Evaluation of various parts of *Stachytarpheta angustifolia* (Mill.) Vahl for phytochemical, proximate, mineral and vitamin constituents

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ABSTRACT

Various parts of *Stachytarpheta angustifolia* (Mill.) Vahl were analyzed for phytochemical, proximate, mineral and vitamin compositions; in order to ascertain its bioactive and nutrient components, including potentially hazardous compounds when used in ethnobotany as food and drug. The plant was collected in the Western Experimental Field Umudike, Abia State, Nigeria, in May 2014, and oven-dried at 65 °C for 48 hours. Standard methods of analyses were employed. Quantitative statistical methods were used for data analyses and the concentrations were considered significantly different (p < 0.05). Levels of alkaloid, flavonoid, phenol, saponin and tannin were highest in the leaf; with flavonoid (3.11 ± 0.04 %) having the highest value. Highest concentrations of carbohydrate (45.41±0.08 %), crude protein (12.31±0.20 %), fat (4.22±0.21 %) and moisture (11.12±0.42 %) were also detected in the leaf. In addition, greatest levels of the vitamins were found in the leaf and vitamin C (92.11±0.96 mg/100g) had the highest concentration. The highest value of hydrogen cyanide, a toxin which was detected in all the plant parts was greatest in the leaf (6.36±0.05 mg/kg). Greatest values of the minerals were found in the root. Oxalate and phytyate which are anti-nutrients were also present in all the plant parts. The plant, therefore, exhibited a high potential for food and drug. However, when ingested raw, it may have serious health consequences due to high value of hydrogen cyanide.


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1. INTRODUCTION

*Stachytarpheta angustifolia* (Mill.) Vahl belongs to the genus *Stachytarpheta* of the family Verbenaceae. This genus is represented by three species in Nigeria and in West Africa as a whole, by *Stachytarpheta angustifolia* (Mill.) Vahl, *S. cayannensis* (Rich.) Vahl and *S. indica* (Linn.) Vahl [1]. Members of this genus are herbs, shrubs or vines and sometimes trees with leaves usually opposite or whorled, simple or palmately compound or exstipulate. *Stachytarpheta angustifolia*, commonly known as devil’s coach is a predominating tropical plant exhibiting a wide range of growth habit and inhabiting diverse habits [2].

A lot of ethnomedical usefulness of *S. angustifolia* has been reported, indicating its popularity in traditional medicine. It has been used as anti-diabetic, anti-asthmatic, abortifacient, emmenagogue, sedative, anti-hypertensive and anti-fever [3]. In addition, the decoction of the whole shrub mixed with natron is taken as remedy for dysentery and also for similar condition for horse [4].

In previous study; high concentration of hydrogen cyanide was observed in various parts of *S. cayannensis* and *S. indica* [5]. This necessitated the investigation of the chemical compositions of different parts of *S. angustifolia*, a complement of the genus in Nigeria and West Africa. The objective of this study, therefore, was to examine the phytochemical, proximate, mineral and vitamin constituents of different parts of this plant, with a view to determining its chemical components. The information could prevent possible human health risk when *S.
2. MATERIALS AND METHODS

2.1 Sources of plant materials

The test plant *S. angustifolia* with collection number ACE-52; was collected from the Western Experimental Field Umudike, Abia State, Nigeria, in May 2014. Voucher specimens were authenticated by Mrs C.A. Ezeabara and deposited in the herbarium of Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

2.1.2 Preparation of plant materials

The test plant after due identification was carefully uprooted in a manner that ensured that the roots did not cut much. The uprooted plants were first washed in running water (tap) to remove soil on the roots as well as dirt and dust on the entire plant. The stem and root were cut into small bits with the aid of a kitchen knife, spread in a separate laboratory trays, and dried in an oven at 65 °C for 48 hours. They were separately ground in a laboratory mill (Model 7044, London) in which they were sieved into small bits with the aid of a kitchen knife, to obtain the firmly ground processed samples used in the analytical studies.

2.3 Quantitative phytochemical determinations

Oxalate and phytate contents were detected by method outlined by Onwuka [6]. Tannin content of each sample was determined by Folin-Denis Colorimetric method [7]. Flavonoid determination was done by the Hydrolysis Gravimetric method of Harborne [8]. Phenol content was determined by the Folin–Denis Spectrophotometric method [9]. Saponin content determination was done by the Double Solvent Extracting Gravimetric method and alkaloid content was determined by the Alkaline Precipitation Gravimetric method [10]. Hydrogen cyanide was determined by Alkaline Picrate Colorimetric method of Trease and Evans [11].

