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Proximate and Phytochemical Analysis of Ethanolic Extracts of Leaves of *Piper guineense* from South-eastern Nigeria

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Abstract: *Piper guineenseis a West African species of Piper known by the Igbos in the South-eastern Nigeria as Uziza. The spice obtained from its dried fruit is ambiguously known as Guinea pepper or West African black pepper. The leaves of this plant have been shown to be useful as spices and in traditional medicine. This study presents the proximate analysis as well as the qualitative and quantitative screening of the phytochemical constituents extracted from the leaves of this indigenous plant by solvent extraction using 95% ethanol. The results of the proximate analysis showed that on dry weight basis, the moisture content is 11.04% (22.08 mg/g), ash content 14.14% (28.28 mg/g), crude protein 17.15% (21.44 mg/g), carbohydrate 36.94% (369.4 mg/g), fats/lipids content 1.74% (3.48 mg/g), while fibre content is 18.99% (37.98 mg/g). The calorific value was estimated at 0.489 mg/g which is equivalent to 378.305 kcal/100g.The qualitative phytochemical analysis of the ethanolic leaf extracts showed strong or intense positivity (+++) for alkaloids, moderate positivity (++) for flavonoids, steroids, tannins, terpenoids, cardiac glycosides and total phenols, and weak positivity (+) for saponins. The quantitative phytochemistry of the leaves showed that the major phytochemicals present are flavonoids (7.86 mg/g), alkaloids (4.48 mg/g), saponins (3.38 mg/g) and tannins (3.20 mg/g) which have therapeutic, pharmacological, anti-oxidant and anti-inflammatory potentials. This study therefore reveals that the leaves of Piper guineense can be harnessed for medicinal purposes due to the presence in them of these phytochemicals.*

Keywords: *Piper guineense, Guinea pepper, West African pepper, uziza, phytochemicals, proximate analysis*

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I. Introduction

Traditional medicine is as old as the history of mankind, and studies have shown that about 80% of the world population presently depend on herbs, roots and seeds medically to satisfy their health needs (Ugoh and Nneji, 2013). The therapeutic and medicinal benefits of some plant extracts can be attributed to the presence of phytochemical constituents in them. Phytochemicals are chemical compounds produced by plants as a result of primary or secondary metabolism. They generally have some biological activity in the host plantand are commonly found in the leaves, roots, bark, fruits, vegetables, nuts, legumes, and grains (Molyneux *et al.,* 2007; Harborne, *et al.,* 1999). Based on functionality,phytochemicals are classified into two categories– phytonutrients and phytotoxins. Phytonutrients are plant chemicals that are recognised to as essential nutrients; they naturally play some roles in the normal physiological functions and are beneficial in human diet. Phyotoxins, on the other hand, specifically refer to plant chemicals that have toxic and detrimental effects on humans(Iwasaki, 1998; Bjeldanes and Shibamoto, 2009). Extraction and characterization of several phytochemicals from herbal products have led to the discovery of new high profile pharmaceuticals, healthcare products as well as preservatives (Ara*et al.,* 2012; Mandal *et al.,* 2007; Ivanova*et al.,* 2005).

Piper guineense, otherwise known as African black pepper is a West African spice plant which possesses some medicinal properties and is widely useful in traditional medicine for the treatment of various ailments (Balogun*et al.,* 2016). Reports by researchers have also revealed that *Piper guineense*finds use in the treatment of intestinal diseases, bronchitis, cough and rheumatism, infertility in woman and low sperm count in men (Sumathykutty*et al.,* 1999; Nwachukwu*et al.,* 2010). This report therefore deals with the proximate as well as the phytochemical compositions of the ethanolic extracts of the leaves which is commonly used in South-eastern Nigeria as vegetable and in trado-medicine.

II. Materials And Methods

Sample Collection and Preparation:The leafy vegetables of *Piper guineense* were harvested from a farm located in Umuika in Isiala-Ngwa LGA of Abia State, South-eastern Nigeria in the month of July 2016. They

were botanically identified at the Botanical garden of Imo State Polytechnic, Umuagwo-Ohaji, washed thoroughly and air-dried for 5 days at room temperature to avoid loss of active compounds.The dried leaves were ground into a powder using the coroner crown machine and preserved for analysis.

