Communication in Physical Sciences 2020, 5(1): 46- 50

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Comparative Studies of the Secondary Metabolites in the root and leaf of *Starchytarpheta cayennennsis* (*L*) *Vahl*

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Received 04 March 2020/Accepted 02 April 2020/Published online: 04 April 2020

Abstract *Secondary metabolites are useful components of plants and they can be distributed differently in different parts of the plant. This study seeks to investigate and compare concentrations of secondary metabolites in the root and leaf of Starchytarpheta cayennennsis (L) Vahl. Samples of the roots and leaf of Starchytarpheta cayennennsis were screened and analyses for secondary metabolites. The results obtained indicated concentrations of alkaloids, saponins, flavonoids, tannins and cardiac glycocides in the root to be 12.631 ± 0.412, 18.141 ± 0.15, 7.589 ± 0.033, 23.110 ± 0.240 and 1.958 ± 0.195 mg/100g respectively. In the leaf of the plant, the corresponding concentrations were 2.75 ± 0.354, 15.25 ± 0.186;1.75 ± 0.354. 3.077 ± 0.186 and 0.001 ± 0.000 mg/100g respectively Concentrations of the secondary metabolites were higher in the root than in the leaf. Subjection of the data to t-test revealed that there is a significant difference (P>0.05) between the concentration of phytochemicals in root of Starchytarpheta cayennennsis than in the leaf.*

Key words: *Starchytarpheta cayennennsis*, secondary metabolites, root and leaf.

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1.0 Introduction

Medicinal plants are of great benefits to human race and the medicinal values of these plants lies in some chemical constituents that produces a definite physiological action on human system. They are natural products which sometimes have pharmacological or biological activity that can be of therapeutic benefit in treating disease (Bello *et al*., 2008). In view of their importance, most medicinal plants are the active components of traditional and modern medicines Medicinal application of medicinal plants is based on the concentrations of primary and secondary metabolites in such plants. Primary metabolites are present in every living cell capable of dividing and are essential to both the plant and human's survival. Secondary metabolites are produced from primary metabolites and are not component of molecular skeleton of the organism, indicating that they do not immediately restrict the survival of an organism but to a good extent, they can weaken some biochemical activities. (Agoha, 2000) Many secondary metabolites are cytotoxic and have been selected and optimized through evolution for use as chemical warfare against prey, predators and competing organisms which is used as traditional treatment (Hill, 2002).

Starchytarpheta cayennesis is an herb commonly found in Nigeria as a weed along some roads, anthropogenic sites, and in fields where crops are cultivated. *S. cayennesis* is known to possess pesticidal activity, it is used locally as a mosquito and insect repellant. It has been used by many localities as remedies to

many ailments such as the treatments of dysentery, gonorrhea, ulcer, eye problem, children ear etc. (Akobundu and Agyakwa, 2002).

Microbial and fungal activities have led to several infection in our environment and account for high proportion of health complications that affect the human population throughout developing countries. It has been reported that the main reason that explains this worsening situation is because of antibiotic resistance of micro-organisms.

According to Pulera and Shazura (2010), micro-organisms have gained and developed their resistance against antibiotics through genetic alteration between themselves and other organisms. However, due to this, immense therapy problems in the treatment of infectious diseases have risen indicating that there is need for improved medical consideration, which may be contributed by herbal medicine through the use of medical plant such as *Starchytarpheta cayennennsis*. (Pulera and Arius Shazura 2010). Scanty studies have been conducted on composition of *Stachytarpheta cayannensis* plant (including leaf, stem and leaf). In the different studies, medicinal roles of this plant is established through preliminary phytochemical screening for identification and not quantitative studies. For example, Ezeabara *et al*. (2015a), carried out comparative studies of phytochemicals, proximate and mineral composition of *Stachytarpheta cayannensis* plants and were able to identified the phytochemicals in the plant without recourse to their concentrations. Iwu *et al*. (2013.) also identified alkaloids, saponins, tannins, cardiac glycosides and flavonoid in samples of some *Stachytarpheta cayannensis* plants. It should be stated that it is not only the presence of phytochemicals that is most paramount in modern medicine but the concentration is also required in order to utilized the extract effectively. Therefore, the present study is aimed at carrying out phytochemical analysis of the root and leaf of *Stachytarpheta cayannensis* plant and to compared the results obtained for each using test statistics.

2.0 Materials and Methods

2.1 Samples preparation

Samples of *Starchytarpheta cayenennis* root and leaf were collected locally from the school botanical farm. The roots and leaves were washed (separately), sliced to pieces and taken to the laboratory for determination of moisture content before drying to constant weight. The dried roots and leaves were grounded into a powder form and each of the samples were digested and stored in different beakers prior to chemical analysis (A.O.A.C. 1990).

