PROXIMATE AND PHYTOCHEMICAL COMPOSITION OF GARCINIA KOLA AND VERNONIA AMYGDALINA

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ABSTRACT

The study on the proximate and phytochemical compositions of Garcinia Kola seeds (Bitter cola) and Vernonia amygdalina leaves (Bitter leaf) was carried out to ascertain an aspect of their nutritive values having compared their therapeutic usefulness. The proximate analysis was carried out for 3 consecutive times for each fraction using AOAC method (1990) whereas the phytochemical screening was done using qualitative chemical tests specific to each phytochemical compound suspected. The result obtained revealed a high percentage proximate fraction in Vernonia amygdalina. However, the percentage nitrogen free extract (NFE) fraction is higher in Garcinia kola seeds than percentage of Vernonia amygdalina leaves reflecting a low percentage of crude protein, crude fibre, ether extract and Ash. The phytochemical constituents detected are similar with respect to phenols and divergent in the case of alkaloids, flavonoids, tannins, saponins, and hemicellulose. Vernonia amygdalina leaves are higher in crude protein, crude fibre, ether extract and ash than Garcinia kola seeds.

KEY WORDS: Proximate, Phytochemical, Garcinia kola, Vernonia amygdalina.

INTRODUCTION

The use of plants and plant parts (root, stem, leaves, fruits, seeds etc) both for food and medicinal purposes is not new in our modern society (Sofowora, 1982). Both Garcinia kola and Vernonia amygdalina share common medicinal properties. Both contain active principle phenols. Vernonia amygdalina contains vernonoside B which has antibacterial and antiparasitic effect (Huffman et al., 2001). Aqueous as well as ethanolic extract of Garcinia kola has antibacterial effect (Ehana et al., 1991).

Pitel, K (1997) reported that Vernonia amygdalina can be used in the management of diabetes mellitus whereas (Iwu et al., 1963) reported that biflavones of Garcinia kola exhibited hypoglycaemic effect.

The antiinflammatory effect of both Vernonia amygdalina and Garcinia kola left much to be desired as it is often seen in use to control wound bleeding as a result of matchet cut. (Igeh et al., 1996).

There seem to be dearth of opinion with respect to their nutritional importance. Vernonia amygdalina has been a leaf of choice in soup making especially in south-Eastern Nigeria but Garcinia kola is often used as a substitute for common cola nut (Kola nitida) Its seed is consumed by many people for various reasons and that is why it is necessary to find its nutrient composition as well as phytochemical constituents so as to advise the consumers properly on its values.

The phytochemical principles of these plants lend credence to their pharmacological uses as medicinal plants. Both are multipurpose plants as they play essential role in the treatment of diseases such as stomach pains, kidney disorders, asthma, menstrual cramps, hiccup etc (Orginma et al. 2003; Braide, 1989).

This study therefore is aimed at providing base line study or primary data, which could be used for further studies relating to the nutritive value of these all important medicinal plants. The phytochemical analysis of these plants will help to elucidate their chemical and biochemical action and hence their therapeutic values.

MATERIALS AND METHODS

Two batches of mature Vernonia amygdalina leaves each weighing 500g were procured from Wall market Calabar whereas 10kg of Garcinia kola seeds were bought from Eko Qua market Calabar by the month of August 2005. One Hundred gram of each sample was taken for dry matter determination while the remaining samples were dried at 80°C for 24 hours. The leaves of Vernonia amygdalina were milled separately and stored in a 500 millilitre conical flask tightly corked to prevent air penetration. The tests of Garcinia kola seeds were removed before milling. The milled sample was stored in an air tight flask until the commencement of chemical analysis.

Proximate Analysis: (Adapted from AOAC 1980)

Fifty (50g) grams of the fresh sample of Vernonia amygdalina was dried in an oven in a controlled temperature of 80°C for 4 hours. The loss in weight due to loss of moisture is calculated as percentage of the original weight of sample before oven drying. This gives the percentage moisture content of the sample milled (Vernonia amygdalina). The same procedure was used to determine the percentage moisture content of milled Garcinia kola seeds.

The percentage crude ash was determined by igniting 50g of milled Vernonia amygdalina in a crucible inside a muffle furnace of a temperature of 600°C. The ashing was terminated on formation of white ash from the sample. The ash formed was cooled in a desicator and weighed. The weight after ashing is expressed as a percentage of sample before ashing and this gives the percentage crude ash of the sample Vernonia amygdalina. The same process was carried out to determine the percentage crude ash of Garcinia kola.

The crude protein content of the sample was determined by kjeldahl method. Twenty milligrams was digested with concentrated tetraoxosulphite V acid using lithium tetraoxosulphate VI as catalyst. This oxidized the organic matter and the nitrogen present in the form of ammonium tetraoxosulphate VI (NH₄SO₄) were distilled with the aid of excess caustic soda (NaOH) during which NH₃ was released. The NH₃ released was allowed to react with 0.1ml HCL in the presence of methyl red and brom-cresol green indicator. The percentage crude protein was estimated by multiplying the titre with 6.25 and expressed as percentage of weight of sample before digestion. The same procedure was repeated for the determination of crude protein content of Garcinia kola.

The ether extract or lipid content of the sample was determined using soxhlet extractor. Five grams of the milled...
sample of Vernonia amygdalina was weighed and placed into a dry soxhlet thimble suspended in a beaker. The beaker was placed in soxhlet condenser attached to a flask containing sufficient ether to fill the thimble. Heat was supplied to the flask by means of electric hot plate so as to keep the ether gently boiling. The ether vapour passes up the side tube of the extractor to the reflux condenser where it is condensed and returned back into the sample in a thimble. When the thimble is practically full, the thimble is returned to the flask by an automatic siphoning device carrying with it some of the fat from the sample.

The siphoning process was terminated after the 24th time just before the next lot of ether entered the point of siphoning over. The flask was dried of in the air and the fat washed out of the soxhlet flask by chloroform. It was dried and weighed again. The percentage crude lipid was estimated by expressing the weight of lipid as percentage of sample weight before extraction.

The crude fiber (structural carbohydrate) was determined by defatting 20g samples of the sample with petroleum ether. The sample was boiled first with 1.25% dilute tetrasodium phosphate VI acid and washed with distilled water. The same sample was again boiled with 1.25% potassium hydroxide (KOH) with each boiling lasting for 30 minutes. The insoluble residue was separated by filtration, washed, dried, weighed and ashed. The loss of weight resulting from ashing was expressed as percentage of the sample weight before ashing.

Phytochemical Screening of Vernonia Amygdalina and Garcinia kola.

An extract each Vernonia amygdalina leaves and Garcinia kola seeds was prepared by macerating a 50g of each fresh sample with redistilled methylated spirit in a blender. The extract of each sample was then suction filtered and the process repeated until all soluble compound has been extracted as judged by loss of colour of the filtrate. The total extract from each plant sample was evaporated to dryness in vacuo at about 45°C and further dried to constant weight of the same temperature in a hot air oven. The yield of residue was noted and a portion of it was used to test for the plant constituents present.

Test For Presence of Alkaloid: (Adapted from Wall et al. 1952 & 1954)

One gramme of each of Vernonia amygdalina and Garcinia kola seed extract was treated with 40% calcium hydroxide solution (Ca(OH)₂) until the extract was distinctly alkaline to litmus paper and then extracted twice with 10ml portion of chloroform. The extracts were concentrated in vacuum to about 5ml. The chloroform extract was then spotted on thin-layer plates. Four different solvents systems of widely varying polarity were used to develop each plant extract. The presence of alkaloids in the developed chromatograms was detected by spraying the chromatograms with freshly prepared dragendorf's spray reagent. This confirmed the presence of alkaloid in the crude extract.

Test For Saponins: (Wall et al. 1952 & 1954)

The ability of saponins to produce frothing in aqueous solution and to haemolysse red blood cells was used as screening test for these compounds. One gram each of the extract of the samples under screening was boiled briefly with 50ml phosphate buffer pH 7.4 and each filtrate was passed for 3 hours through an asbestos disc which had been previously soaked with 3 drops of 1% cholesterol in ether and dried. After filtration, the disc was washed with 1.0ml of distilled water, dried and boiled in 20ml of oxylol for 2 hours. The disc was then washed in ether, dried and placed on a 7% blood nutrient agar. There was haemolysys of red blood cells around the disc after 6 hours confirming the presence of saponins in the crude extracts.

Test For Tannins: (Treas & Evans 1978)

Five grams each of the crude extracts of Vernonia amygdalina and Garcinia kola seed were stirred with 10ml of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue-black precipitate was formed which indicates the presence of tannins in the plant extracts.

Test For Anthraquinones: (Courtesy of Treas & Evans 1978)

Five grams of each plant extract was shaken with 10ml of benzene, filtered and 5ml of 10% ammonia solution was added to the filtrate. The mixture shaken and there was violet colour in the ammonical (lower) phase which indicates the presence of anthraquinones.

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<tr>
<th>Table 1: Chemical Composition (% of Dry Matter)</th>
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<td>Components</td>
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<td>Moisture</td>
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<td>Crude protein</td>
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<td>Ether extract</td>
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<td>Crude Ash</td>
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<td>Nitrogen free extract</td>
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<th>Table II: Phytochemical Constituents</th>
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<td>Vernonia amygdalina</td>
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<td>---------------------</td>
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<tr>
<td>Saponin</td>
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<td>Phenol</td>
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<td>Tannin</td>
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<td>Hemicellulose</td>
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RESULT AND DISCUSSION

The result of proximate chemical compositions of Vernonia amygdalina and Garcinia kola seeds are presented in table I. The result obtained showed a general high percentage proximate fractions (CP, CF, EE and Ash) in Vernonia amygdalina. Vernonia amygdalina leaves have high nutrient composition. Their use in soup making is therefore justified. In the proximate fraction (CP, CF, EE and Ash) of Garcinia kola seeds rather revealed low percentage composition. It is however, very high in NFE fraction indicating its relatively low contents of (CP, CF, EE and crude ash). The appreciably high content of crude protein and fiber in Vernonia amygdalina leaves as obtained in this analysis agrees with that of (Ije et al. 1996).
The low levels of crude protein and crude fiber (indices of nutritive value) in *Garcinia kola* seeds lend credence to their use as an anti-obese agent (Iwu et al. 1990). Nobou (2001) also reported anti-adipogenic effect of *Garcinia kola* extract, which inhibits the accumulation of lipid droplets in fat cells. His report lends weight to the low level of ether extract 3.3% as obtained in this study.

The predominant phytochemical principles detected in the study samples elucidate their roles in trado-medicine and are presented in table I. The phytochemical principle or active principle of any plant contributes directly or indirectly to their therapeutic usefulness in herbal medicine. *Vernonia amygdalina* contains tannins, phenols, saponins and hemicelluloses. The presence of saponins in *Vernonia amygdalina* supports its use as natural antibiotics (Riley et al. 2000). Its cholesterol lowering ability confirms its use in the treatment of hypercholesterolemia (Rene et al. 1995). The disinfecting property of phenol is utilized when *Vernonia amygdalina* is used in wound treatment. This explains the reason why people applied the squeezed out moisture in open wound or fresh cuts (Ijesi et al. 1998).

The presence of alkaloids and biflavonoids in *Garcinia kola* seeds account for their numerous uses in traditional medicine. Biflavonones of *Garcinia kola* exhibited hypoglycemic effects comparable to those of tolbutamide (Iwu et al. 1990). The crude flavonoid extract of *Garcinia kola* seeds contains, hydroxyl citrate which inhibits fatty acid synthesis and lipid accumulation in the rat liver (Lowenstein 1971; Sullivan et al. 1974). Ibekewe et al. (2006) reported a significant (P < 0.05) reduction in serum level of testosterone of Albino rats treated with crude flavonoid extract of *Garcinia kola*. This finding agreed with that of Braide et al. (2003) who equally reported a decreased serum level of testosterone of rats treated with crude alkaloid extract of *Garcinia kola* seeds. Whereas *Garcinia kola* seeds have little or no nutritional value, their medicinal values are tremendous. *Vernonia amygdalina* leaves have both nutritional, (though limited) as well as medicinal value.

**CONCLUSION**

*Vernonia amygdalina* (bitter leaf) though consists a high percentage of crude protein and fiber, its nutritive value as animal feed resources might be hindered by the presence of tannins which is one of its active principles. *Garcinia kola* seeds (Bitter kola) on the other hand have poor nutritive composition and as such very poor nutritive value but highly valued in trado-medicine due to its useful active principles or phytochemical composition. In conclusion therefore, both bitter leaf (*Vernonia amygdalina*) and bitter cola (*Garcinia kola*) have divergent herbal usefulness but limited nutritional values, if used as livestock feed.

**REFERENCES**

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