Extraction of Phytochemicals: The powder (100 g) was soaked in 500 ml absolute ethanol in a corked conical flask and heated in a water-bath (40-60^{0}C) for 24 hours to extract the phytochemicals. The content was then filtered and the filtrate collected for qualitative phytochemical analysis.

Proximate Analysis

Determination of Moisture Content and Total Solids: The sample (5 g) was put into a pre-weighed, pre-dried and cooled crucible and then dried in the oven at 80^{0}C for 2 hours and at 105^{0}C to obtain a constant weight. The crucible and its content was cooled in a desiccator for some time, after which it was reweighed. The moisture content and total solids werecalculated and expressed as % moisture and % total solids respectively.

Determination of Ash Content*:* The crucible was washed, cleaned and placed in a hot-air circulation oven for 2 hours and then transferred into a muffle furnace to burn off all organic matter and stabilize the weight of the crucible at a temperature of 550^{0}C, after which it was cooled to room temperature in a desiccator. The sample (5 g) was weighed into the crucible placed in the muffle furnace, ashed at 600^{0}C for 3 hours, cooled in a desiccator at room temperature and re-weighed. The ash content was calculated and expressed as % ash content.

Determination of Crude Fats/Lipids*:* The sample (5 g) was extracted for lipids with 300 ml of petroleum ether (40-60^{0}C) for 3 hours in a Soxhlet extractor. The content of the thimble (extracted lipids) was dried in an oven at 80^{0}C for a few minutes, cooled in a desiccator and then reweighed. The drying and cooling was continued to obtain a constant weight and the percentage lipid was calculated and expressed as % crude lipid (AOAC, 1984).

Determination of Dietary Fibre Content*:* The sample (2 g) was dissolved in 100 ml of 0.25N $H_2S0_4$and heated for 1 hour in a water bath, filteredand the residue obtained. The residue was dissolved in 100 ml of 0.3 N NaOH solution, heated under reflux for additional 1 hour.The mixture was filtered through a fibre sieve cloth and 10 ml of acetone was added to dissolve any organic constituent.The residue was washed with about 50ml of hot water twice on the sieve cloth before it was finally transferred into the crucible. The residue in the crucible was oven-dried overnight at 105^{0}C to drive off moisture, then cooled in a desiccator and weighed to obtain the weight (w_1). The crucible with content was transferred to the muffle furnace for ashing at 550^{0}C for 4 hours. The crucible and its ashedcontent was cooled in the desiccator and reweighed to obtain the weight (w_2). The difference $w_1 – w_2$ gives the weight of fibre (AOAC, 1999). The result was expressed as % fibre content.

Determination of Crude Protein Content using Kejhdal Method*:* The sample (8 g) was mixed with 3g of copper sulphate catalyst and 25 ml of concentrated sulphuric acid. The mixture was heated over a Bunsen flame in a fume cupboard to expel any poisons gas. It was then heated with shaking at intervals for 1hour until the mixture becomes clear. Distilled water (400 ml), 2% boric acid (50 ml) andmethyl red indicator (1 ml)were added. The solution was made alkaline by the addition of 75ml of 50% NaOH solution. The ammonia was distilled into the Boric acid solution and 250ml of the distillate was collected and titrated with 0.1 M Hydrochloric acid. The percentage crude protein was calculated from the % nitrogen of the sample.

Determination of Total Carbohydrate*:* The total carbohydrate content was conveniently calculated using the difference method i.e. Total carbohydrate = [100 – (% lipid + % ash + % moisture + % protein)]%.

Determination of Caloric Value*:* The % crude protein, fat, and carbohydrate were used to estimate the calorific value of the sample. Thus, the value of protein content was multiplied by 4; that of lipid by 9 and that of total carbohydrate by 4. The sum of these values gives the calorific value expressed in kcal/100g sample (Oneyike*et al.,* 2000).

Qualitative Phytochemical Analysis

The presence of alkaloids, flavonoids, saponins, steroids, tannins, terpenoids, glycosides and phenolic compounds in the leaf extracts were determined using standard analytical methods stipulated by the Association of Official Analytical Chemists (AOAC).

Quantitative Phytochemical Analysis

Determination of Alkaloids:The sample (5 g) was dissolved in 100ml of 20% acetic acid in ethanol in 250 ml beaker, covered and allowed to stand for 4 hours at 25^{0}C, filteredand the filtrate was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the filtrate until the precipitate was collected and washed with 1 % NH_4OH solution.The solution was filtered and the residue obtained (the alkaloid) was oven-dried at 80^{0}C, after which it was allowed to cool and then re weighed. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analysed (Harborne, 1995).

