Comparative studies on nutrient and antinutrient composition of *Eremomastax polysperma* (Benth.) Dandy varieties in Akwa Ibom State, Nigeria

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Accepted 07 August, 2014

Abstract

The varieties of *Eremomastax polysperma* investigated for the presence of nutrient and anti-nutrient. Standard analytical methods were used for these analyses. The varieties possessed high moisture content with *Eremomastax polysperma* purple leaf bark (EP) (81.90±3.28 %) being higher than the 79.13±1.57 % contained in the EG variety. There is a similar trend in the various aspects of the proximate composition of the plant, except the ash and fibre contents which were significantly different at p < 0.05 confidence level. Generally, the plants are rich in all the classes of nutrient. The presence of phytochemicals in these plant species suggests a possibility of medicinal usage of these plants. Tannins are present in the range of 52.23 ± 1.43 – 62.33 ± 2.75 mg/100g with EP exhibiting a higher level than the *Eremomastax polysperma* green leaf bark (EG) variety, there is a high level of phenols in both varieties with EG having a higher level than the EP sample. Alkaloids and saponins are also present in the ranges of 2.94±0.05 – 3.65±0.05 and 4.19±0.16 – 4.28±0.02 respectively. EP has a higher level of alkaloid while EG had higher levels of saponins. There were significant differences in zinc (Zn), magnesium (Mg) and calcium (Ca) contents of the two varieties. These sources of local plants could be a good source of trace elements essential to man. Despite the presence of useful nutrients in the plant varieties, there is likely to be safety concerns on the consumption of these botanicals due to the presence of antinutrients such as oxalate, thiocyanate and phytate.

Keywords: Nutrient, Antinutrient, Phytochemicals, Safety, Consumption

INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.*, 1999; Okpuzor *et al.*, 2008) opined that the practice of traditional medicine using medicinal plants is as old as the origin of man, Evans, (2002) described such a practice as herbalism or botanical medicine. Medicinal plants are a source of great economic value all over the world (Joshi *et al.*, 2011). Nature has endowed us with a very rich botanical wealth and a large number of diverse types of plants grow in different parts of Nigeria. The country is very rich in all three levels of biodiversity namely; species diversity, genetic diversity and habitat diversity. A vast array of species is known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times.
Herbal medicine is still the mainstay of about 75-80% of the whole population, and a major part of traditional therapy involves the use of plant extract and their active constituents (Akerere, 1993). A review, by Elujoba, et al., (2005) reiterated that the use of traditional medicine cannot fade out in the treatment and management of an array of diseases in the African continent. This was attributed to our socio-cultural, socio-economic heritage, lack of basic health care and personnel to take charge of every nook and cranny of rural communities.

The bioactive ingredients that have the therapeutic activity in plants used in traditional practice are mostly unidentified and traditional healers believe in the holistic nature of their treatment. Active components differ from one plant species to another, examples of active components present in plants include; anthraquinones, flavonoids, glycosides, saponins, tannins, alkaloids. Plant also contain other compounds such as morphine, atropine, codeine, steroids, lactones and volatile oils, which possess medical values for treatment of different diseases (Chevalier, 2000). Since most plants have medicinal properties, it is of utmost importance that their efficacy and toxicity risk are evaluated.

There is a paucity of information on the plant materials selected for this study. However, literature on their taxonomies has it that *Eremomastax polysperma* is a stout erect much branched herb of the forest zone; it is a weed on cacao farms, dispersed from Guinea to Western Cameroons and widespread in tropical Africa (Heine, 1966). *Eremomastax polysperma* belongs to the Plantae family of Acanthaceae. In Nigeria, the plant is commonly known as a blood tonic while the Akwa Ibom people of Nigeria identified *Eremomastax polysperma* as Edem Iduduo meaning purple bark. They are of two species, one with purple coloured bark and the other green one.

Generally, the plant is used as an analgesic, as well as for the treatment of cutaneous and subcutaneous parasitic infections (venomous stings and bites). The plant has been used in Ghana by the Akuapem people as a fish poison while the Asanti/Fante and Twi people of Ghana used the botanical in soap making for bathing. This is perhaps due to the presence of saponins in the plant. The plant is cultivated in many home gardens in Akwa Ibom state because of its medicinal properties. Propagation is by cutting the stem which is feasible while stump regrowth is natural. Indeed, the plant is an invasive species after a mere few years in an area.

**Sample collection**

Fresh leaves of *Eremomastax polysperma* (purple and green bark), were collected in the wild in Uyo and Ibibio Local Government Areas with the aid of a herbalist. The samples were taken to the University of Uyo and identified by the taxonomist in the University Herbarium, located in the Botany and Ecological Studies department.

**Proximate analysis**

The proximate composition of the leaves was determined using standard methods of AOAC (1990) while carbohydrate was calculated by difference (Onyeka et al., 2010; James, 1995).

**Phytochemical analyses**

Quantitative analyses were carried out to ascertain the presence of the different phytochemical and antinutrient in the leaves.

**Estimation of Alkaloids**

Ten grams of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10. The mixture was allowed to stand for 4h at 28ºC. It was later filtered using whatman No 42 grade filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with dropwise addition of conc. Aqueous NH₄OH until the alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1% NH₄ solution and dried in the oven at 80ºC. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

\[
\% \text{ Alkaloid} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100
\]

**Estimation of Tannin**

Five grams of sample was dispersed in 50ml of distilled water and shaken. The mixture was allowed to stand for 30min at 28ºC before it was filtered through Whatman No.42 grade of filter paper. Extract (2ml) was dispersed into a 50ml volumetric flask. Similarly, 2ml standard tannin solution and 2ml of distilled water were put in separate volumetric flasks to serve as standard and the reagent was added to each flask and the 2.5ml of standard Na₂CO₃ solution added. The content of each flask was made up to 50ml with distilled water and allowed to incubate at 20ºC for 90min. their respective absorbance was taken in a spectro- photometer at 260nm using the reagent blank to calibrate the instrument at zero.

**Estimation of saponins**

Fifty grams of the dried sample was introduced into a conical flask and 50cm³ of 20% aqueous ethanol added.
The sample was thereafter placed in the water bath and stirred continuously at a constant temperature of 55°C for 12hrs. The sample solution was filtered using Whatman No.42 filter paper and the residue was extracted again with another 100cm³ of 20% ethanol. The extracts were combined and concentrated to 20cm³ in a water bath. The concentrated solution was transferred into a 250ml separating funnel and 20ml of diethylether was added and shaken vigorously. The aqueous layer was kept while the ether layer was discarded. The purification process was repeated two more times; 2.0g of N₂Cl was added to adjust the PH of the remaining solution to 4.5. the solution was shaken with 30cm³ and 15cm³ portions of ethanol respectively. The combined ethanol extracts were washed twice with 10cm³ of aqueous N₂Cl. The remaining solution was evaporated to dryness in the water bath and dried to constant weight in the oven. The final weight of saporins extracted and expressed as a percentage.

\[
\% \text{saponins} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100
\]

**Estimation of flavonoids**

Five gram of sample was boiled in 50ml of 2MHCL 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 wise addition until finally all the volume. The flavonoid precipitated was recovered. The resulting weight difference gave the weight of flavonoid in the sample.

**Estimation of phenols**

Spectrophotometric method was used to determined the total phenols in the sample. The fat free sample was boiled with 50cm³ of ether for 15mins. The extract (5cm³) was pipette into 50cm³ flask and then 10cm³ of distilled water added.2cm³ of NH₄OH solution and 5cm³ of concentrated amyl alcohol were also added. The sample was left to react for 30mins for colour development. The absorbance of the solution was read using spectrophotometer at 560nm wavelengths.

**Estimation of phytates**

Sample was extracted with 100ml of 0.2HCL solution. This was done by shaking the mixture at room temperature for 30mins. Then 1ml portion of the supertant was treated with 1.5ml portion bipyridine solution. Standard phytic acid solution was prepared and 1ml of it was put in separate test tubes with 1ml of distilled water kept as reagent blank. Then the absorbance of each was measured at 519nm in a spectrophotometer with reagent blank.

**Estimation of cyanide**

One gram of the sample was dissolved in 50ml of distilled water in a corked flask and was allowed to stay overnight. The solution was filtered and the extract used for cyanide determination. One millimetre of the filtrate was corked in a test tube and 4ml of alkaline picrate was added and incubated in a water bath for 5mins after colour development. The absorbance was read in a spectrophotometer at 490nm with the blank.

**Estimation of Oxalate**

2.5g of sample was digested with 95:5% distilled water in 6NHCL. The digest was filtered and diluted to 125ml with distilled water. A duplicate of 50ml was taken into a beaker and then 4 drops of methyl red indicator added and was evaporated to 25ml and filtered to remove precipitate containing ferrous ion. Then the filtrate was treated with 5ml of conc NH₄OH and heated at 90°C and 10ml of 5% caclc solution was added while being stirred constantly. After heating, it was cooled and left overnight at 90°C. the solution was then centrifuged at 2500rpm for 5min. the supernatant was decanted and the precipitate obtained was washed into a beaker with 10ml of 20% (v/v) H₂SO₄ solution and total volume was diluted to 125ml with distilled water. Aliquots of 125ml of the solution was heated near 90°C and then titrated against 0.05N KMN₄ solution to a faint pink solution which persist for 10sec. The calcium oxalate concentration calculated. Note: 0.05NMnO₄ = 2.2mg oxalate.

**Estimation of elemental composition**

Standard methods of A.O. A. C, (1990) were used for the estimation of elemental composition of the plant samples.

**RESULTS AND DISCUSSION**

The result of the proximate analysis of the leaves of the two varieties of *Eremomastax polysperma* (green and purple bark) denoted by EG and EP respectively is presented in Table 1. From the result presented, it is evident that the both varieties were rich in nutrients. There is a similar trend in the various aspects of the proximate composition of the plant, except the ash and fibre contents which were significantly different at p<0.05 confidence level. Generally, the plants are rich in all the classes of nutrient.
Table 1. Proximate composition of EG and EP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EG</th>
<th>EP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>79.13 ± 1.57</td>
<td>81.90 ± 3.28</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>3.11 ± 0.78</td>
<td>2.10 ± 0.18</td>
</tr>
<tr>
<td>Fibre (%)</td>
<td>0.20 ± 0.03</td>
<td>1.32 ± 0.13</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.67 ± 0.70</td>
<td>3.95 ± 0.81</td>
</tr>
<tr>
<td>Lipid (%)</td>
<td>0.51 ± 0.10</td>
<td>0.63 ± 0.11</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>10.61 ± 0.67</td>
<td>12.80 ± 1.21</td>
</tr>
<tr>
<td>Caloric Value (Kcal)</td>
<td>61.68 ± 4.02</td>
<td>72.67 ± 4.02</td>
</tr>
</tbody>
</table>

Eremomastax polysperma (green and purple leaf bark) varieties denoted by EG and EP respectively. Values expressed as mean ± S.E.M, n=3, * Significant difference

Table 2. Result of quantitative phytochemical analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>EG (mg/100g)</th>
<th>EP (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannin</td>
<td>52.23±1.43</td>
<td>62.33±2.75</td>
</tr>
<tr>
<td>Phenol</td>
<td>297.40±5.71*</td>
<td>215.50±5.00*</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>3.05±0.10</td>
<td>3.37±0.12</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>3.65±0.05</td>
<td>2.94±0.05</td>
</tr>
<tr>
<td>Saponin</td>
<td>4.19±0.16</td>
<td>4.28±0.02</td>
</tr>
<tr>
<td>Oxalate</td>
<td>296.90±0.59*</td>
<td>726.00±1.60*</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>281.00±0.90*</td>
<td>426.00±2.00*</td>
</tr>
<tr>
<td>Phytate</td>
<td>11.27±0.10</td>
<td>9.14±0.02</td>
</tr>
</tbody>
</table>

Values expressed as mean ± S.E.M, n=3, * Significant difference

Table 3. Result of elemental analysis of EG and EP

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>EG (mg/Kg)</th>
<th>EP (mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>3.10±0.03</td>
<td>2.50±0.11</td>
</tr>
<tr>
<td>Mg</td>
<td>700.00±2.20*</td>
<td>511.31±0.31*</td>
</tr>
<tr>
<td>Se</td>
<td>0.62±0.08</td>
<td>0.98±0.08</td>
</tr>
<tr>
<td>Ca</td>
<td>531.80±31.2</td>
<td>413.70±10.2</td>
</tr>
<tr>
<td>Fe</td>
<td>2.59±0.06</td>
<td>2.48±0.07</td>
</tr>
</tbody>
</table>

