

Research Article

Comparative Study of Phytochemical, Proximate and Mineral Compositions of *Stachytarpheta cayannensis* (L.C. Rich.) Schau and *Stachytarpheta indica* (Linn.) Vahl

Chinelo A Ezeabara*, Lovina A Orachu, Okeke CU, Chinyere V Ilodibia, Adaeze N Emeka and Ekwealor KU

Department of Botany, Nnamdi Azikiwe University, Nigeria

***Corresponding author**

Chinelo A Ezeabara, Department of Botany, Nnamdi Azikiwe University, P.M.B. 5025 Awka, Anambra State, Nigeria, Email: e.chinelo5@yahoo.com

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Abstract

Phytochemical, proximate and mineral compositions of leaves and stems of *Stachytarpheta cayannensis* (L.C. Rich.) Vahl and *S. indica* (Linn.) Vahl were evaluated using standard methods and compared. Protein, fat and minerals as well as certain bioactive agents: alkaloid, flavonoid, saponin, sterol and tannin were detected in various concentrations. Higher concentrations of fat and protein as well as all the micronutrients were present in the stems of *S. indica*. Concentrations of alkaloid were higher in the leaves and stems of *S. cayannensis* where as higher level of protein was detected in the leaves. Greater level of hydrogen cyanide was found in the leaves of the two plants. Data were considered statistically significant at $P \leq 0.05$. However, high concentration of hydrogen cyanide, an acute toxin, detected in both the leaves and stems of the plants render them inedible without proper processing. Thus, the utilization of these plants in ethno medicine as food and drug could be fatal; therefore, it is highly discouraged. Nonetheless, the bioactive compounds and nutrients could be extracted for development of drugs and food supplements.

INTRODUCTION

Stachytarpheta cayannensis (L.C. Rich.) Schau. and *Stachytarpheta indica* (Linn.) Vahl. are members of the genus *Stachytarpheta* of the family, Verbenaceae. They have been used extensively in traditional medicine. *Stachytarpheta indica* is one of the ancient plants in the world, which is used in the traditional system of medicine for diabetes and liver components [1]. In addition, *S. indica* leaves were reported to possess antibacterial ability and the active principle responsible for the antibacterial activity is a phenolic compound [2]. The plant is used in parts of Southern Nigeria and Peru for the treatment of malaria [3,4]. *Stachytarpheta cayannensis* in the other hands, has been reported

to be anti-inflammatory, antinociceptive, antiulcerogenic [5-7] as well as anti diarrhoeal [8]. These therapeutic activities can only be possible as a result of the bioactive compounds present in these plants.

Use of plants and plant extracts in treatment of diseases is an age-long practice. In the last decade, demand in the search of bioactive compounds in plants is on the increase since plants are the fundamental sources of new drugs. However, there are some plants that have toxic constituents that are considered harmful to man. Although *Stachytarpheta cayannensis* and *S. indica* have been reported to be used extensively in traditional medicine, yet there is need to investigate the leaves and stems of these plants

for phytochemical, proximate and mineral constituents with the view of ascertaining their usefulness as food and drug as well as determine whether they have poisonous constituents.

MATERIALS AND METHODS

Collection of Plant Materials

Stachytarpheta cayennensis and *Stachytarpheta indica* were collected from the Forestry Research Institute of Nigeria (FRIN) and Dugbe area both in Ibadan, Oyo State, respectively. The collection was done in September, 2012. The voucher specimens of the plant samples were deposited in the herbarium of the Forestry Research Institute of Nigeria, Ibadan. The voucher specimen numbers are FHI-109820 and FHI-109821 for *Stachytarpheta indica* and *Stachytarpheta cayennensis*, respectively.

Preparation of Samples

Leaves and stems of *Stachytarpheta cayennensis* and *S. indica* were picked separately and packed in sample envelopes properly labelled and were oven dried at 65 °C for 5hrs. The samples were ground into a powder. The powdered samples were kept in an air tight container until use.

Quantitative Phytochemical Determinations

Determination of Alkaloid: This was done by the alkaline precipitation gravimetric method described by Harborne [9]. A measured weight of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4h at 28°C. It was later filtered via whatman No 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of conc. aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1% ammonia solution dried in the oven at 80°C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

Determination of Flavonoid: This was determined according to the method of [9]. Five grams of the sample was boiled in 50ml of 2M HCl solution for 30min under reflux. It was allowed to cool and then filtered through whatman No 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with drop.