Determination of Flavonoids:The sample (10 g) was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered into a pre weighted beaker and transferred to a water-bath to evaporate the solution to dryness. The residue is weighed and the flavonoid content is calculated and expressed as % flavonoids (Harborne, 1995).

Determination of Saponins:The sample (5 g) was dissolved in 100ml of 20% acetic acid in ethanol and allowed to stand in a water-bath at 50^{0}C for 24 hours.The mixture was filtered and the extract was concentrated using the

water-bath to one-quarter of the original volume. Concentrated NH_4OH solution was added drop-wise to the extract until the precipitation was complete. The solution was allowed to settle and the precipitate was collected by filtration, oven-dried and weighed (Harborne, 1973). The saponin content was calculated and expressed as % saponin.

Determination of Steroids by Liebermann-Burchardt Method:A 1 ml portion of the leaf extract was made up to 5 ml using chloroform in a test tube. Liebermann-Burchardt reagent (2 ml) was added and the test tube covered with black paper and kept in the dark for 15minutes. Using cholesterol as the standard, the green colour complex formed was spectrophotometrically measured at 640nm and the % steroid was evaluated.

Determination of Total Tannin by Titration method*:*TheFolin-Dennis method described by Pearson (1974) was used. Petroleum ether (100ml) was added to the sample (10 g) in a conical flask which was then covered and left to stand for 24 hours. The mixture was then filtered and allowed to stand for 15 minutes to allow the petroleum ether to evaporate. It was re-extracted by soaking in 100ml of 10% acetic acid in ethanol for 4 hours. The solution was filtered and 25 ml of NH_4OH solution was added to the filtrate to perpetrate the tannins. The solution was then heated in a water-bath as to remove some of the NH_4OH still present. The solution (2ml) was taken and mixed with 20 ml of ethanol, and was titrated with 0.1 MNaOH solution using phenolphthalein as indicator until pink end-point was reached.The tannin content was then calculated and expressed as % tannic acid.

Determination of Terpenoids:The sample extract (1 ml) was put into the colorimetric cuvette to read the absorbance at 538 nm. The blank used was 95% (v/v) methanol. For the standard calibration curve, 200µl of previously prepared Linalool solution in methanol was added to 1.5 ml Chloroform and serial dilution was done (dilution level – 100mg/200µl to 1mg/200µl Linalool Conc.). In case of serial dilution total volume of 200µl was made up by addition of 95% (v/v) Methanol. The calibration curve was used to determine the % terpenoid present (Idumathi*et al.,* 2014).

Determination of Cardiac Glycosides:Cardiac glycosides were quantitatively determined according to Solich*et al.*(1992) by some modifications. A 10% extract of the leaves was mixed with 10 ml freshly prepared Baljet's reagent (95 ml of 1% picric acid + 5 ml of 10% NaOH). The mixture was diluted after an hour with 20 ml distilled water and the absorbance was measured at 495 nm by Shimadzu UV/VIS spectrophotometer model 160A (Kyoto, Japan). The standard curve was prepared using 10 ml of different concentrations (12.5-100 mg/L) of securidaside. Securidaside was isolatedfrom Securigerasecuridacaextract (Tofighi, 2016). Total glycosides from triplicate determinations was expressed as mg of securidaside per g of dried sample.

Determination of Total Phenolic Contents**:** Folin-Ciocalteuassay was carried outfor determination of total phenols in the cells (Singleton *et al.,* 1999).The methanol solution of the sample(0.2 mL, 100 µg/mL) was mixed with Folin-Ciocalteureagent (2 mL, 1:10 diluted with distilled water). Saturated NaHCO3solution (1.5 mL, 60 g/L distilled water) was added after 5 minutes. The blend wasincubated in the dark for 90 minutesat room temperature, and the absorbance of the blue-coloured solutionwas measured at 725 nm using a spectrophotometer. The same procedure was repeated for different concentrationsof gallic acid solutions (0–100 mg/mL) as standard and the calibration curve was plotted. The total phenoliccontents was expressed as mg of gallic acid equivalents (GAE)per g of dry weight of sample powder.

III. Results And Discussion

Proximate Analysis

Table 1: Proximate analysis of the leaf extracts of *Piper guineense*

S/N	Parameter	Amount (%)	Amount (mg/wt. of sample)	Amount (mg/g)
1	Moisture content	11.04	110.4 mg/5 g	22.08
2	Total solids	88.96	88.96 mg/5g	17.792
3	Ash	14.4	141.4 mg/5 g	28.28
4	Crude protein	17.15	171.5 mg/8 g	21.44
5	Crude fats/lipids	1.74	17.4 mg/5 g	3.48
6	Crude dietary fibre	18.99	189.9 mg/2 g	37.98
7	Carbohydrate	36.94	369.4 mg/g	369.4
8	Calorific value	4.899	48.992 mg/100 g	0.489

Table 2: Qualitative phytochemical analysis of the leaf extracts of *Piper guineense*

S/N	PARAMETER	RESULT
1	Alkaloids	+++
2	Flavonoids	++
3	Saponins	+
4	Steroids	++
5	Tannins	++
6	Terpenoids	++
7	Glycosides	++
8	Phenols	++

Key: +++ = High (Strong), ++ = Scanty (Moderate), + = Low (Weak).

Table 3: Quantitative phytochemical analysis of the leaf extracts of *Piper guineense*

S/N	Phytochemical	Amount (%)	Amount (mg/g)
1	Alkaloids	2.24	4.48

2	Flavonoids	7.86	7.86
3	Saponins	1.69	3.38
4	Steroids	19.33	0.193
5	Tannins	3.20	3.20
6	Terpenoids	45.76	0.458
7	Cardiac glycosides	32.69	0.327
8	Total Phenols	56.561	0.565

IV. Discussions

The proximate analysis revealed that leaves of *Piper guineense*is constituted by 11.04% (22.08 mg/g) moisture content, 14.14% (28.28 mg/g) ash content, 17.15% (21.44 mg/g) crude protein, 36.94% (369.4 mg/g) carbohydrate, 1.74% (3.48 mg/g) fats/lipids content, and 18.99% (37.98 mg/g) fibre content (Table 1). The calorific value was estimated at 0.489 mg/g which is equivalent to 378.305 kcal/100g. The high ash content of the leaves is an indication of the level of inorganic elements or minerals such as calcium, zinc, magnesium, copper and potassium in the vegetable. The high crude fibre content suggests that the leaves could aid digestion, absorption of water from the body, bulk stool and also prevent constipation. The leaves may therefore be very useful in the control of body weight, reduction of serum cholesterol level since it contains very low level of fat/lipids.The high crude protein content of the leaves makes it a good source of plant protein. The consumption of leaves of*Piper guineense* could also provide the body with fuel and energy that is required for daily activities since it contains the highest value in terms of carbohydrate and caloric content.

In the qualitative phytochemical analysis, the ethanolic extracts of the leaves showed strong or intense positivity (+++) for alkaloids, moderate positivity (++) for flavonoids, steroids, tannins, terpenoids, cardiac glycosides and total phenols and weak positivity (+) for saponins (Table 2).The quantitative analysis of phytochemicals showed that the major phytochemicals present are flavonoids (7.86 mg/g), alkaloids (4.48 mg/g), saponins (3.38 mg/g) and tannins (3.20 mg/g) (Table 3).Igile*et al.* (2013) reported that saponins at low level less than 10% are safe for human consumption. Therefore, the low level of saponins in these leafy vegetables(3.38 mg/g) which accounts for 1.69% of the sample weight conforms well to the report.Alkaloids are one of the most efficient therapeutically significant bioactive substances in plants.Hence, the level of alkaloid in the leaves of *Piper guineense* supports its therapeutic relevance.The observed presence and content of tannins shows that these leafy vegetables possess good antioxidant properties (Okwu, 2001). The report by Okwu also reveals that flavonoids have good inhibitory effects onthe formation of plaques and streaks in arteries and as such hinder hypertension and other related cardiovascular diseases. Studies have shown that phenols and terpenoids are effective hydrogen donors, hence they have potentials as antioxidants (Igile*et al.,* 2013).Steroids also play a vital role in the regulation of carbohydrate and protein metabolism and also possess anti-inflammatory properties.

V. Conclusion

This studyhas reported the proximate and phytochemical compositionsof the leaves of *Piper guineense* (uziza) and has really proven that uziza leaves are very important for their physiological and pharmacological effects in humans. Therefore, consumption of this leafy vegetable is encouraged both as food and as a herbal medicine.

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