Values expressed as mean ± S.E.M, n=3, * Significant difference

The results of the phytochemical screening of the extracts of the two varieties of *Eremomastax polysperma* are presented in table 2 and 3 above. Phlobatannins and cardiac glycosides were absent in the plant species as indicated by a negative sign (-), while the positive sign (+) showed the presence of the phytochemical. Further analyses quantified the different phytochemicals and anti-nutrient as presented in table 4. Tannins are present in the range of 52.23 ± 1.43 – 62.33 ± 2.75 mg/100g with EP exhibiting a higher level than the EG variety, there is a high level of phenols in both varieties with EG having a higher level than the EP sample. Alkaloids and saponins are also present in the ranges of 2.94±0.05 – 3.65±0.05 and 4.19±0.16 – 4.28±0.02 respectively. EP has a higher level of alkaloid. On the other hand, EG has a higher level of saponins. There were significant differences in zinc (Zn), magnesium (Mg) and calcium (Ca) contents of the two varieties.

Proximate composition is a key determinant of the edibility or otherwise of any plant species. The varieties of *Eremomastax polysperma* investigated possessed high moisture content with EP (81.90±3.28 %) being higher than the 79.13±1.57 % contained in the EG variety. Moisture content in plants can affect the concentration of the secondary metabolites as well as the keeping quality of such materials. In the same vein, both species are not quite rich in proteins. Proteins are made up of amino acids which are essential in body building, it can help boost ones immunity thereby improving the health outcomes the consumers. The presence of protein also help to replace worn out and aged tissues. The presence of some levels of carbohydrate is of great significance meeting the energy needs of the person’s physical and mental activities. Fibre is very important for gastro-intestinal tract function as well as preventing and managing a variety of diseases (Grooper et al., 2009).
Generally the proximate analysis revealed that the leaves of these plants investigated possessed nutritive content hence their consumption is encouraged barring the anti-nutrient composition of the herbs.

The presence of phytochemicals in these plant species suggests a possibility of medicinal usage of these plants. Typically, medicinal actions of plants result from the secondary metabolites which are distinct to a particular phytomaterial, consistent with the concept that the combination of secondary products in plants is taxonomically distinct (Parekh et al., 2010). Phytochemicals are non-nutritive chemicals that contain protective, disease preventive compounds (Howard and Rosi, 2005; Anderson, 2004; Liu, 2004). They are naturally occurring compounds in fruits, vegetables, legumes and grains. These compounds are associated with prevention and treatment of diseases such as cancer, cardiovascular diseases and hypertension (Leverand, 1990; Booth et al., 1992). The results of qualitative and quantitative analyses presented in tables 2-4 clearly indicate the presence of tannins, alkaloids, saponins, phenols and flavonoids. Tannins have been reported to provide protection against microbial degradation of dietary proteins (Abulude et al., 2010), tannins also act as antioxidants; may inhibit enzymes that activate carcinogens (Boyle and Long, 2002). Similarly, alkaloids in plants may have pharmacological effects on animals and humans, such effects include blood clotting, neuroblocking (Clause et al., 1971) and increased oxygen carrying capacity (Guyton and Hall, 2002). Moreso, saponins have been reported to cleanse and purify blood (Yusuf and Ekanem, 2010), control human cardiovascular disease and reduce cholesterol levels (Abulude et al., 2010).

Despite the presence of useful nutrients in the plant species, there is likely to be safety concerns on the consumption of these botanicals due to the presence of antinutrients such as oxalate, thiocyanide and phytate. Oxalate and phytate are known to militate against the consumption of certain minerals. Luckily, the levels of these antinutrients are within the safety limits. The presence of minerals such as copper, iron, magnesium and selenium has been found to relieve oxidative stress. Zinc, iron and copper have been reported to be beneficial in the control of anaemia. These sources of local plants including fruits could be a good source of trace elements essential to man (Isong et al., 1996).

REFERENCES


