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Screening, Quantification and Storage Studies with Selected Phytochemicals Constituents of *Piper guineense* Leaves

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ABSTRACT

In this work, the screening and quantification of alkaloids, saponins, tannins and cyanogenic glycosides constituents of raw *Piper guineense* leaves were undertaken. Also post processing and storage studies with the alkaloids, saponins, tannins and cyanogenic glycosides constituents of the sun dried and grated *Piper guineense* leaves were investigated. The *Piper guineense* leaves samples used in this study were obtained from some open markets in Benin City, Edo State. Storage facilities used were the open laboratory wherein samples were kept separately in opened and closed containers, as well as at water activities (a_w) of 0.23, 0.52 and 0.97. All storages were carried out at ambient conditions and the storage duration was two months. The presence of alkaloids, saponins, tannins and cyanogenic glycosides in the studied *Piper guineense* leaves were established by the results of the qualitative tests. Findings showed that in the various solvents extracts used, variations occurred in the levels of the phytochemicals examined. Results for the quantitative investigations carried out using raw *Piper guineense* leaves indicated that among the phytochemicals examined, alkaloids occurred most with a concentration of 6.73±1.25mg/g. In a decreasing order, the sequence of occurrence of the other phytochemicals examined is tannins (2.32±0.18mg/mg), saponins (1.24±0.51mg/g), and cyanogenic glycosides (0.20±0.05mg/g). Further deducible from results was that processing and storage duration positively affected the values of the phytochemicals examined. The noted positive effects were statistically significant ($P<0.05$) with respect to storage conditions. It is hoped that handlers and users of *Piper guineense* leaves would find this work useful. Additionally, this work would be relevant to policy makers in the formulation of standard methods for the processing and storage of *Piper guineense* leaves.

Keywords: *Piper guineense*, phytochemical, storage, water activity (a_w).

INTRODUCTION

Foods are materials which in their naturally occurring, processed or cooked forms, are consumed by humans as nourishment and for enjoyment (Belitz *et al*., 2009). According to Carper (1988) there is a rich heritage in the medical uses of foods. Nonetheless, Quillin (1987) noted that the lack of proper fertilization is one of the causes of marginal nutrient intake, poor health and the need for supplemental nutrition. Additionally, Johnson (2002) posited that it is becoming increasingly difficult to produce soybean meal with high protein content because increasing farm yields are depressing protein content. Thus, it would appear that there is increasing imbalance between human effort to produce and process foods and the availability of quality foods to consumers. It is pertinent to mention that every consumer deserves to eat safe and quality foods, especially as the consequences of eating foods with reduced quality indices could be disastrous even in the short run. Significantly, Lean (2006) remarked that adequate and well balanced diet is essential for the enjoyment of good health.

Presently in Nigeria, efforts by relevant authorities to ameliorate the situations where unsafe foods are marketed to consumers appeared to be more on processed and packaged food. It is pertinent to say that the little or no attention paid to many of the traditional foods sold in the Nigerian open markets is worrisome, particularly as some of them are processed and stored based on the utilizations of unstandardized method. There is need for these other classes of foods to be investigated for their compositional chemistry, especially as larger segment of Nigerian populace depend on the open markets for their foods supplies. The relevance of the present study could be viewed from this perspective.

It is imperative to mention that detailed nutritional and toxicological values of food materials would be better established if they are investigated postharvest, post processing and in their stored forms. Significantly, Ukhun and Dibie (1991) posited that in computing and compounding food intakes aimed at meeting recommended daily allowances for various nutrients, food composition must be considered as it exists naturally and as it exists post-processing and during storage under

various conditions, if reliable nutritional information is to be obtained. The problem of inadequate food supply, especially in the developing countries, is related not only to the total output of raw agricultural production but also to post-harvest factors such as processing and storage; these two post-harvest factors must be taken into consideration in evaluating the adequacy in terms of total quantity and nutritional wholesomeness of food supplies in developing countries like Nigeria (Ukhun, 1986).

The uses of *Piper guineense* leaves in many diets preparations and in some herbal medicine formulations have long historical dating. Apparently, the relevance of *Piper guineense* leaves in the aforementioned aspects of human needs is due to their compositional chemistry. It is imperative to mention however, that biological materials such as *Piper guineense* leaves are composed of numerous chemicals. Significantly, biological materials are composed of a whole ray of chemicals (Josly, 1970). In particular, processing and/or storage could positively or negative affect these chemicals with respect to their relevance in humans needs. This is consistent with the view of Ukhun (1984) that although food processing sometimes represents a form of food preservation, certain food processing operations aimed at enhancing certain food quality factors, may, in fact, promote the loss of other quality parameters, following processing, in short and long term storage. This obviously calls for routine investigations of the quality indices of marketed foods.