2.4 Proximate determinations

Ash content was determined with the Furnace incineration gravimetric method [12]. The crude protein content of the samples was determined by Kjeldahl method; carbohydrate content was determined by estimation using the Difference method; crude fibre was determined by the Weende method; determination of calcium and magnesium was done by Versenate Complexometric Titration method; phosphorus by the Vanado–Molybdate colorimetric method; sodium and potassium by Flame Photometry; moisture and fat by methods described by James [13]. Determination of vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), and vitamin C (ascorbic acid) contents were done by the methods outlined by Kirk and Sawyer [7].

2.5 Statistical analyses

All data were checked for normality (Kolmogorov–Smirnov Test) and tested for homogeneity (Leven Median Test) before they were analyzed using F-Test at confidence limit of 95% (p<0.05). Least Significant Difference (LSD) was used to compare every possible pair of treatment means and data were presented as mean ± standard deviation of triplicate determinations.

3. RESULTS

The results obtained from the analyses of the different parts of *S. angustifolia* revealed that the plant synthesized and accumulated a wide range of phytochemicals including alkaloids, phenol, flavonoids, saponins, tannins, and glycosides. Alkaloid, flavonoid, saponin and tannin contents were highest in the leaf and least in the root. Highest percentage of phenol was detected in the leaf (0.59±0.00 %) and root (0.51±0.00 %). Greatest values of phytate and oxalate were found in the leaf; meanwhile there was no significant difference in the values present in the stem and root. Hydrogen cyanide was detected in the leaf (6.36±0.05 mg/kg), stem (3.52±0.03 mg/kg) and root (3.71±0.71 mg/kg) (Table 1).

Carbohydrate, crude protein, fat and moisture contents were highest in the leaf and least in the root. There was no significant difference between the moisture content of the stem and root. Highest value of crude fibre was found in the root (50.69±1.16 %) and least in the leaf (21.93±0.15 %). The ash content of the root (5.95±0.09 %) was the highest (Table 2).

Highest values of calcium, magnesium, phosphorus, potassium and sodium were present in the root, whereas the least values were present in the stem (Table 3). Levels of vitamins B1 and C were highest in the leaf and least in the root. Highest vitamin B2 content was found in the leaf (0.051±0.06 mg/100g), whereas the least was present in the stem (0.026±0.06 mg/100g) and root (0.022±0.02 mg/100g); and there was no significant difference between them. Vitamin B3 value was greatest in the leaf (0.340±0.03 mg/100g) and least in the stem (0.086±0.01 mg/100g) (Table 4).
Saponins are antibacterial action of saponins. Sand jaundice [15, 16]. This is probably due to the whole plant is taken as a remedy for gonorrhea on animals [14]. Saponin content was highest in the root (0.93 %). The infusion of the plant at a concentration range of 0.27 % (leaf) to 0.42 % in the root. Plant leaves with high tannin content have been used successfully as hops alternative in beer foam forming in nature and have been implicated as a bioactive antibacterial agent of plant [17]. Saponins are also potential, sometimes for utilization in foods that need sustained foam volume such as ice-creams. The phenol content was between 0.35 % and 0.42 % in the stem and 0.51 % in the leaf. Flavonoids have been shown to be highly effective scavengers of most oxidizing agents. Another phytochemical of health benefits found in S. angustifolia was flavonoids. The concentration of flavonoids was highest in the leaf (3.11 %) and least in the root (0.93 %). Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules [19]. In addition, tannin was found in the plant at a concentration range of 0.27 % (root) to 0.42 % (leaf). Plant leaves with high tannin content have been used successfully as hops alternative in beer.

### Table 1. Phytochemical compositions (%) of different parts of *Stachytarpheta angustifolia*.

<table>
<thead>
<tr>
<th>Compositions</th>
<th>Leaf</th>
<th>Stem</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>2.79±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.15±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80±0.18&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>3.11±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.89±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.93±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.59±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.51±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Saponin</td>
<td>0.87±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.35±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.29±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tannin</td>
<td>0.42±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.27±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phytate</td>
<td>0.36±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.28±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oxalate</td>
<td>0.37±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HCN (Mg/kg)</td>
<td>6.36±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.52±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.71±0.71&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

HCN=Hydrogen cyanide. Values are means of triplicate determination ± standard deviation. Figures with different superscripts along the row are significantly different at (p<0.05).