The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

Determination of Phenol: This was determined by the Folin-ciocatean spectrophotometer [10]. The total phenol was extracted in 200mg of the sample and 10ml concentrated methanol. The mixture of centrifuge at 500rpm for 15minutes and the supernatant (extract) was used for the analysis. One millilitres portion of the extract from each sample was treated with equal amount of volume of Folin-ciocatean reagent followed by the addition of 2ml of 2% sodium carbonate solution. The intensity of the resulting blue coloration was measured (absorbance) in a spectrophotometer at 560nm wavelength. Measurement was made with a reagent blank at zero.

Determination of Steroids: This was determined by the method described by Okeke and Elekwa [11]. A measured weight of each sample was dispersed in 100ml freshly distilled water and homogenized in a laboratory blender. The homogenate were filtered and the filtrate was eluted with normal ammonium hydroxide solution ($\text{P}^{\text{H}} 9$). Two millilitres of the eluate were put in test tube and mixed with 2ml of chloroform. Three millilitres of ice-cold acetic anhydride were added to the mixture in the flask and 2 drops of conc. H_2SO_4 were cautiously added to cool. Standard sterol solution was prepared and treated as described above. The absorbance of standard and prepared sample was measured in a spectrophotometer at 420 nm.

Determination of Tannin: Tannin content was determined by the Folin-Denis colorimetric method described by Kirk and Sawyer [12]. Five grams of the sample was dispersed in 50mls of distilled water and shaken. The mixture was allowed to stand for 30min at 28°C before it was filtered through whatman No. 42 grade of filter paper. Two millilitres of the extract was dispersed into a 50ml volumetric flask. Similarly 2ml standard tannin solution (tannic acid) and 2ml of distilled water were put in separate volumetric flasks to serve as standard and reagent was added to each of the flask and the 2.5ml of saturated Na_2CO_3 solution added. The content of each flask was made up to 50mls with distilled water and allowed to incubate at 28°C for 90min. Their respective absorbance was measured in a spectrophotometer at 260nm using the reagent blank to calibrate the instrument at zero.

Determination of Hydrogen Cyanide (HCN): This was determined by Alkaline Pikrate Colorimeter method of Trease and Evans [13]. Using 25ml conical flask, 1.02g of the sample was dispersed in 50ml of distilled water. An alkaline Pikrate paper was hung over the sample mixture and the blank in their respective flasks. The set up were incubated overnight and each Pikrate paper was eluted (or dipped) into a 60ml of distilled water. A standard cyanide solution was prepared and diluted to a required concentration. The absorbance of the eluted sample solution and of the standard were measured spectrophotometrically at 540nm wavelength with the reagent blank at zero.

Nutrient Determinations

Ash, moisture and protein contents of each plant sample were determined according to the method of AOAC [10]. The ash content was determined by incineration in a muffle furnace at 550°C for 48hrs; moisture content by drying in an oven at 100°C until constant weight; protein content by Nitrogen determination using Kjeldahl method and conversion of Nitrogen to protein by the factor 6.25. The fat content was determined by the continuous solvent extraction method as described by James [14].

Mineral contents of these samples were done following the dry ash extraction method [12,14]. A measured weight of these samples were burnt to ashes (as in ash determination) thereby remaining all the organic materials leaving the organic ash. The resulting ashes were each dissolved in 5mls of dilute (0.1M) hydrochloric solution and then diluted to 100mls in a volume flask. This extract was used in specific analysis for the different mineral elements.

Statistical Analysis

T-test was used to analyse the variance and significantly different treatment means determined at 5% level using SPSS software version 20. Data were expressed as mean \pm standard deviation of triplicate determinations.

RESULTS

The results are presented in Tables 1 - 3. The leaves of *S. cayennensis* have higher composition of alkaloid (3.46 \pm 0.017%) and sterol (0.44 \pm 0.012%) whereas saponin (2.85 \pm 0.006%), flavonoid (1.89 \pm 0.046%), tannin (1.83 \pm 0.011%), phenol (0.31 \pm 0.012%) and hydrogen cyanide (6.93 \pm 0.017%) were higher in *S. indica* at $P \leq 0.05$ (Table 1). The stems of *S. cayennensis* has higher alkaloid (2.36 \pm 0.000%), whereas high level of tannin and hydrogen cyanide were present in the stems of the two plants.