2.2 Determination of saponins

Saponins content of the sample were determined by the double solvent extraction gravimetric methods. 2 g of the extract was mixed with 50 ml of aqueous solvent (20% ethanol). The mixture was incubated with periodic agitation in a water bath at 55 ^{o}C for 3 hours and filtered. Both extracts were pooled together and the combine extracts was enriched by evaporating to about 40 ml and then transferred to a separating funnel, Equal volume of diethyl either was added and shaken vigorously. Separation was done by partitioning during which the non aqueous layer was discarded leaving behind the aqueous layer. Re-extraction by partition was done repeatedly until the aqueous layer became clear in colour. The pH was adjusted to 4.5 using dilute sodium hydroxide and the saponin was finally extracted with successive portion of 60 ml and 10 ml of normal butanol. The combine extract was washed with 5% sodium chloride and evaporated to dryness in a pre-weighed beaker (W_1) in an oven at 60 ^{o}C and reweighed (W_2). Saponin content was calculated using the following formula,

$$Saponin = \frac{W_2 - W_1}{Total\ weight\ of\ sample} \times \frac{100}{1} \quad (1)$$

where W_1 is the weight of the dried beaker, W_2 is the weight of the dried beaker with sample

2.3 Determination of flavonoid

Flavonoid content in the extracts was determined spectrophotometrically. 0.05 mg/L of Rutin stock solution was prepared in methanol. Serial dilutions were done to obtain 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.78 mg/L concentrations respectively. 1 mL of 2% $AlCl_3$ in methanol was separately added to methanol solution of the extract (1 mL of 0.05 mg/mL) and 1 ml of the stock solution. The mixture was incubated at room temperature for 1 hour. The absorbance was measured at 415 nm, using UV/VIS spectrometer. The sample, blank (methanol) and standards were prepared in triplicate for each analysis and the mean value of absorbance obtained and expressed in terms of rutin equivalents (mg of RU/g of extract). Using the calculated formular below:

$$\%flavanoid = \frac{Weight\ of\ the\ extracted\ flavanoid}{Weight\ of\ dsmple} \times \frac{100}{1} \quad (2)$$

2.4 Determination of alkaloid

5 g of sample was weighted into 2 ml beaker and dispersed into a beaker of 10 % acetic acid solution in ethanol. The mixture was shaken and allowed to stand for 4 hours. This was filtered and the filtrate was concentrated to one-quarter of its original volume using a water bath. Concentrated ammonia hydroxide (NH_4OH) was added drop-wise to precipitate the alkaloid. The precipitate was filtered washed with 1 % NH_2O_4 solution and dried at 60^0 c then reweight.

2.5 Determine of cardiac glycoside

1 g of grinned sample was weighted into a conical flask and 50 ml of distilled water was added to the extract, then the extract was allowed to stand overnight. The solution obtained was filtrated and 1 m of the filtrated was added to a test tube containing 4 ml of alkaline pricrate, 5 g of sodium carbonate 1 g of pricrate acid and 100 ml of distilled water were also added and the resulting system was allowed to stand in a water bath for 5 minutes. Absorbance was read at 490 nm using a spectrophotometer (Sofowora , 1980).

2.6 Determination of tannins

Concentration of tannin was estimated using Folis-Denis Colorimetric method. 2 g of sample was dispersed in 50 ml of distilled water and shaken. The mixture was allowed to stand for 30 minutes at 28 ^oC and filtered through whatman No.42 grade of filter paper. 2 ml of the extract was dispersed into 50 ml volumetric flask. Also, 2 ml of standard tannins solution (tannic acid) and 2 ml of distilled water were added to a separate volumetric flask to serve as a standard while the reagent was also added to each of the flask. 2.5 ml of the standard sodium carbonate solution was also added. The content of each flask was made up to 50 ml with distilled water and allow to stand at 20^oC for 90 minutes. Their respective absorbance was measured in spectrophotometer at 560 nm.

3. Results and discussion

Table 1 presents concentrations of alkaloids, saponins, flavonoids, tannins and cardiac glycosides in root and leaf of *Starchytarpheta cayennensis* plant.

Table 1 Phytochemical constituents of the roots and leaves of *Starchytarpheta cayennensis* .

Phytochemicals	Concentration (%)	
	Root	**Leaves**
Alkaloids	12.631 ± 0.412	2.75 ± 0.354
Saponins	18.141 ± 0.015	15.25 ± 0.186
Flavonoids	7.580 ± 0.033	1.75 ± 0.354
Tannins	23.110 ± 0.240	3.077 ± 0.186
Cardiac glycosides	1.958 ± 0.195	0.01 ± 0.000