### Table 2. Proximate compositions (%) of different parts of *Stachytarpheta angustifolia*.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Leaf</th>
<th>Stem</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>45.41±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.75±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.93±1.35&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude protein</td>
<td>12.31±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.14±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.97±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>4.22±0.21&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.41±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.74±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>21.93±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.53±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.69±1.16&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>5.05±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.35±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.95±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture content</td>
<td>11.12±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.65±0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.71±0.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means of triplicate determination ± standard deviation. Figures with different superscripts along the row are significantly different at (p<0.05).

### Table 3. Mineral contents mg/100g of different parts of *Stachytarpheta angustifolia*.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Leaf</th>
<th>Stem</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>122.9±2.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.9±12.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>154.9±2.32&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Magnesium</td>
<td>144.8±1.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.53±1.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>162.4±1.39&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>244.4±3.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>201.8±0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>312.1±0.46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Potassium</td>
<td>130.3±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.0±0.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>143.7±0.61&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sodium</td>
<td>137.1±1.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.0±0.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>153.7±0.46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are means of triplicate analyses ± standard deviation. Figures with different superscripts along the row are significantly different at (p<0.05).

### Table 4. Vitamin contents (mg/100g) of *Stachytarpheta angustifolia*.

<table>
<thead>
<tr>
<th>Compositions</th>
<th>Leaf</th>
<th>Stem</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.121±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.100±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.053±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.051±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.026±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.022±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.340±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.086±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.118±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>92.11±0.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.85±1.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>21.71±1.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means of triplicate determination ± standard deviation. Figures with different superscripts along the same row are significantly different at (p<0.05).

### 4. DISCUSSION

The chemical constituents of plants are shown through phytochemical determination; and this lead to the revelation of presence and concentration of active biomolecules. The alkaloid content of *Stachytarpheta angustifolia* varied from 0.80±0.18 % in the root to 2.79±0.31 % in the leaf. Alkaloids are vast and vary a lot in their activity when ingested by man and livestock. Some alkaloids are useful and important in medicine and constitute most of the valuable drugs currently used by humans. They are reported to have marked physiological effect on animals [14]. Saponin content was highest in the leaf (0.87±0.01 %). The infusion of the whole plant is taken as a remedy for gonorrhea and jaundice [15, 16]. This is probably due to antibacterial action of saponins. Saponins are...
production as well as in the textile industry. Phytates and oxalates were also found in the plant with concentration range of 0.26±0.00 % (stem) to 0.36±0.00 % (leaf) and 0.19±0.01 % (stem) to 0.37±0.01 % (leaf), respectively. Both phytochemicals are considered detrimental in the nutrition industry as they bind with mineral thus interfering with their absorption during digestion of foods [6]. They can therefore, give the same effect as caloric restriction by inhibiting uptake of protein, vitamins and minerals. It is known that lower protein intake and caloric restriction, protects against many chronic diseases and delays the process of aging [20]. However, oxalates and phytates can be reduced considerably even normally eliminated by heat treatment involved in food processing.

Hydrogen cyanide, a glycoside product of hydrolysis, also a known toxic substance was detected. Its content in the plant ranged from 3.52±0.03 mg/kg to 6.35±0.05 mg/kg. The greatest level was found in the leaves and the least in the stem. Highest concentrations of hydrogen cyanide were also detected in the leaf of S. cayennensis (5.64±0.104 ml/kg) and S. indica (6.93±0.017 ml/kg) [5]; and the values present in the stem of the three species fell within the same range, (Stachytarpheta angustifolia, 3.52±0.03 mg/kg), S. cayennensis (3.12±0.036 ml/kg) and S. indica (3.18±0.006 ml/kg). This indicated that the leaf of these species of Stachytarpheta accumulate high level of hydrogen cyanide more than the stem and root; and this is probably as a result of need to protect the aerial parts of the plants against herbivores. Plants use cyanide as a poison to deter predators and the concentration differs in different plants, as well as plant parts. Moreover, the toxicity effect of hydrogen cyanide is dose, body weight and duration dependent. Several workers have reported both external and internal fatal doses of hydrogen cyanide. Dermal exposure lethal dose was reported to be 100 mg/kg of body weight [21, 22]; and acute oral lethal dose, 0.5 - 3.5 mg/kg [5, 23]. Therefore, the levels of hydrogen cyanide detected in parts of this plant were considered toxic, for they fell above safe limit. It has been reported that the toxic effects of cyanide ion in humans and animals are generally similar and are believed to result from inactivation of cytochrome c oxidase and inhibition of cellular respiration and consequent histotoxic anoxia [24]. The primary targets of cyanide toxicity in humans are the cardiovascular, respiratory, and central nervous systems. However, extensive processing could remove the concentration of hydrogen cyanide from food.

