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Phytonutrients Composition of Sour Soup Leaves from Five States in Nigeria

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ABSTRACT: This investigation was undertaken to examine the phytochemicals in the leaves of sour soup harvested from five States of Nigeria. Standard methods for the quantitative assessment of the phytochemicals were adopted in the analysis. Results obtained indicated that the phytochemicals were in varied concentrations in the leaves from each of the States. The range of the concentration of alkaloid, tannin, flavonoid, saponin, phytate, steroid, phenol and oxalate were 4.08-2.11%, 30.20-10.50%, 1.52-0.61%, 1.66-0.69%, 0.16-0.08%, 3.80-3.20%, 3.60-3.19% and 0.26-0.24% respectively. Additionally, the leaves from the various States appeared to be good sources of these phytochemicals which are highly employed in preventive and curative treatments in man. In conclusion most of the phytochemicals detected in this study have significant *in vivo* roles, thus acting as antioxidants which are capable of scavenging the free radicals and thus preventing the cause of some ailments in humans.

Keywords: Alkaloid, Tannin, Flavonoid, Saponin, Phytate, Phenol, Steroid, Oxalate.

Introduction

Annona is a genus of the tropical fruit trees belonging to the family Annonaceae, of which there are approximately 119 species. Seven species and one hybrid are grown in Nigeria for domestic and commercial use (ICUC, 2002). *Annona muricata* L. is known as sour soup in English-speaking countries and is referred to by numerous common names (Morton, 1987; Thompson, 2003). After the arrival of Spanish in the Africa, the *Annona* species were distributed throughout the tropics (Popenoe, 1920). Sour soup trees are widespread in the tropics and frost-free subtropics of the world (Morton, 1973; Samson, 1980) and are found in the West Indies, North and South America, lowlands of Africa, Pacific Islands and Southeast Asia. The sour soup fruit and other parts of the tree are considered to be underutilized.

Information on the composition, nutritional value, medicinal uses and toxicology of the sour soup fruit and plant has not been properly documented. In the tropics alone, it has been estimated that 25,000 plants species are used in traditional medicine. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952).

Many of these indigenous medicinal plants are used as sources of food. They are also sometimes added to food meant for pregnant and lactating mothers for medicinal purposes (Okwu, 2001). The family Annonaceae commonly known as the sour soup has for long been utilized by communities in the forest areas for all forms of medicinal treatment (Letoucey, 1985). The economic importance of the Annonaceae is derived from the considerable range of non-timber product obtainable from its species (Suleiman *et al.*, 2008). The plant is used in folk medicine for the

treatment of several ailments like guinea worm, diarrhea, snake bite, headache and respiratory infection. The leaves are used for treatment of pneumonia, white gum from tree bark is used in sealing cuts and wounds (Orwa *et al.*, 2009).

The sour soup is an upright, low-branching tree reaching 8 to 10 meters (Popenoe, 1920, Mowry *et al.*, 1953). The tree has green, glossy evergreen leaves. The flowers appear anywhere on the trunk or any branch (Salazar, 1965). It is usually grown from seeds (Lopes *et al.*, 1981) which can be stored for several months before planting. Germination of seeds usually takes 3 weeks, but under suboptimal conditions can be delayed for up to 2-3 months. Alternatively, propagation of the *Annona* species is achieved by cuttings for rapid multiplication of new genotypes and for the elimination of viral and disease infection (Frey, 1981). With the exception of a few cultivars, clonal propagation of the *Annona* species by cutting or air layering has not been very successful (Rasai *et al.*, 1995). Vegetative propagation of rootstocks or cultivars of known agronomic potential could eliminate tree to tree variability in growth and reproduction (George and Nissen, 1987). However, the seedling rootstocks are highly variable in vigour and disease resistance and consequently scion growth and productivity are also variable.

This study was focused on the quantitative estimations of the phytonutrients in the leaves of sour soup from five states of Nigeria in view of the increasing applications of these bioactive in medicine. Hitherto, screening methods for the detection of phytonutrients in these leaves have been widely reported in literature.

Materials and Methods

Quantitative Estimation of Phytonutrients

Sampling methods: 10 g each of the fresh young leaves were harvested from five sour soup plants per state. The leaves were dried until constant weight was achieved.