****Mean ± SD of three replicates**

Concentrations of alkaloids, saponins, flavonoids, tannins and cardiac glycosides (%) in the root and leaves of *Starchytarpheta cayennensis* are presented in Table 1. The results revealed that mean concentrations of alkaloids in the root of *Starchytarpheta cayennensis* (12.631 ± 0.412 %) was higher than the concentration in the leaves (2.75 ± 0.354 %). Similar differences were observed for the concentrations of saponins, flavonoids, tannins and cardiac glycosides (18.141 ± 0.015, 7.580 ± 0.033, 23.110 ± 0.240 and 1.958 ± 0.195 %) in the root, which were higher than their corresponding concentrations in the leaves (15.25 ± 0.186, 1.75 ± 0.354, 3.077 ± 0.186 and 0.001 ± 0.000 %). However, as shown in Table 1, although the concentrations of the studied phytochemicals were higher in the roots of *Starchytarpheta cayennensis* than in the leaves, the variability in concentrations (measured by standard deviation) was higher in the leaves than in the root. Such differences are not uncommon. For example, Senguttuvan *et al.* (2014) studied the levels of phytochemicals in *Hypochaeris radicata L* and found that concentrations in the roots were significantly higher than concentrations of phytochemicals in the leaves of the same plant. Ezeabara *et al* (2015b), also reported alkaloid content of *Starchytarpheta cayennensis* root as high as 3.46 ± 0.17 % and remarked lower concentration in the root. Sai Prasanna *et al.* (2015) reported significant and higher concentrations of phytochemicals in *Micrococca mercurialis (L) Benth* stem and root than in the leaf but did not provide reason for the difference. It is however believe that the leaves are the major photosynthetic part of plant and the process of photosynthesis starts from the leaves, indicating that it is the centre for numerous biochemical reactions and the primary source of primary metabolites. Once synthesized

in the leaves, they will be transported to the root and stem where they can be stored in the plant.

In order to compare the results obtained for phytochemical contents of the root and leaf of Starchytarpheta *cayennesis*m, a t-test statistics was used. This can be expressed as,

$$t_{exp} = \frac{\overline{X_A} - \overline{X_B}}{S_{AB}\sqrt{\frac{1}{n_A} + \frac{1}{n_B}}} \quad (3)$$

where $\overline{X_A}$ *and* $\overline{X_B}$ are mean of sample set A and B having n_A and n_B data set respectively, S_{AB} is the pooled standard deviation expressed as,

$$S_{AB} = \sqrt{\frac{(n_A-1)S_A^2 + (n_B-1)S_B^2}{n_A + n_B - 2}} \quad (4)$$

Calculated S_{AB} and t_{exp} values for the estimated phytochemicals are presented in Table 2.

Table 2: Experimental t values for estimated phytochemicals in root and leaf of *Starchytarpheta cayennesis*m plant

Phytochemicals	$\overline{X_A} - \overline{X_B}$	t_{exp}
Alkaloids	9.881	48.34572
Saponins	2.891	26.92127
Flavonoids	5.839	28.56904
Tannins	20.033	186.5492
Cardiac glycosides	1.957	33.89622

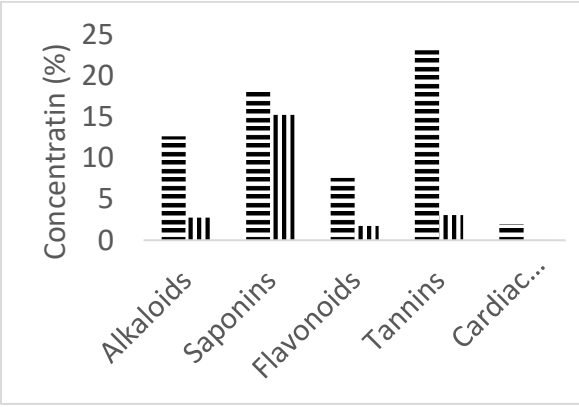

Fig 1: Bar chart showing concentrations of phytochemicals in leaf and root of *Starchytarpheta cayennesis*m plant

(**Root is represented by horizontal draft and leaf, vertical draft)

The t-values obtained are greater than the theoretical t value, hence there is a significant difference between concentration of alkaloids, saponins, flavonoids, tannins and cardiac glycosides in the root and stem of *Starchytarpheta cayennesis*m plant. This information is also depicted in the bar chart (Fig. 1) which indicates taller peaks for phytochemicals in root than those in leaf.

4.0 Conclusion

The study reveals that root and leave of *Starchytarpheta cayennesis*m plant are rich in tannin, saponin, cardiac glycoside, alkaloid and flavonoid. However Tannin is most abundant in the root while saponin is the most abundant secondary metabolite in the leaf. Cardiac glycoside had the least concentration in both the root and leaf of the plant . Generally, concentrations of tannin, alkaloid, cardiac glycoside, saponin and flavonoid in the root were significantly higher than their respective concentration in the leaf.

5.0 Acknowledgement

The authors are grateful to the almighty God for his grace. We acknowledge with thanks Tertiary Education Trust Fund (TETFUND) for sponsoring the research and all technical staff in the Chemistry unit of Science Technology department in Akwa Ibom State Polytechnic.

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