Moreover, a quite number of nutrients were detected in parts of this plant. The crude protein contents were 3.97±0.10 % in the root; 5.14±0.1 % and 12.31±0.20 % values in the stem and leaf, respectively. Vegetables are good sources of protein which are generally responsible for cell repairs and regeneration. The plant also had low fat content which ranged from 1.71±0.12 % in the root to 4.22±0.21 % in the leaf. Fats are energy sources which maintain good body temperature. Fibre was found to be generally high in the plant. The leaf contained 21.93±0.15 % crude fibre which was the least concentration, while the stem and root contained 48.53±0.12 % and 50.69±1.16 %, respectively. The higher fibre content of the stem and root was attributed to the presence of structures containing hemicelluloses and cellulose which are hard fibres. Dietary fibre forms a vital part of the diet by adding mass to the stool, which eases elimination [25]. It also helps in weight control and reduces the risk of developing obesity and cardiovascular diseases.

There was significant variation in mineral contents of the different parts of S. angustifolia. In addition, the highest values of all the minerals present were detected in the root. This contributed to the high ash content of the root (5.95±0.09%), which represented the whole mineral in a food. The ash content was 5.95±0.09 % in the root which was the highest level, while the least was detected in the stem (4.35±0.03 %). The root of the plant takes up nutrients from the soil including minerals. This was considered the possible reason for the high ash content of the root, relative to the other parts of the plant. In addition, calcium content was highest in the root and the least in the leaf. Calcium diet enhances good bone and teeth formation in growing children. The leaf contained magnesium (144.8±1.39 mg/100g) and phosphorus (244.4±3.39 mg/100g). Potassium content was also high with a range of 106.0±0.40 mg/100g to 143.7±0.61 mg/100g with the highest concentration found in the root. The same trend was seen in the sodium content where the least concentration of 96.0±0.69 mg/100g was obtained in the stem, while the highest concentration of sodium was in the root (153.7±0.40 mg/100g). Sodium, potassium and magnésium are important electrolytes which work to maintain a good balance in the concentration of body fluids and regulate body physiology through osmotic processes. Phosphorus is involved in energy transfer and also works as co-enzyme in many physiological reactions in the body [3].

Furthermore, variations of significant difference were recorded in the vitamin content of parts of S. angustifolia. Greatest levels of all the vitamins were found in the leaf, with vitamin C having the highest concentration. Vitamin B1 was of the range, 0.053±0.02 mg/100g in the root, to 0.121±0.03 mg/100g in the leaf. Vitamin B2 values were 0.051±0.06 mg/100g in the leaf and least (0.022±0.02 mg/100g) in the root, while vitamin B3 content was 0.340±0.03 mg/100g in the leaf. Vitamins A and E were not detected in the plant, but may be presented in minute quantities. Generally, vitamins are important in diets, because of the various roles they play and their high health benefits. Niacin, riboflavin and thiamine work as co-enzymes, as well as boost body immunity; while vitamin C is
strong antioxidant and protects the body from all damage by oxidative reactions [17]. It also helps in wound healing and reduction of allergies. The leaf of this plant contained relatively high level of these vitamins, and hence represents a potential good source for extraction and manufacture of dietary supplements.

5. CONCLUSION ND RECOMMENDATION

Based on the findings of this study, *S. angustifolia* synthesized and accumulated many different phytochemicals, which were distributed at varied concentrations in the parts; with the greatest levels detected in the leaf. In addition, some of the phytochemicals have health benefits, while some are known to be toxic. The nutrient status of the leaf was found to be moderate with high protein, ash and crude fibre levels, but low fat content. Minerals and vitamins were also found at various concentrations in the different plant parts. Therefore, the plant has high potential ethnomedical usefulness. However, utilization of raw parts of *S. angustifolia* in ethnombotany as food and drug may not be safe, due to toxic effects of high concentration of hydrogen cyanide; hence, in vitro and in vivo demonstrations of blood hydrogen cyanide level, after administration of these raw parts is highly recommended.

AUTHOR CONTRIBUTIONS

CAE designed the study and wrote the first draft of the manuscript. CAE authenticated the voucher specimens, and conducted the phytochemical and proximate evaluations, whereas CME performed the mineral and vitamin determinations. Both authors managed the literature searches and statistical analyses; read and approved the final manuscript.

REFERENCES