There was no significant difference between the phenol and sterol concentrations of the stems of *S. cayennensis* and *S. indica* (Table 1). Higher moisture content (4.75 \pm 0.029%), ash (10.03 \pm 0.006%) and protein (16.73 \pm 0.006%) were found in the leaves of *S. cayennensis* whereas *S. indica* has higher fat content (4.97 \pm 0.006%) (Table 2). Level of proximate compositions were higher in the stems of *S. indica*; moisture content (3.83 \pm 0.006%), ash (6.35 \pm 0.006%), fat (4.77 \pm 0.006%) and protein (12.66 \pm 0.006%) (Table 2). Higher value of phosphorus (5.22 \pm 0.006%), magnesium (0.25 \pm 0.116%) and potassium (2.62 \pm 0.006%) were observed in the leaves of *S. cayennensis* whereas higher calcium content (1.16 \pm 0.006%) as well as significant high concentrations of all the micronutrients was observed in *S. indica*. There was no significant difference between the nitrogen content of the leaves of the two plants (Table 3). Higher level of nitrogen (2.68 \pm 0.006ppm) was detected in the stems of *S.*

Table 1: Phytochemical composition of leaves and stems of *Stachytarpheta cayennensis* and *S. indica*.

Constituents (%)	Leaves <i>S. cayennensis</i>	<i>S. indica</i>	Stems <i>S. cayennensis</i>	<i>S. indica</i>
Alkaloid	3.46 \pm 0.017*	2.81 \pm 0.026*	2.36 \pm 0.000*	1.94 \pm 0.017*
Flavonoid	1.70 \pm 0.015*	1.89 \pm 0.046*	0.84 \pm 0.000*	0.90 \pm 0.006*
Phenol	0.25 \pm 0.000*	0.31 \pm 0.012*	0.15 \pm 0.012	0.13 \pm 0.006
Saponin	2.77 \pm 0.026*	2.85 \pm 0.006*	0.92 \pm 0.029*	0.75 \pm 0.012*
Sterol	0.44 \pm 0.012*	0.40 \pm 0.021*	0.22 \pm 0.200	0.25 \pm 0.006
Tannin	1.79 \pm 0.012*	1.83 \pm 0.011*	1.59 \pm 0.023*	1.71 \pm 0.012*
		(ml/kg)		
Hydrogen cyanide	5.64 \pm 0.104*	6.93 \pm 0.017*	3.12 \pm 0.036*	3.18 \pm 0.006*

Values are Mean \pm Standard deviation of triplicate determinations.* Significantly different at $P \leq 0.05$

Table 2: Proximate composition of leaves and stems of *Stachytarpheta cayennensis* and *S. indica* (% dry matter).

Constituents (%)	Leaves <i>S. cayennensis</i>	<i>S. indica</i>	Stems <i>S. cayennensis</i>	<i>S. indica</i>
Ash	10.03 \pm 0.006*	9.22 \pm 0.006*	6.30 \pm 0.006*	6.35 \pm 0.006*
Fat	1.04 \pm 0.000*	4.97 \pm 0.006*	0.27 \pm 0.006*	4.77 \pm 0.006*
Moisture content	4.75 \pm 0.029*	4.52 \pm 0.006*	3.72 \pm 0.006*	3.83 \pm 0.006*
Protein	16.73 \pm 0.006*	12.65 \pm 0.006*	5.22 \pm 0.006*	12.66 \pm 0.006*

Values are Mean \pm Standard deviation of triplicate determinations.* Significantly different at $P \leq 0.05$

Table 3: Mineral composition of leaves and stems of *S. cayennensis* and *S. indica*.

Constituents (%)	Leaves <i>S. cayennensis</i>	<i>S. indica</i>	Stems <i>S. cayennensis</i>	<i>S. indica</i>
N	0.83 \pm 0.006	0.80 \pm 0.006	2.68 \pm 0.006*	2.02 \pm 0.006*
P	5.22 \pm 0.006*	0.09 \pm 0.006*	0.44 \pm 0.006	0.11 \pm 0.006
Ca	0.84 \pm 0.116*	1.16 \pm 0.006*	1.08 \pm 0.006*	2.68 \pm 0.006*
Mg	0.25 \pm 0.116*	0.22 \pm 0.006*	0.19 \pm 0.006	0.22 \pm 0.006
K	2.62 \pm 0.006*	2.57 \pm 0.006*	2.53 \pm 0.006	2.57 \pm 0.006
		(Ppm)		
Na	37.32 \pm 0.006*	76.02 \pm 0.006*	2.33 \pm 0.006*	78.01 \pm 0.006*
Mn	8.93 \pm 0.006*	117.01 \pm 0.006*	87.06 \pm 1.732*	120.01 \pm 0.006*
Fe	21.48 \pm 0.006*	71.61 \pm 0.006*	17.53 \pm 0.006*	75.53 \pm 0.006*
Cu	6.38 \pm 0.116*	15.08 \pm 0.006*	10.81 \pm 0.116*	19.04 \pm 0.006*
Zn	4.88 \pm 0.116*	24.96 \pm 0.006*	6.62 \pm 0.006*	26.81 \pm 0.006*