Alkaloid determination: 5 g of the sample was weighed (W_1) and transferred into a conical flask after which 50 mL of 10 % acetic acid in ethanol was measured and transferred into the flask. The solution was left to stand for 4 h at 28 °C. It was then filtered using filter paper. The filtrate was then concentrated to $\frac{1}{4}$ of its original volume by evaporation, concentrated aqueous NH_4OH was added into the evaporated solution before it was precipitated. The precipitate was filtered through a weighed filter paper into another conical flask. The precipitate was washed with 1 % ammonia solution after which it was dried in an oven at 80°C. The dried filter paper was again weighed (W_2) and the alkaloid content was calculated.

Calculation

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100$$

Flavonoid determination: 5 g of the sample was weighed (W_2) into a conical flask after which 50 ml of 2 M HCl was then measured and transferred into the conical flask. The resulting solution was boiled on a heater for 30 mins, it was then cooled and filtered using a filter paper and a funnel. 5ml of the sample was pipetted into a flask, after which 5ml of ethyl acetate was added drop-wise until a precipitate was formed (Sofowora, 1982). A weighed filter paper (W_1) was used to filter the precipitate which was then dried in the oven and reweighed (W_2) again. The flavonoid content was then calculated.

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100$$

Phenol Determination: 0.02 g of the sample was weighed into a conical flask. 100ml of distilled water was measured with a measuring cylinder and transferred into the flask. 0.2 ml of folin C was pipetted and transferred into the flask, after which 2 ml of distilled water and 1ml of 15 % Na_2CO_3 were measured and transferred into the same flask. The solution was left for 2 hours until a blue color was observed. The sample was read on the UV spectrophotometer at a wavelength of 765nm the value was recorded and phenol was calculated for.

F.I. Obahiagbon and C.P. Mbamalu

Calculation

$$\text{Phenol (ppm)} = \frac{\text{Slope gradient} \times \text{absorbance} \times \text{extract ratio}}{\text{Weight of Sample}}$$

Tannins Determination: 0.01g of the sample was weighed into a flask, 20 ml of cold methanol extract was measured using a measuring cylinder and was transferred into the flask. The solution was shaken and then centrifuged for 10 min in a centrifuge. (1-5 ml of the filtrate was measured into a 50 ml flask. 0.3 ml of Folin D was measured and transferred. 0.6 ml of 15 % Na₂CO₃ solution was also measured and was transferred into the same flask. The solution was allowed to stand for 25-30 mins until a blue colour was observed and this was then read on a spectrophotometer at 760 nm. The value was recorded and the tannin content was calculated.

$$\text{Tannins (ppm)} = \frac{\text{Absorbance} \times \text{extract ratio} \times \text{slope gradient}}{\text{Weight of Sample}}$$

Saponin Determination: 10 g of sample was weighed using a weighing balance; this was transferred into a conical flask. 30 mL of n – hexane was measured with a measuring cylinder and transferred into the conical flask. 30 mL of methanol extract was measured into the flask. The resulting solution was filtered with a filter paper into another conical flask. The residue was put in a flask and another 30mL of methanol was measured and transferred into it, this was filtered and the procedure was repeated for the 3rd time to the residue until it was finally filtered. The filtrate was heated and concentrated to 1/3 of the original concentration. 100 mL of cold acetone was measured and transferred into the concentrated filtrate, it was put in the refrigerator for 50 mins, and was filtered with a weighed filter paper. The filter paper was then reweighed after drying. The saponin content of the sample was calculated.

Calculation

$$\text{Saponin (\%)} = \frac{W_2 - W_1}{W_0} \times \frac{100}{1}$$

Phytate Determination: 2 g of the sample was weighed and soaked into a conical flask containing already measured 100 ml 2 % HCl. It was left for 3hrs and was filtered with a filter paper. 25 mL of the filtrate was measured and transferred into a conical flask. 5ml of 0.03 % ammonium thiocyanate solution was measured and added to the filtrate as an indicator. 10ml of distilled water was added, after which the solution was titrated with ferric chloride until a faint yellow color appeared indicating its endpoint. The blank was used as a sample. The titre value was recorded and the phytate content was then calculated for.

