria. The leaves were destalked, washed and Ovendried at 60°C for 24 hours. After drying, the leaves were ground into a fine powder using mortar and pestle, and then sieved and stored in a well labeled air-tight container for analysis.

The proximate analysis for the various constituents was carried out based on the recommendation of the Association of Official Analytical Chemist (A.O.A.C., 1975).

Moisture content determination involved washing a known weight of sample with clean and distilled water and drying to a constant weight at 60° C in an oven (Gallen Kamp hot box).

Determination of ash involved incineration in a muffle furnace (Gallen Kamp hot box) at 550°C for 24 hours. Crude fat determination involved using exhaustive soxhlet extraction of a known weight of sample with petroleum ether (b.pt $40-60^{\circ}$ C) and methanol mixed properly in the ratio 1:1. Crude fibre was obtained from the loss of weight on ignition of dried residue remaining after digestion of fat-free samples with 1.25% each of sulphuric acid and sodium hydroxides under specified condition. % Fibre = Loss of weight on ignition

_____X 100 Weight of sample used

Determination of crude protein was done using the microkjeldahl nitrogen method which involves the digestion of a given weight of the sample with concentrated H_2SO_4 and a catalyst to convert any organic nitrogen to ammonium sulphate in solution, followed by the decomposition of ammonium sulphate with NaOH. The ammonia liberated was distilled into 5% boric acid. The nitrogen from ammonia was reduced from titration of the trapped ammonia with 0.05N HCl using methylene red and methylene blue (double indicator solution) indicators. The value of nitrogen obtained was multiplied by 6.25 to give the % crude protein.

The carbohydrate content was determined by subtracting the total crude protein, crude fibre, ash and lipid from the total dry matter. The caloric value estimation was done by summing the multiplied values for crude protein, fat or lipid and carbohydrate (excluding crude fibre) by their respective ATWATER factors (4 9,4). Mineral element composition was determined using the AAS after acid digestion of the samples (A.O.A.C. 1984). Hydrogen cyanide was determined by alkaline titration method (A.O.A.C, 1984), total oxalate was by the permanganate titration method by Dye (1956) and Tannin were estimated by the alkaline method of titration (A.O.A.C, 1984)

Results and discussion

The data reveals that the moisture content of Sida acuta (54.82%) is lower than the reported range (81.4-90.3%) in some Nigerian green leafy vegetables (Agbo 2004; Gupta et al., 2005). The moisture content is of great value in the preservation of food materials (Ravindran, 1993). The ash content which is an index of mineral contents in biota, is low (7.94%) compared to the values reported in leaves of Talinum triangulare (20.05%) (Ifon and Bassir, 1980; Ladan et al., 1996) and Amaranthus hybridus (13.80%) (Akubugwo et al., 2007). It is comparasble with Hibiscus esculentus (8.00%) (Akindahunsi and Saalawu, 2005). The ash content constitute the total organic matter in the leaf sample, that is the fraction of the leaves rendered carbonfree as a result of burning away of the organic proportion. Clinically, the level of ash in a food sample could be an indication of adulteration. A level much higher than expected may indicate the presence of other materials that may contain higher inorganic materials than the food sample in question, while a level lower than expected may indicate dilution of the food sample with substances having lower inorganic level (Ravindran, 1993).

The crude protein content of *Sida acuta* was determined to be 17.85% higher than the protein content of *Momor-doca foecide* (4.6%) leaves consumed in Nigeria and Swaziland (Ogle and Grivetti, 1985; Isong and Idiong, 1997; Hassan and Umar, 2006), but comparable with *A. hybridus* leaves (17.92%) (Akubugwo *et al.*,2007). It is lower than those of *I. batatas* (28.85%), *Amarathus candatus* (20.5%), *Piper guineeses* (29.78) and *T. triangulare* (31.0%) (Etuk *et al.*, 1998; Akindahunsi and Salawa, 2005; Antia *et al.*, 2006). According to Pearson (1976), plant food that provides more than 12% of its calorific value from protein is considered a good source of protein).

The crude fat (lipid) content of S. acuta was determined to be 2.70%, this is lower compared to reported values (8.3-27.0%) in some vegetables consumed in West Africa (Ifon and Bassir, 1980; Sena et al., 1998). However, it compares favorably with some edible green leafy vegetables (1.85-8.71%) of south India and Nigeria (Agbo, 2004; Gupta et al., 2005). Nevertheless, a daily intake of 80 to 160g is recommended in United Kingdom. Excessive intake of fat beyond the calorie needs of the body may lead to obesity. Excessive intake of fat high in cholesterol may result in atherosclerosis, thrombosis and consequently myocardaic infection (Vasudevan and Sreekumari, 2007). The crude fibre content was determined to be 5.30% lower when compared to A. hybridus (8.61%), T. triangulare (6.20%) and I. batatas leaves (7.20%) (Akubugwo et al., 2007; Akindahunsi and Salawu, 2005; Antia et al., 2006). Adequate intake of dietary fibre can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Ishida et al., 2000; Rao and Newmark, 1998). High fibre content may be attributed to low protein utilization values and low fibre content often results in high protein utilization value (Ravindran, 1993).

The carbohydrate content of S. acuta determined to be 66.21% is higher than 20.00, 23.70, 39.05, 51.95 and 52.18% reported for Sanna obtusfolia, Amaranthus incurvatus, M. balsamina. Amaranthus hybridus and I. batatas leaves respectively (Faruq et al., 2002; Hassan and Umar, 2006; Antia et al., 2006; Akubugwo et al., 2007). It is however, lower than reported values for Corchorus tridens (75.00%) (Asibey-Berko and Tayie, 1999). Carbohydrate serves as carbon sources for the synthesis of energy and other bi-molecules. It is produced by plants during photosynthesis (Vasudevan and Sreekumari, 2007). The recommended daily intake by United Nations Organization is between 200-500g. Excessive intake of carbohydrate usually results in hyperglycaemia and leads to storage of large amount of fats in the adipose tissues, resulting in obesity. On the other hand, insufficient carbohydrate intake may lead to hypoglycaemia; ketone bodies may be elaborated from fats, resulting in ketosis (Ravindran, 1993).

The caloric value (360.54kcal/100g) in S. acuta is higher than the reported values (248.307.1 Kcal/100g) in some Nigerian vegetables (Isong et al., 2006), but close to 384.33 kcal reported for B.coricea seed (Aaechi 2009). The energy value of food is usually expressed in calories (a calorie is the amount of heat required to raise the temperature of a gram of water by 1°C). The chemical energy bound in the molecule of carbohydrate, fat and protein is usually converted into heat and mechanical energy needed to perform work, maintain body temperature, and for growth. The rate at which the body uses calories for the basic life processes such as cellular activity, heartbeat maintenance of body temperature and respiration is termed the basal metabolic rate (BMR) (Ravindran, 1993). It is usually measured at post absorptive period (12-14 hours after meal). The BMR for a healthy 65kg man is 63kcal/hour and that for a woman is 54kcal/hour. BMR figures are usually expressed as percentage of the standard value.

Mineral Element Composition

The result of the mineral elements composition is reported in Table 3. From the result potassium has the highest value (117.40mg/100g) and iron the lowest (1.01mg/100g).

The sodium content of *Sida acuta* determined to be 81.90mg/100g is higher than reported values for *I. baatatas* (4.23mg/100g) (Antia *et al.*, 2006) and *A. hybridus* (7.43mg/100g) (Akubugwo *et al.*, 2006), lower than that reported for *Carica papaya* (178.2mg/100g) (Ayoola and

Adeyeye, 2010). Sodium is a macro element mainly found in the body as body fluid (Na⁺). The average amount in an adult is 80g. The daily recommended intake in adult is 5 to a much as 15g NaCl. Sodium aids in regulation of phosphorus, osmotic pressure and water balance, transmission of nerve impulses, active transport of glucose and amino acids. Daily Sodium needed for basic physiological fix is 1/8 tablespoon (250mg); daily sodium limit for seniors is ½ tablespoon (1200mg); while the daily sodium limit for Adult is 1 tablespoon (2300mg) (USDA Dietary Guideline 1999). This is the reason behind the leave being used as a cleanser in herbal remedy (Atta, 1999).

The data reveals that the potassium content is *S. acuta* (117.40mg/100g) is higher than that reported for *Aa. Hybridus* (54.20mg/100g) and *I. batatas* leaves (4.50mg/100g) (Akubugwu *et al.*, 2007; Antia *et al.*, 2006). It is also lower than that of the leaf content of *carica papaya* (288.90mg/100g). Potassium is a macro element mainly found in the body as cellular fluid (K^+). The average amount in an adult is 135g. The daily recommended intake for adults is 800-1300mg. Potassium aids in regulation of osmotic pressure and acid-base balance, activation of a number of intracellular enzymes, regulation of nerves and muscles instability. Deficiency of potassium in the body results in symptoms like weakness, anorexia, abdominal distension and tachycardia. This is reason while it is used as antibacterial in herbal remedy (Atta, 1999).

The calcium content was determined to be 44.32mg/100g. It compares favourably with values reported in some green leafy vegetables consumed in Nigeria and some wild edible leaves grown in Eastern Anatolia, Turkey (Ladan *et al.*, 1996; Turan *et al.*, 2003) and with *A. hybridus* leaves (44.15%) (Akubugwo *et al*, 2007). The total Calcium in the human body is about 1 to 1.5kg, 99% of which is seen in the bones and 1% in extracelluar fluid (Vasudevan and Sreekumari, 2007). Calcium is found in the body as calcium salts. The average amount in an adult is 1000-1500g. The daily recommended intake for adults is 800mg and children 1200 mg per day. Calcium mediates excitation and contraction of muscle fibres. The bulk quantity of calcium is used in bone and teeth formation. It is also used for the transmission of nerves impulses. Calcium deficiency causes tetany (Vasudevan and Sreekumari, 2007).

The phosphorus content in *S. acuta* was determined to be 1.15mg/100g lower than that reported for *A. hybridus* (34.91mg/100g) and *I. batatas* (37.82mg/100g) (Akubugwo *et al.*, 2007; Antia *et al.*, 2006). Phosphorus is found in the bones as calcium phosphate (Ca₃(PO₄)₂. The average amount in an adult is 600-900mg. The daily recommended intake for adult is 800mg (Vasidevan and Sreekumari, 2007).

The Iron content of *S. acuta* was determined to be 1.01 mg/100) (Antia *et al.*, 2006; Akubugwo *et al.*, 2007). Iron is an essential trace element for haemoglobin formation and normal functional of the central nervous system and in the oxidation of carbohydrates, protein and fats (Adeyeye and Otokiti, 1999). The body needs iron in a trace quantity. The average amount in an adult is 4g. The daily recommended intake is 10mg for men and 12mg for women. Deficiency of iron results in anaemia (iron deficiency anaemia) (Vasuadevan and Sreekumari, 2007).

The zinc content in *S. acuta* determined to be 1.070mg/100g is lower than that reported for *A. hybridus* (3.80mg/100g) and higher than that reported for *I. batatas* (0.08mg/100g) (kubugwo *et al.*, 2007 and Antia *et al.*, 2006). The human body requirement is 1.2μ g/ml. The daily recommended intake for adult is 10-20mg/day. Zinc deficiency results in poor appetite (growth retardation) hepatosplemegally dwarfish hypogonadism

in males, mental cathargy and delay in wound healing (Vasudevan and Sreekumari, 2007).

Magnesium content of *S. acuta* was determined to be 14.40mg/100g lower than that reported for *I. batatas* (30mg/100g) and *A. hybridus* leaves (231.22mg/100mg) (Antia *et al.*, 2006 and Akubugwu *et al.*, 2007), but higher than that reported for *Carica papaya* (6.78mg/100g) (Ayoola and Adeyeye, 2010). Magnesium is mainly seen in intracellular fluid. Total body magnesium is about 25g, 60% of which is complexed with calcium in bone. RDA is 400mg for men and 300mg for women. Doses above 600mg may cause diarrhea. Magnesium assists the assimilation of phosphorus. Its supplementation improves glucose tolerance (Vasudevan and Sreekumari, 2007).

Phytochemical and Anti-nutrient Composition

The hydrocyanate, oxalate, tannin and phytate content (mg/100g dry weight) of *S. acuta* are shown in Table 3. The tannin content is the highest (603.68mg/100g) while hydrocyanic content is the lowest (98.25mg/100g).

The HCN content in *S. acuta* was determined to be 98.25mg/100g, higher than the values reported for *A. hybridus* (16.99mg/100g) and *I. batatas* (30.24mg/100g) leaves (Akubugwu *et al.*, 2007 and Antia *et al.*, 2006). The toxic effects of cyanide ion in humans and animals are generally the same (Ansell and Lewis, 1970) and are believed to result from the inactivation of cytochrome oxidase and inhibition of cellular respiration and consequence histotoxic anoxia. The lowest reported oral lethal dose for humans is 0.54mg/kg body weight (calculated as hydrogen cyanide) (Ansell and Lewis, 1970). The major defence of the body to counter the toxic effect of cyanide is its conversion to thiocyanate mediated by the enzyme rhodanese (Lang, 1933). The primary targets of cyanide toxicity in humans and animals are the cardiovascular, respiratory and the central nervous system (Vasudevan and Sreekumari, 2007).

The phytate content in *Sida acuta* was determined to be 210.073mg/100g higher than values reported for *A. hybridus* (1.32mg/100g) and *I. batatas* (1.44mg/100g) (Akubugwo *et al.*, 2007; Antia *et al.*, 2006). Phytate is endogenous toxicants. Phytate causes toxicity by chelating with dietary calcium, which when absorbed into the kidney, accumulates to cause kidney stones, which lead to kidney dysfunction and intoxication. Phytate also decrease iron absorption by 5% (Christain and Greger, 1994; Natow and Heslin, 2004) and thus can lead to impaired oxygen transport and anoxia.

The oxalate content in *S. acuta* was determined to be 140.800mg/100g. This value is higher than the value reported for the same plant by Enemor et al. (2013), and high when compared to 95.50mg/100g for *Vernonia amygdalina* and 59.80mg/100g for *Tlferia occidentalis* (fluted pumpkin) (McGraw Hill, 1987), but lower than that reported for *I. batatas* (308mg/100g) (Antia *et al.*, 2006). Calcium oxalate is insoluble and produces kidney stones. This implies that only insoluble fraction of oxalate causes toxicity, as it is the smallest proportion of oxalate. Approximately, 75% of all kidney stones are predominantly calcium oxalate (Curhan, 1999).

The tannin content of *S. acuta* was determined to be 603.682mg/100g. This agrees with the earlier report on the same plant (Enemor et al 2013), but higher than that reported for *I. batatas* (0.21mg/100g) and *A. hybridus* L. 90.49mg/100g). Tannins at 0.5% level and above in diet cause a marked reduction in growth and available energy value of food, decreased availability of protein, and severe mortality at higher levels (4% and above).

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Parameters	% Weight
Moisture content	54.82
Ash content	7.94
Crude protein	17.85
Crude fat (lipid)	2.70
Carbohydrate	66.21
Crude fibre	5.30
Caloric value (Kcal/100g)	360.54

Table 1: Result of Proximate Composition of Sida acuta Leaves

Table 2: Mineral Element Composition of S. acuta leaves

Parameters	Mg/100g
Sodium	81.90
Potassium	117.40
Calcium	44.32
Phosphorus	1.15
Iron	1.01
Zinc	1.07
Magnesium	14.40

Table 3: Phytochemical and Anti-nutrient Compositions of S. acuta Leaves

Parameters	Mg/100g
Hydrocyanic acid (HCN)	98.25
Phytate	210.07
Oxalate	140.80
Tannin	603.68
Alkaloids	523.00
Flavonoids	310.00
Saponin	650.00

Alkaloids content of *S. acuta* (523.0mg/100g) is higher than the values reported for the leafy vegetables *Aspilia Africana*, *Bryophyllum pinnatum*, *Cleome rutidosperma* and *Emilia coccinea* consumed in Nigeria (Edeoga *et al.*,2005; Okwu and Josiah, 2006). Alkaloids are known to play some metabolic role and control development in living system and have a protective role in animals (Edeoga *et al.*, 2006; Edeoga and Eriata, 2001). Alkaloids like morphine and codeine are narcotic, analgesis and codeine is also used as stimulant. Alkaloids being found among the malvaceae of which *Sida acuta* belongs could be traced as an effective wounds healer (Edeoga *et al.*, 2006; Saganuwan and Gulumbe, 2006).

The flavonoid content (310.0mg/100g) and saponin content (650.0mg/100g) compare flavourably with the value reported for some medicinal plants used in Nigeria (Edeoga *et al.*, 2005; Oku and Josiah, 2006), but at variance with the value reported for the same plant by Enemor et al.(2013). Flavonoids play a vital role in symbiosis and defense in plant and also act as chemo-attractants and nod-gene inducers in some plant (Stafford, 1997). Flavonoids have been known from literature for its anti-inflammatory, anti-allergic effects, anti-thrombitic and vasoprotective properties. Many flavonoids containing plants are diuretic or antiplasmodic (Hrazdina, 1997). Saponins are generally known for their antibacterial and anti-fungal properties (Odebiyi, 1978), and hence, are likely responsible for the therapeutic value of the leaves of *Sida acuta*.

Conclusion

The result obtained revealed that the leaves of *S. acuta* contain appreciable amount of proteins, fat, fibre, carbydrate and high calorific, mineral elements, vitamins and moderately high concentration of toxicant. Consumption of these leaves over a long period of time will lead to bioaccumulation of toxicants. However, *S. acuta* can be detoxified by soaking and boiling. The chemical constituents of *S. acuta* predicate that it may not only

be useful due to its dietetic value but also medicinally and pharmacologically.

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