Values are Mean \pm Standard deviation of triplicate determinations.* Significantly different at $P \leq 0.05$.

cayennensis whereas higher calcium content ($2.68 \pm 0.006\%$) was detected in the stems of *S. indica*. All the micronutrients were found in higher concentrations in the stems of *S. indica*; sodium ($78.01 \pm 0.006\text{ppm}$), manganese ($120.01 \pm 0.006\text{ppm}$), iron ($75.53 \pm 0.006\text{ppm}$) and copper ($19.05 \pm 0.006\text{ppm}$). There was no significant difference between the phosphorus, magnesium and potassium contents of the stems of these plants (Table 3).

DISCUSSION

The results revealed that high levels of phytochemicals and nutrients; especially alkaloid, saponin, tannin, fat, protein and minerals were present in leaves and stems of *Stachytarpheta cayennensis* and *S. indica*. Generally, high values of bioactive and nutrients were found in parts of *S. cayennensis* but most of them were higher in *S. indica*. This indicated that these plants are rich in bioactive and nutrient compositions.

In addition, these bioactive compounds possess therapeutic activities of which probably account for the folk use of these plants in some parts of the world. Juice extracted from leaves and stems of *Stachytarpheta indica* is taken with sugar for treatment of Leucorrhoea in women in Vitbilila village, Sujanagar Sub-District of Pabna District, Bangladesh [15]. In Kalahandi District, Orissa; leaves of *Stachytarpheta indica* when pounded with black pepper is applied on ulcerated tongue surface for cure of tongue and mouth sore [16]. The leaves of *S. cayennensis* are used ethnomedically in Nigeria and other parts of the world for insomnia and anxiety [17]. In Brazil, an infusion of the entire *S. cayennensis* plant is used to treat malaria [18] and against respiratory diseases, it is drunk as tea until the symptoms disappear [19].

However, high level of hydrogen cyanide found in the leaves ($5.64 \pm 0.104 \text{ ml/kg}$) and stems ($3.12 \pm 0.036 \text{ ml/kg}$) of *S. cayennensis* as well as in the leaves ($6.93 \pm 0.017 \text{ ml/kg}$) and stems ($3.18 \pm 0.005 \text{ ml/kg}$) of *S. indica*; suggested that they are toxic. Although the concentrations in the stems of the two plants were lower than those of the leaves; with leaves of *S. indica* having the greatest, yet they are all poisonous. The hydrogen cyanide has long been considered a poison. It is a deadly substance and effects of a high dose of cyanide are quick, and death occurs within minutes [20]. Some studies have proposed potential lethal doses of hydrogen cyanide for humans. Lethal dose for adults was estimated to be 1.0mg/kg of body weight [20]; intravenous lethal dose, 1.0 mg/kg of body weight; dermal exposure lethal dose, 100 mg/kg of body weight [20, 21]; and acute oral lethal dose, $0.5 - 3.5\text{mg/kg}$ of body weight [22]. Exposure to lower levels of cyanide over a long period of time resulted in increased blood cyanide levels in rats, which can result in weakness and a variety of symptoms, including permanent paralysis and nervous lesions [23]. Therefore, the presence of high concentration of hydrogen cyanide in these parts of these plants, especially the leaves, suggested that they are toxic and hence, not suitable for human consumption.

CONCLUSION

The extensive use of *Stachytarpheta cayennensis* and *S. indica* in ethnomedicine for food and treatment of various ailments, in almost all areas of the world, is presumably as a result of their rich bioactive and nutrient constituents. However, high level

of hydrogen cyanide content in them poses a potential health risk to consumers. This work, thus, raised awareness that there is high lethal level of hydrogen cyanide in leaves and stems of *Stachytarpheta cayennensis* and *S. indica*; as a result, utilization of these plants in ethnomedicine as food and drug is highly discouraged. Nonetheless, they could be used in food supplement and drug formulations but isolation of hydrogen cyanide from the extracts, is of paramount health importance.

In addition, final conclusion cannot be drawn from this study alone; hence, there is need for further research on parts of these species of *Stachytarpheta* from other regions, in order to ascertain whether the high hydrogen cyanide concentration was as a result of soil composition of the areas from where they were collected. In vivo toxicity assay is also recommended, in order to determine the blood hydrogen cyanide level, when these plants are ingested.

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