Derived products of *Piper guineense* leaves obtained via the processes of sun drying and grating are currently sold in many Nigerian open markets. It would appear however that there are scares if any literature reports on the compositional chemistry of sun dried, grated and stored *Piper guineense* leaves. In particular, literature reports on the responses of the alkaloids, glycosides, saponins and tannins constituents of *Piper guineense* leaves to sun drying, grating and in storage, seems nonexistence.

It should be emphasized that the heavy utilizations of *Piper guineense* leaves create necessity for research works into their compositional chemistry especially in their postharvest, post processed and stored conditions. Significantly, such research works are needed to safeguard the local consumers, in addition to being a possible means of stimulating wider interests especially from the more industrialized nations, in the use of *Piper guineense* leaves. There is no gainsaying that significant economic gains are attached to such findings.

All storages investigated in this work were at ambient conditions. There is dearth of information on the effect of a_W on the compositional chemistry of *Piper guineense* leaves. Significantly, there is increasing acknowledgement of the central role of water activity in food systems (Dibie, 2019). Also, Coultate (2002) posited that the concept of water activity is nowadays universally adopted by food scientists and technologists to quantify availability. Additionally, Belitz *et al* (2009) remarked that the storage quality of food does not depend on the water content, but on water activity (a_w). Some other researchers (Acker, 1969; Schoebel *et al*., 1969; Labuza, *et al*., 1970; Lajollo, *et al*., 1971; Eichner and Karel, 1972; Ukhun, 1986; Ukhun and Uwatse, 1988; Ukhun and Dibie, 1991; Dibie and Ukhun, 2020) have also shown that food stability, safety and other properties will be better predicted from a_w than from water content. Water activity is a measure of moisture availability for chemical reactions, microbial growth and activity (Troller and Christian, 1978). Clearly, the relevance of water activity in food stability studies cannot be over emphasized. Therefore, the choice of water activity as aspects of storage studies in this work is important.

In this work *Piper guineense* leaves heavily consumed by many Nigerians for their dietary and therapeutic values, in their postharvest, post processed and stored conditions was investigated for its alkaloids, glycosides, saponins and tannins constituents. These parameters though nonnutritive, are bio-active with significant therapeutic physiological and biochemical relevance. Spectrophotometric methods would be used in the quantitative determinations of the parameters examined in this work.Furthermore, data that will be generated in this study will be statistically analyzed. In particular, aspects of descriptive statistical evaluation of data and statistical evaluation of the relation between variables (ANOVA) will be carried out. International Business Machine (IBM), Statistical Package for Social Sciences (SPSS) will be used in statistical evaluation of data.

MATERIALS AND METHODS

Sample Collection

Samples Inspection and Cleaning

The *Piper guineense* leaves were pretreated in order to free them from contaminants and microbial infection. Thus any contaminating plants part was identified, removed and healthy leaves were used.

Samples Preparation

Samples of *Piper guineense* leaves used in this work were initially sun dried to constant weight. Thereafter, the sun dried samples were grated with the aid of Black and Decker 650W, BX550 blender. Subsequently, the grated samples were sieved using a 16 – mesh standard sieve (Pascall Eng. Co. Ltd. Sussex, England).

Samples Storage

The method of Rockland (1960) was used to establish a_w of 0.23, 0.52 and 0.97 in three separate air tight desiccators. Thereafter, three hundred grams of sun dried and grated *Piper guineense* leaves were weighed in triplicates into different 500ml glass beakers (Pyrex glass) and kept in the separate air tight desiccators. The storage desiccators were placed on laboratory bench at ambient conditions. On a monthly basis, samples were investigated for the parameters examined in this work. Samples storage duration was two months.

Qualitative Phytochemical Screening of Samples

In this study, all the chemicals used were BDH chemicals England; and they were of analytical grade.

Extraction of Samples for Phytochemical Screening

The methods of Oluduro *et al.* (2011) were used to obtain the various *Piper guineense* leaves crude extracts. *Piper guineense* leaves samples used for extraction were previously air – dried at room temperature for three weeks and subsequently ground using Black and Decker 650W blender. The extraction methods entailed weighing separately, 100g each of the test powdered samples into separate 500ml conical flasks containing 250ml of distilled water (aqueous extraction), 250ml of 99.5% methanol (methanol extraction), 250ml of absolute ethanol (ethanol extraction), 250ml of 85% n-hexane (hexane extraction), 250ml of 90% acetone (acetone extraction) and 250ml of 95% ethyl acetate (ethyl acetate extraction). Thereafter, the mixtures were covered. They were subsequently stirred every 24h using a sterile glass rod. With respect to the aqueous extraction, storage time was 3 days and 5 days for each of methanol, ethanol, hexane, acetone and ethyl acetate extractions respectively. Subsequently, the respective mixtures were separately filtered through Whatman filter paper No. 1 (Whatman limited, England). The respective filtrates obtained were concentrated at 40^0C after which they were used for qualitative phytochemicals screening carried out in this work.

Test for Saponins

The test for saponins was carried out by the addition of 5ml distilled water to 1ml of test extract in a test–tube. Thereafter, the test–tube with its contents were shaken vigorously. Saponins were indicated by the formation of stable foam which persisted for 20 minutes (Edeoga *et al*., 2005)

Test for Tannins

The method described by Earnsworthy *et al*. (1974) was used for qualitative tannins screening. The method entailed the addition of 1ml of 3% $FeCl_3$ to 1 ml of the test extract in a test–tube. The presence of blue–black green precipitate indicated the presence of tannins.

Test for Alkaloids

Qualitative determination of alkaloids was carried out in accordance with the method described by Mandal *et al*. (2000). The test entailed an initial evaporation of portion of the various extracts to dryness. Subsequently, 0.5g of each of the dried extract was separately dissolved in 10ml acidified alcohol (8ml of ethanol/2ml of HCl), thereafter boiled and filtered. Subsequently, 2ml of NH_4OH was added to a measured 5ml of the filtrate in a test tube. This was followed by the addition of 5ml of chloroform and then shaken gently. The chloroform layer was subsequently extracted with 10ml of acetic acid, and thereafter, the extract obtained was divided into two portions. To one portion was added Meryer's reagent, on the hand, Draggendoff's reagent was added to the second portion. In the portion wherein Meryer's reagent was added, the formation of a cream indicated positive test for the present of alkaloids. With respect to the second portion to which Draggendoff's reagent was added, the formation of reddish brown precipitate indicated positive test for alkaloids.

Test for Glycosides.

The method of Egwaikhide *et al*. (2007) was used in the qualitative glycosides screening. The qualitative test entailed the addition of 1ml of the test extract to 1ml of glacial acetic acid to which one drop of $FeCl_3$was previously added. Subsequently but gently, 1ml of conc. H_2SO_4 was added along the side of the test tube. The formation of a blown ring indicated the presence of glycoside.

In all qualitative tests for the phytochemicals determined, the absence of investigated phytochemical was indicated by "-"sign; whereas the presence of any investigated phytochemical was indicated by either "+", or "++" depending on the intensity of occurrence.

Quantification of Phytochemicals

Extraction of phytochemicals

The phytochemicals examined in *Piper guineense* leaves were extracted with methanol using the soxhlet extractor. 100g of ground *Piper guineense* leaves was weighed and then uniformly distributed in a thimble. Thereafter, 400ml of methanol (material: solvent ratio is 1:4) was measured in the extraction flask, and the extraction was subsequently carried out for 20h (it was observed that the solvent in the siphon tube of the extractor became colourless after 18h of extraction, though the extraction was continued for another 2h to ensure that exhaustive extraction has been carried out). Subsequently, the methanol was removed with the aid of rotary evaporator (model: RE52-3 SEARCH TECH INSTRUMENTS).

Determination of Total Tannins Content

Total tannins content determination was carried out colorimetrically in accordance with the method described by Siddhuraju and Manian (2007).

To 0.5ml of the different methanol extracts of samples in separate test tubes, 100mg of polyvinyl polypyrrolidone and 0.5ml of distilled water were added. Subsequently, the test–tubes were shaken properly, after which the solutions in the respective test – tubes were incubated at 4°C for 4h. Thereafter, they were centrifuged at 5,000rpm for 5min and allowed to settle, then, 0.2ml of the supernatant was measured out [the supernatant has only simple phenolics free of tannins, the tannins would have been precipitated along with the polyvinyl Polypyrrolidone (Senguttuvan *et al.*, 2014). Blank was also prepared. The phenol content of the supernatant was determined by the method given below. Thereafter, tannins content was obtained by difference.

Determination of Total Phenols Content

Total phenol was determined spectrophotometrically by the Folin – Ciocalteau method as described by Kujala *et al*. (2000).

To a 1ml of the methanol extract of sample and standards (gallic acid solutions of concentration: 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10mg/l prepared by dissolving gallic acid in a 1:1, v/v mixture of methanol: water) in separate test tubes, were added 5ml each of Folin–Ciocalteau reagent (1:10 dilution with distilled water) and mixed thoroughly. Thereafter, 4ml of 1M Na_2CO_3 was added to each of the test tubes and again, mixed thoroughly. Subsequently, the solution was allowed to stand for 30min in the dark at room temperature. Blank was also prepared. This was followed by absorbance reading at 765nm, using a Uv/visible spectrophotometer. The total phenol content was calculated from the standard graph of gallic acid, and the results were expressed as gallic acid equivalent (mg/g), which is a common reference compound.

Tannins (mg GAE/g extract) = Total phenols (mgGAE/g extracts) - Free phenols (mgGAE/g extract)
Where: GAE = Gallic acid equivalent.

Determination of Total Saponins Contents

Total saponins contents of the various *Piper guineense* leaves extracts were spectrophotometrically determined in accordance with the method described by Makkar *et al*. (2007). Half milliliter of the methanol extract was measured into a test tube after which 0.5ml of distilled water was added. Thereafter, 0.25ml of vanillin reagent (prepared by dissolving 800mg of vanillin in 10ml of 99.5% ethanol) was added. This was followed by the addition of 2.5ml of 72% sulphuric acid (v/v). The reaction mixture was mixed thoroughly. Subsequently, the test-tube was immersed in a water bath maintained at 60^0C for 10min. Blank was prepared alongside. At the completion of 10min of immersion in the water bath, the reaction mixtures were cooled in ice cold water bath for 4min. The absorbance was subsequently read at 544nm against the prepared blank, using a Uv/vis spectrophotometer. The total saponins contents were calculated from the standard graph of diosgenin, and the results were expressed as diosgenin equivalent (mg/g).

Determination of Total Alkaloids Content

The determination of total alkaloids contents of the various methanol extracts of *Piper guineense* leaves was spectrophotometrically carried out in accordance with the method described by Shamsa *et al*. (2008).

Standard curve of atropine was used to calculate the total alkaloids content of the various methanol extracts. Stock atropine solution was prepared by dissolving 2mg of atropine in 10ml of distilled water to give 0.2mg/ml. To a 1ml of the methanol extract of the test sample was added 5ml of 2M HCl. The mixture was shaken thoroughly and allowed to stand for 1min. Thereafter, the mixture was filtered through a Whatman No. 41 filter paper, and the pH of the extract neutralized with 0.1M NaOH. Subsequently, 1ml of this solution was transferred to a separating tunnel after which 5ml of bromocresol green solution was added, followed by addition of 5ml of phosphate buffer. Subsequently, the contents of the separating funnel were mixed properly, after which 5ml of chloroform was added. Thereafter, the separating funnel was shaken vigorously. Subsequently, it was allowed to stand, after which the chloroform layer was collected in a 10ml volumetric flask, and made up to volume with chloroform. The standard and blank were subjected to the same treatment. Absorbance reading of the complex in the various chloroform extracts was taken at 470nm, using a Uv/vis spectrophotometer against a blank. The total alkaloid content was calculated from the standard graph of atropine, and the results were expressed as atropine equivalent (mg/g).

Determination of Cyanogenic Glycosides

Cyanogenic glycosides in *Piper guineense* leaves samples studied were determined spectrophotometrically using the alkaline picrate method as described by Onwuka (2005), in which, to a 5.0g of ground *Piper guineense* leaves sample that is contained in a conical flask was added 50ml of distilled water. Thereafter, the conical flask was stoppered and allowed to stand for 16h under ambient conditions. Subsequently, the content of the conical flask was filtered, and the filtrate used for subsequent determination. 1ml of the filtrate was measured into a test tube, after which 4.0ml of alkaline picrate (prepared by dissolving 1g of

picrate and 5g of Sodium carbonate in 50ml of distilled water and heated to a temperature of 35^{o}C; after cooling, the solution was transferred to a volumetric flask and made up to 200ml with distilled water) was added to the test-tube containing the filtrate. Thereafter, the content of the test-tube was shaken thoroughly and the tube with its content, were subsequently incubated in a thermostatically controlled water bath where the temperature had been maintained at 30°C. Subsequently, absorbance reading against a blank was taken at 490nm, using an Ultraviolet/visible spectrophotometer. From the standard curve obtained with absorbance values of various KCN standard solutions, extrapolation was made, and values obtained were used in calculating the cyanide content of sample.

RESULTS AND DISCUSSION

Phytochemical screening

The results of phytochemical screening of samples of *Piper guineense* leaves obtained from some markets in Benin City are presented in Table 1.

Table 1: Qualitative Analysis of Selected Phytochemicals Constituents of samples of Piper guineense leaves

S/N	Phytochemicals/ family	*Piper guineense* Leaves Extracts					
		Aqueous	Methanol	Ethanol	n-Hexane	Acetone	Ethyl acetate
1	**Glycosides**						
	*sample 1	+	+	+	-	+	+
	*sample 2	+	+	+	-	+	+
	*sample 3	+	+	+	-	+	+
	*sample 4	+	+	+	-	+	+
	*sample 5	+	+	+	-	+	+
2	**Saponins**						
	*sample 1	++	++	+	-	-	-
	*sample 2	++	++	+	-	-	-
	*sample 3	++	++	+	-	-	-
	*sample 4	++	++	+	-	-	-
	*sample 5	++	++	+	-	-	-
3	**Tannins**						
	*sample 1	++	++	++	++	++	++
	*sample 2	++	++	++	++	++	++
	*sample 3	++	++	++	++	++	++
	*sample 4	++	++	++	++	++	++
	*sample 5	++	++	++	++	++	++
4	**Alkaloids**						
	*sample 1	++	++	++	-	+	+
	*sample 2	++	++	++	-	+	+
	*sample 3	++	++	++	-	+	+
	*sample 4	++	++	++	-	+	+
	*sample 5	++	++	++	-	+	+

+ = Slightly present, ++ = Largely present, - = Absent

Findings indicated that qualitatively, glycosides content of samples 1,2,3,4, and 5 showed no noticeable variation from each other. It seemed therefore, that the various cultivars of *Piper guineense* leaves marketed in Benin City, do not have their glycosides content significantly influence by location. Suffice it to say that *Piper guineense* traditionally, is not cultivated as it is the case with farm crops. Rather, *Piper guineense* is more of wildly grown tropical forest plant. Thus the reduced variations in agronomic practices could even if partly, account for the findings obtained in the qualitative determinations of the glycosides content of *Piper guineense* leaves samples obtained from some markets in Benin City. Additionally, postharvest handling time before *Piper guineense* leaves are sent to the market for sale is short, hence the occurrence of reactions associated with storage is minimal before *Piper guineense* leaves get to the consumers. These factors are speculated to have effects on the results obtained. It is also discernible from results that in *Piper guineense* leaves investigated, glycosides were noted to be slightly present in the aqueous, methanol, ethanol, acetone and ethyl acetate extracts, but absent in n-hexane extract. The variation in the pattern of glycosides occurrence in the various solvents extracts of *Piper guineense* leaves is worthy of note, as it could be relevant in the selection of solvents for the processing of *Piper guineense* leaves.

The observed pattern of occurrence of saponins in *Piper guineense* leaves was such that, predominant occurrence of saponins was observed in the aqueous and methanol extracts of *Piper*

guineense leaves, but in the ethanol extract, findings indicated slight presence of saponins. Additionally, findings did not reveal presence of saponins in the n-hexane, acetone and ethyl acetate extracts of *Piper guineense* leaves studied. It does appear that solvent type has effect in the extraction of saponins from *Piper guineense* leaves. Significantly, the more polar solvents used in this study, that is, aqueous and methanol, extracted saponins from *Piper guineense* leaves, more than the less polar ones. Results obtained in this study further indicated that Alkaloids occurred predominantly in the aqueous, methanol, and ethanol extracts of the leaves of *Piper guineense*, but slightly in the respective acetone and ethyl acetate extracts. On the other hand, in the n-hexane extract of the leaves of *Piper guineense*, alkaloids were not detected.

Quantitative Phytochemical Determinations

The results of quantitative analysis of alkaloids, cyanogenic glycosides, saponins and tannins levels in raw, sun dried, grated and stored *Piper guineense* leaves are presented in Table 2.

Table 2: Quantification of Selected Phytochemicals Contents of Raw, Sun Dried, Grated and Stored *Piper guineense* Leaves

S/N	Parameter	Raw (fresh) sample	Sun dried and pre-stored sample	Stored samples									
				Storage conditions/time (months)									
				a_w0.97		a_w0.52		a_w0.23		Open Laboratory			
										Covered container		Opened container	
				2-months	1-month	2-months	1-month	2-months	1-month	2-months	1-month	2-months	1-month
1	Tannins (mg/g)	2.32 ± 0.18	2.45 ± 0.11	4.52±0.93	3.18±0.26	6.02±1.13	3.85±0.91	8.46±1.37	4.02±0.85	4.86±1.00	3.36±0.41	3.93±0.64	2.86±0.39
2	Alkaloids (mg/g)	6.73 ± 1.25	6.89 ± 1.31	14.15±2.63	7.52±1.09	18.77±1.64	9.16±1.25	26.35±2.41	12.59±1.61	16.03±2.09	8.64±0.91	7.22±1.08	6.93±1.25
5	Saponins (mg/g)	1.24 ± 0.51	1.30 ± 0.42	13.21±2.14	4.98±0.63	17.52±2.10	6.38±1.12	24.60±1.49	9.53±1.28	14.93±2.18	5.71±1.00	6.74±1.15	2.56±0.37
6	Cyanogenic glycosides (mg/g)	0.20 ± 0.05	0.20 ± 0.03	0.46±0.11	0.28±0.04	0.61±0.13	0.38±0.09	0.86±0.21	0.46±0.85	0.49±0.06	0.35±0.09	0.24±0.07	0.21±0.03

It is deducible from the results (Table 2) that among the phytochemicals examined, alkaloids with a concentration of 6.73±1.25mg/g occurred most in raw *Piper guineense* leaves. The sequence of occurrence of the phytochemicals examined in decreasing order is tannins (2.32±0.18mg/mg), saponins (1.24±0.51mg/g), and cyanogenic glycosides (0.20±0.05mg/g). Also discernable from the results presented in Table 2 was that sun drying of *Piper guineense* leaves led to increases in the concentrations of the phytochemicals examined. Significantly, the values of the phytochemicals examined in sun dried *Piper guineense* leaves were: alkaloids (6.89±1.31mg/100g), tannins (2.45±0.11mg/100g) and saponins (1.30±0.42mg/g). It would appear from the results that the value of cyanogenic glycosides (0.20±0.03mg/g) remained the same as it was in raw *Piper guineense* leaves. Clearly, the reported increases in alkaloids, tannins and saponins levels following sun drying of *Piper guineense* leaves were indications of continued biosynthesis of these secondary metabolites postharvest and under sunlight. As earlier noted from the results presented in Table 2, there was no increase in the concentration of cyanogenic glycosides following sun drying of *Piper guineense* leaves. Several factors could be responsible for this observation. In particular, it could be that the initial low concentration of cyanogenic glycosides in raw *Piper guineense* leaves contributed to the result obtained for cyanogenic glycosides in the sun dried samples. Additionally, in *Piper guineense* leaves, if neutral interactions occurred with respect to cyanogenic glycosides biosynthesis and sunlight, then sun drying of raw *Piper guineense* leaves will not lead to increase in its cyanogenic glycosides content. Furthermore, it is imperative to mention that simultaneous occurrence of synthesis and degradation reactions can take place in chemical systems. Therefore, with respect to sun drying of *Piper guineense* leaves, if the rate of cyanogenic glycosides biosynthesis and that of their degradations are equal, then the value of cyanogenic glycosides will remain unaffected following sun drying. Hence, it is suggested, that the findings of this work with respect to the relationship between the cyanogenic glycosides levels in raw *Piper guineense* leaves and the corresponding sun dried form be viewed from these points of view.

It is discernible from the open laboratory storage studies with sun dried and grated *Piper guineense* leaves separately stored in opened and closed containers, that at the end of the two months storage time, storage increases occurred in the levels of the phytochemicals examined. