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Antimicrobial properties of *Sacrocephalus latifolius* (Sm.) Bruce

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Sacrocephalus latifolius **(Sm.) Bruce (Rubiaceae) formerly known as** ***Nauclea latifolia*** **(Sm.), is a highly valued medicinal plant used in ethnomedicine in the treatment of various infectious diseases due to its insecticidal and antiparasitic properties. The proximate, mineral composition and antimicrobial properties of the leaves were analyzed with a view to evaluate its nutritional and medicinal potentials. The proximate analysis revealed that the moisture content, ash, lipid, crude fibre, crude protein and the carbohydrate content of the leaves were 56.36 ± 0.50, 5.93 ± 0.2, 0.28 ± 0.50, 4.48 ± 0.20, 6.55 ± 0.10 and 3.21 ± 0.30%, respectively. Mineral compositions of the leaves showed that the vitamins content, potassium, calcium, iron and zinc content of the leaves were 3.41 + 0.30, 1.52 + 0.20, 2.28 + 0.30, 3.51 + 0.20 and 2.71 + 0.20%, respectively. The phytochemical screening revealed the presence of glycosides, alkaloids, flavonoids, tannins, triterpenoids and saponins in the methanolic extracts of the sample. Aqueous, choloroform and methanol extracts exhibited broad spectrum activity against** ***Staphyococcus aureus*** **and** ***Escherichia coli*** **by using disc diffusion method. The results indicate that** ***S. latifolius*** **contains substantial amounts of organic constituents and minerals, suggesting its potential for nutrient composition. As such, the results of phytochemical screening could justify observed antimicrobial herbal medicine.**

Key words: *Sacrocephalus latifolius,* nutrients, ethnomedicine, Antimicrobial, phytochemicals.

INTRODUCTION

Medicinal plants are valuable and renewable sources of active substances in pharmacology. Approximately 20% of the plants in the world have been subjected to pharmacological and/biological evaluation and as such substantial number of new antibiotics are being introduced into the market obtained from natural or semi synthetic sources (Mothana and Lindequist, 2005; Aliyu et al., 2011). Many species in the genus *Sacrocephalus* have been reported to have extensive history in traditional medicine system, widely used for the treatment of malaria,

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of malaria, hypertension, diarrhea, tuberculosis, dysentery and also as a laxative (Benoit-Vicalet.al., 1998). A number of researchers have reported the anti-bacterial (Fakae et-al., 2001) and antidiabetic properties of the plant. Phytochemical analysis revealed that the *Sacrocephalus latifolius*. contain alkaloids saponins, polyhenols and tannins (Tona et al., 2000; Boakye-Yiadom et al., 1977).

S. latifolius (Sm.) Bruce formerly known as *Nauclea latifolia* is a savanna shrub sometimes found in undisturbed fringing forest and closed savanna woodland. It belongs to the family Rubiaceae commonly called pin cushion tree, locally known as 'Egbo egbesi in Yoruba, 'Ubalu inu in Igbo and 'Tabasiya' in Hausa in Nigeria. It has many branches varying up to a height of 12 m with an open canopy. Flowers possess terminal spherical head like cymes of small whitish Flowers (Benoit-Vical et al., 1998). The flowers are found conjoined with their calyces. The fruit is syncarp and ripens during July to September. However, the baboons eat the fruits and disperse the seeds. It is further reported that livestock eat shoots and the leaves (Deeni and Hussain, 1991).

In West and Central Africa, *S. latifolius* is prominent for its insecticidal and antiparasitic properties. In Gabon, Congo and Nigeria, infusions of the leaves and bark are used to cure fevers (Di Giorgio et al., 2006). In Kinshasa, DR Congo extracts and preparations, along with other plants, are administered for diagnosing diarrhea (Tona et al., 2000). *S. latifolius* is used in medicine and as food, thus serving the dual purpose of food and medicine (Etkin, 1996). This study evaluates the nutritional and medicinal properties of *S. latifolius* since it serves both as food and medicine.

MATERIALS AND METHODS

Plant

Fresh leaves of *S. latifolius* were collected in bushes located around, Abuja, Nigeria. The plant was authenticated at the Herbarium unit of the Department of Biological Sciences, University of Abuja, Abuja, Nigeria. The leaves were air-dried at room temperature and grounded to fine powder, using a laboratory mill and stored in air-tight containers for laboratory analysis. All analysis was carried out in triplicates.

Proximate and mineral analysis

The proximate analysis of *S. latifolius* powdered leaf sample was tested to obtain values for the moisture content, crude fat, crude protein, crude fibre, ash and carbohydrates following the procedures as described by official methods of Analysis of Official Analytical chemists (AOAC, 1995). The moisture content was determined by air oven drying that exhibited a weight difference at 130°C for 1 h. The crude protein content by micro Kjeldahl method is given as 1% total nitrogen × 6.25 (AOAC, 1990). The crude fibre content was determined using dilute acid and alkali hydrolysis. Crude fat was extracted by exhaustively extracting 10 g of each sample in a soxhlet apparatus using dimethyl ether (boiling range 30 to 60°C) as the solvent. Ash was also determined by the incineration of 10 g of each sample placed in a muffle furnace maintained at 550°C for 5 h. The total carbohydrates content (on dry weight basis) was calculated by the difference: 100 - (crude protein + crude fat + ash + crude fiber).

The mineral constituents and vitamin analysis

The ash solutions were prepared by weighing 5 g of each of the powdered samples and dried at 550°C in muffle furnace for about 5 h and the resultant residues were dissolved in 100 ml of deionized water. A standard solution of the minerals (potassium, calcium, iron and zinc) to be analyzed were prepared. The atomic absorption spectrophotometer (model 200 – A, Buck Scientific) was set with power on for ten minutes to stabilize and the standard mineral solutions were injected to calibrate the atomic absorption spectrophotometer (AAS) using acetylene gas. Finally, an aliquot of ash solutions were injected and the concentrations obtained from the AAS.

Vitamin analysis

Vitamin C in the sample was determined as follows; 100 g of the fresh leaves of *S. latifolius* were cut into small pieces and grounded with a mortar and pestle. 10 cm^3 of distilled water was added several times and decanted into a 100 ml. volumetric flask. The grounded leaves was strained through a cheese cloth, filtrate were collected and washed in volumetric flask. The extracted solution was made up to 100 ml with distilled water. 20 ml aliquots of the sample solution were pipette into a 250 ml conical flask, 150 ml distilled water and 1 ml starch solution was added. The sample was titrated with 0.005 m iodine solution. The end point of the titration was identified as the first permanent trace of a dark blue colour due to the starch iodine complex. The titrations were repeated two times with further aliquot of sample solution, with a constant result obtained (Adeboye, 2008).

Phytochemical analysis

Extraction of plant material: In order to extract plant material, an equal amount of aqueous, chloroform and methanol were used as solvents for the extraction of bioactive compounds. The extracts so obtained were completely evaporated by using vacuum rotary evaporator. The resultant concentrated extracts were used for antibacterial activity.

Phytochemical screening: The phytochemical properties of the dried powdered plant leaf parts were determined using standard methods described by Harborne (1993) and Nabavi et al. (2008). The leaf extract was screened for glycoside, anthraqiunones alkaloids, tannins, terpenoids, flavonoids and saponins.

Test microorganisms: The test organisms used to detect the presence of antibacterial assay are *Staphylococcus aureus* and *Escherichia coli*. Cultures of these bacteria were maintained on the slopes of the nutrient agar at 37°C which were collected from Microbiology Laboratory of University of Abuja Teaching Hospital,

Table 1. Proximate composition of *S. latifolius*.

Organic composition	Value (%) ± SD
Moisture	56.36 ± 0.50
Ash	5.93 ± 0.20
Crude fat	0.28 ± 0.50
Crude fibre	4.48 ± 0.20
Crude protein	6.55 ± 0.10
Carbohydrate	3.21 ± 0.30

Table 2. Mineral composition of the leaves.

Mineral composition	Value (%) ± SD
Vitamin C	3.41±0.30
Potassium	1.52±0.20
Calcium	2.28±0.30
Iron	3.51±0.20
Zinc	2.71±0.20

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Antibacterial assay

The agar well diffusion technique as described by Jombo and Enebebeaku (2007) was employed for the determination of the zone of inhibition of the extract. The minimum inhibiting concentration (MIC) was determined after Baron and Finegold (1990) while the minimum bactericidal concentration was determined as described by Jombo and Enebebeaku (2007). The same procedure was followed to know the frequency of occurrence of standard antibiotics ampiclox for *Staphylococcus aureus* while chloramphenical was used for *Escherichia coli* to know the zone of inhibition as controls.

RESULTS AND DISCUSSION

Table 1 shows the result of the proximate analysis of *S. latifolius.* The concentration of organic compounds analyzed followed a sequential order of moisture > protein > ash > fibre > carbohydrate > fat. In *Sacrocephalus* species, the moisture content of the leaves (56.30%) is higher than the reported value of *Phyllanthus pentandrus* (Aliero and Shehu, 2010). A high moisture content of *Sacrocephalus* indicate that the plant is susceptible to microbial attacks during storage. The low ash content of 5.93% reflects low mineral and high organic content which are comparable to those recorded by Dike (2010). A very low content of fat (0.28%) was found similar when compared with the leaves of some higher plants by Dike (2010). However, the low fat content of the leaves of *Sacrocephalus* revealed that this plant is not an oil species and as such cannot be used either in industrial or domestic purposes, for industrial or domestic purposes. The fibre content of *S. latifolius* leaves was found to be 4.48% which validates the findings of Ujowundu et al. (2010) who reported the presence of fibre content in the leaves of higher plants. It is revealed that the protein content (6.55%) was found to be lower than the values of *Vernonia amygdalina* (Dike, 2006) carbohydrates content. However, the value of *S. latifolius* was 3.21% similar to those recorded for some leafy vegetables (Mensah et al., 2008).

The results showing presence of mineral composition are presented in Table 2. The presence of major elements like potassium, calcium, iron and zinc in the leaves correlates with the reports of Gafar et al. (2011). Potassium along with sodium is responsible for maintaining proper acid-base balance and nerve transmissions in the body (Akinyeye et al., 2010). Calcium plays an important role in building strong and healthy bones and teeth throughout life (Ojo and Ajayi, 2009). Iron helps in the formation of hemoglobin and myoglobin (Gafar et al., 2011). Zinc speeds up the healing process after injury and vitamin C content was relatively high when compared to lettuce (USDA, 2005). However, the deficiency in vitamin C leads to the disease scurvy and the primary cause of vitamin C deficiency is a poor diet (king, 2008). Thus the vegetable leaves can be used as a supplement for vitamin C.

Table 3 shows the qualitative phytochemical analysis of leaves of *S. latifolius.* The results showed that the aqueous leaf extract of S. *latifolius* contains tannins, terpenoids, flavonoids and saponins. The chloroform leaf extract contains glycoside, tannin, terpenoids flavonoid and saponins. The methanolic leaf extract contains glycoside, alkaloids, tannins terpenoids, falvonoids and saponins.

The antimicrobial activities of higher plants are attributed to the presence of alkanoids and flavonoids (Cordell et al., 2001). The role of tannins and other compounds of phenolic nature as active antimicrobial compounds has been reported (Rojas et al., 1992). The presence of these phytochemicals could to some extent justify the observed antimicrobial activities exhibited by the extracts in the present study. The result of antibacterial assay of aqueous, chloroform and methanol extracts are presented in the Table 4. The extracts of *S. latifolius* exhibited varying activities against *S. aureus* and *E. coli* as evidenced by its zone of inhibition when compared with the standard antibiotics of ampliclox for *S. aureus* and choramphenical of *E. coli.*

The highest zone of inhibition was observed at 200 mg/ml against *S. aureus* (18.0 mm) with methanol extract, while *E. coli* exhibited highest zone of inhibition at 21.0 mm with methanol extract. Aqueous extract has the least inhibition for both organisms at all concentrations.

Table 3. Phytochemical compositions of leaves of *S. latifolius*.

Phytochemical composition	Aqueous	Chloroform	Methanol
Glycoside	-	+	-
Anthraquinones	-	-	-
Alkaloids	-	-	+
Tannins	+	+	+
Terpenoids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+

Table 4. Zone of inhibition of the aqueous choroform and methanolic leaf extract of *S. latifolius* against the microorganisms.

Test organism	Conc. (mg/ml)	Mean diameter of zones of inhibition (mm)			
		Aqueous	Chloroform	Methanol	Control
Staphylococcus aureus	200	11±0.6	14±1.4	18±1.3	22±2.4
	100	10±1.5	12±1.3	15±1.5	19±3.6
	50	9±1.4	11±1.6	13±1.4	16±2.1
Escherichia coli	200	13±1.3	16±1.3	21±2.8	24±2.5
	100	11±1.8	15±1.2	19±1.5	22±3.3
	50	9±0.5	12±2.2	14±1.7	18±2.7

For Control: Ampliclox was used for *Staphylococcus aureus* while choramphenicol was used for *E. coli.*

Table 5. Minimum inhibitory concentration (MIC), minimum bacterial concentration (MBC) of *S. latifolius* leaf extracts of the micro-organisms (mg/ml).

Microorganism	Solvent	MIC	MBC
Staphylococcus aureus	Aqueous	100	200
	Choroform	25	50
	Methanol	12.5	25
Escherichia Coli	Aqueous	50	100
	Choroform	12.5	25
	Methanol	6.3	12.5

Although, gram-negative bacteria tend to have higher intrinsic resistance to most antimicrobial agents (Ndukwe et al., 2005). However, there is impressive activity against this gram negative bacterium in gastrointestinal and urinary tract of the human body. The susceptibility of *E. coil* to the extracts is an indication to the therapeutic potentials of these extracts against such disease in Table 5. The growth of *S. aureus* was inhibited by the extracts with greatest inhibition in methanol. *S. aureus* is a human pathogen whose infection is difficult to treat with conventional antibiotics. In animals, *S. aureus* is encapsulated by fibrotic tissue and forms microabsceses to protect it from the usual antibiotics (Giesecke et al., 1994). Therefore, the activity of *S. latifolius* against *S. aureus* may prove to be a worthy alternative in search of new antibiotics.

The phyotochemical screening of *S. latifolius* extracts shows the presence of glycoside, alkanoids, tannins, terpenoid, flavonoids and saponins in Table 3. Differences in antimicrobial activity of medicinal plants

have been compared with the contents of active compound (Boakye-Tiadom and Konning, 1975). However, the antimicrobial activities of higher plants are attributed to the presence of alkaloids and flavonoids (Cordell et al., 2001). The role of tannins and other compounds of phenolic nature as active antimicrobial compound has been reported (Rojas et al., 1992). As such, presence of these phytochemicals could therefore justify the observed antimicrobial activities exhibited by the extracts of *S. latifolius*.

However, it was concluded from the present study that *S. latifoliu* contain substantial amounts of organic substances and minerals, representing the prospective nutrient composition. The result of phytochemical screening justified the antimicrobial activities and strongly confirm its use in herbal medicine for the treatment of bacterial infection

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Conflict of Interest

Authors have not declared any conflict of interest.

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