Calculation

$$\text{Phytate (mg|100g)} = \frac{T \times \text{molarity of ferric chloride} \times \text{volume of HCl}}{\text{Weight of Sample} \times \text{Aliquot taken}}$$

Oxalate determination: 2g of the sample was weighed into a conical flask and digested with 10 ml of 6 M HCl for 1hr. The solution was filtered and made up to 50mL mark with distilled water. The pH of the solution was adjusted by adding Conc. NH₄OH solution until a faint yellow color was observed in the solution. 10 ml of 5% calcium chloride (CaCl₂) was measured and added to the solution until a precipitate was formed. The solution was filtered, the residue was dissolved in 10mL of 20 % H₂SO₄ after which the solution was filtered into a conical flask with a filter paper into a conical flask and made up to 60ml with distilled water. An aliquot of 25 mL of the filtrate was measured and heated on a heater to near its boiling point. The resulting solution was titrated against 0M of standardized KMnO₄ solution until a pink color was observed indicating its end point. The color persisted for 30mins. The titre value was recorded while the blank was also used and treated similarly as the sample. The oxalate content was then calculated

Calculation

$$\text{Oxalate (\%)} = \frac{\text{Molarity of KMnO}_4 \times T \times 60}{\text{Weight of Sample} \times \text{aliquot taken}}$$

Terpenoid determination: 100 mg of sample was weighed into a conical flask; 40 mL of distilled water was measured and transferred into the flask. The content was taken into the oven and heated for 15min at 100 °C. The

solution was centrifuged and the supernatant was decanted. The supernatant was extracted with 5 ml of petroleum ether. The absorbance was read on the UV-VIS spectrophotometer at 450 nm. The value was recorded and the content of terpene was calculated for.

Calculation

$$\text{Terpenoid (ppm)} = \frac{\text{Slope gradient} \times \text{Absorbance} \times \text{Extract ratio}}{\text{Weight of Sample}}$$

Statistical analysis: The results obtained were statistically analysed using ANOVA. The results were expressed as mean SEM of duplicate determinations

Results

The results of phytochemical composition of the leaves of Sour soup from five States of Nigeria are presented in Table 1.

Table1: Phytochemical composition of the leaves of Sour soup from five States of Nigeria (Mean)

| States | Alkaloid (%) | Tannin (%) | Flavonoid (%) | Saponin (%) | Phytate (%) | Steroid (%) | Phenol (%) | Oxalate (%) |
|---------|--------------|------------|---------------|-------------|-------------|-------------|------------|-------------|
| Benin | 4.08±0.00 | 13.40±0.00 | 1.40±0.20 | 0.71±0.00 | 0.16±0.00 | 3.80±0.30 | 3.60±0.40 | 0.25±0.00 |
| Delta | 3.53±0.20 | 20.10±0.00 | 0.78±0.20 | 0.69±0.10 | 0.08±0.00 | 3.40±0.00 | 3.46±0.20 | 0.26±0.01 |
| Ondo | 3.05±0.00 | 10.50±0.20 | 0.87±0.00 | 1.03±0.00 | 0.16±0.00 | 3.40±0.00 | 3.31±0.00 | 0.26±0.00 |
| Anambra | 2.11±0.00 | 10.50±0.20 | 0.61±0.10 | 1.14±0.00 | 0.16±0.01 | 3.30±0.00 | 3.28±0.04 | 0.24±0.00 |
| Enugu | 3.33±0.20 | 30.20±0.00 | 1.52±0.20 | 1.66±0.20 | 0.11±0.00 | 3.20±0.00 | 3.19±0.00 | 0.24±0.00 |

Alkaloid: The results of the alkaloid concentrations in the leaves indicated a variation from one state to the other among the five states. The alkaloid concentration ranged between 4.08 and 2.11%. The highest concentration was detected in the leaves from Edo State, while the least concentration was detected in the leaves from Anambra State.

Tannin: The result of the analysis showed that the concentrations of tannin in the sour soup leaves varied in the different States. The tannin concentrations ranged between 30.20 and 10.50 %. The sour soup leaves from Enugu State had the highest tannin concentration while the least tannin concentration was detected in the leaves from Anambra State.

Flavonoid: The flavonoid concentration in the sour soup leaves varied from one State to another between the range of 1.52 and 0.78 %. The highest flavonoid concentration in the leaves was detected from Enugu State and the least concentration was in the leaves from Anambra State.

Saponin: Variations in the saponin concentration among the five States were also observed. The concentrations ranged between 1.66 and 0.69 %. The sour soup leaves from Enugu State had the highest saponin concentration while the sour soup leaves from Delta State had the lowest saponin concentration.

Phytate: The concentration of phytate in the sour soup leaves varies among the five different States and was between the range of 0.16 and 0.08%. Sour soup leaves from Edo, Delta and Anambra States had the highest phytate concentration with the same range (0.16 %) while Delta State had the lowest phytate concentration. The phytate was detected as having least concentration among the phytochemicals assayed for.

Steroid: The concentrations of steroid in the leaves from the five States were in varied concentrations. The concentration range was between 3.80 and 3.20 %. The highest concentration was detected in the leaves from Enugu State.

Phenol: The concentrations of phenol in the sour soup leaves from the five States ranged between 3.60-3.19 %. The sour soup leaves from Edo State had the highest phenol concentration.

Oxalate: The oxalate concentration in the sour soup leaves varied among the different States and ranged between 0.26 and 0.24 %. The highest oxalate concentrations were detected in the sour soup leaves from Delta State and Ondo States with equal oxalate concentrations while sour soup leaves from Anambra and Enugu States had the lowest oxalate concentration with the same values.

Discussion

Alkaloid: The variation in the alkaloid concentration of the sour soup leaves from the different States could be said to be due to some natural and biological factors affecting the plant which include the rate of bio-synthesis and bio-availability of the phytochemicals in the plant, enzymes activities in the plants, genetic makeup of the plant, etc. Alkaloid function in defending plants against herbivores and pathogens and are widely exploited as pharmaceutical stimulants, narcotics and poisons due to their powerful biological activities. They are also employed in clinical uses as an analgesic, muscle relaxant, antibiotics, anti cancer, anti rhythmic, pupil dilator, active stimulant, etc. (Okwu, 2005). The alkaloids are the largest group of phytochemicals made from ammonium compounds comprising basically of Nitrogen bases synthesized from Amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring. Alkaloids are regarded as bioactive compounds which is responsible for the medicinal value of the respective plant foods (Edeoga *et al.*, 2005).

Tannin: The concentration of tannin in the sour soup leaves varied from one State to the other due to some factors affecting the biological activities in the plants as well as the production of the phytochemicals by the plant including the rate of biosynthesis and bioavailability of the phytochemicals and the enzymatic activities, the genetic makeup of the plant and other factors. Tannin are polyphenols, capable of forming complexes with metal ions, proteins and polysaccharides. Dietary tannin has been found to be capable of reducing feed efficiency and weight loss in chicks (Oluremi *et al.*, 2007). There has been positive evidence on some phytochemicals improving fertility or reproduction capacity in a number of animals (Bendich, 1998). Some phytochemicals can penetrate the blood brain barrier in an *in vitro* model (Yano *et al.*, 1999; Del-Rio *et al.*, 1997). Phytochemicals can reduce the symptom hemophilia and can prevent edema of the legs (Rasai *et al.*, 1995). Many physiological activities such as stimulation of phygocytic cells, host mediated tumor activity and wide range of anti-infective action have been assigned to tannins (Okwu and Okwu, 2004).

Flavonoid: The different flavonoid concentrations of the sour soup leaves from the five different States may be due to the genetic makeup of the plants, enzymatic activities of the plants, bio synthesis and availability of the phytochemical in the plants, growth rate as well as other factors that are capable of reducing plant nutrients. Flavonoids have inherent ability to modify the body reaction to allergens viruses and carcinogens. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity (Okwu, 2005). The flavonoid can be said to be responsible for the sour taste in sour soup fruits, leaves and have also demonstrated significant inflammatory activity because of direct inhibition of several processes of inflammation. For example, it inhibits both the production of histamine and other allergic inflammatory mediators (Rasai *et al.*, 1995; Del-Rio *et al.*, 1997). Flavonoids are said to carry out the following activities; anti-oxidant, anti-tumor and can combat prostatitis, heart disease, cataract, bronchitis, asthma and can reduce cholesterol (Yano *et al.*, 1999; Del Rio 1997; Rapisarda *et al.*, 1999). Flavonoid are highly necessary in the human diet due to its physiological role as free radical scavengers (anti oxidants), diuretic, anti-viral, anti-bacterial, anti- tumor, anti- allergic, anti- microbial, anti- inflammatory and anti- platelets agents (Hertog *et al.*, 1993; Middleton and Kandaswami, 1992; Soetan and Aiyelagbe, 2009; Omale, 2009). More of this leaves should be included in the diets to be taken as fruits but in order to get maximum effects. They can as well be added to confectionaries such as cakes, sweet, custards, juice, chocolate, ice cream, etc (Parle, 2012).

Saponin: The different values of saponin concentrations detected may be linked to the different biosynthetic rates of the plants and bioavailability of the phytochemicals. The genetic makeup of the plant may be additional reasons which affected the growth rate of the plant and thus the availability of the phytochemicals in the plants. Saponin is one of the anti nutrients commonly found in plant foods which have both adverse effects and good health benefits.

Several workers have documented the health benefits of saponin to include antidiabetic (Soetan and Aiyelagbe, 2009). Saponin have hypertensive and cardiac depressant properties (Olaleye, 2007), including antinematocidal, mulluscidal, insecticidal and anti-oxidant properties (Argentieri *et al.*, 2007). Saponins are classified as glycosides, and this includes steroid saponins and triterpenoidsaponins (Del-Rio *et al.*, 1997). Excess saponin can lead to hypocholestraemia due to its binding ability with cholesterol thus making saponin unavailable for absorption (Soetan and Aiyelagbe, 2009).

Phytate: The variation observed in the phytate concentrations in the leaves from the five States may be attributed to the genetic makeup of the plant including the ability of the plant to preserve already synthesized phytochemical and other factors affecting the plants. The use of sprouted grains will reduce the quantity of phytic acids in feed, with no significant reduction of nutritional value (Malleshi *et al.*, 1986). Phytates also have the potential to be used in soil remediation, to immobilize uranium, nickel and other inorganic contaminants (Samso, 1980). Phytates have therapeutic uses; it is reported as being able to prevent colon cancer due to its production of butyric acid through fermentation thus leading to reduction in gut and bile acid metabolism (Shamusuddin, 2002). Phytate act on HIV and HIV specific antigen at an early noticeable stage. Phytate can reduce the incidence of fatty livers by reducing the activities of hepatic enzymes involved in lipogenesis. It can also reduce the incidence of diabetes mellitus by being able to lower glucose response and regulate insulin secretion through its effects on calcium channel activity (Larsson *et al.*, 1997; Baker and Berggren, 1999).

Steroid: The variation in the steroid concentration of the sour soup leaves samples may also be attributed to genetic, biosynthetic, bioavailability and the rates the plant phytonutrients are being consumed. Some of the health benefits of steroid are that they act as sedatives, anti-spasmodic and recommended for persons suffering from nervousness, insomnia, palpitation, migraine and asthmatic patients (Rasai, 1995). Steroids also have invigorating properties for the digestive systems and it is required for those suffering from lack of appetite, heavy digestion and poor functioning of the stomach (Okwu, 2008). Steroids have vermifuge effect in being able to expel parasites from the intestine (Rasai, 1995).

Phenol: Significant variations in the concentrations of phenols were observed in the sour soup leaves from the five States which may be traceable to some biological factors which affected the plants. These factors could also be genetic, bio synthetic and bio availability and climatic conditions. Phenols have the highest phytochemicals concentration compared to the other detected phytochemicals and also share the same health benefits with the flavonoids. Additionally, phenol strengthens the capillaries and reduces haemophilia. Phenol also has the ability to reduce edema of the legs and cyto-toxicity of oxidized low oxygen lipoprotein (LDL) and cholesterol (Del-Rio *et al.*, 1997).

Oxalate: Like the above phytonutrients, the variation in the oxalate concentrations from one state to the other may not be far from their soil type, climatic conditions and other biological factors. The plant oxalate are classified as specific anti-nutrients, having the capability of binding with calcium to form calcium oxalate crystals which later deposits as urinary calcium known as stones. These stones are said and believed to have the ability of blocking the renal tubules. The presence of oxalate could cause a variety of disorders, such as hyperoxaluria, calcium oxalate stones (Oluremi, 2007). Restriction of dietary oxalate helps to prevent formation of calcium oxalate stones in individuals who have previously formed such stones..

In conclusion, the phytonutrients detected in the leaves of the sour soup from the five states were in varied concentrations due genetic, soil, climatic factors and the different rates of biosynthesis of the phytonutrient. Additionally, most of the phytonutrients detected are well documented for their antioxidant roles in man

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F.I. Obahiagbon and C.P. Mbamalu

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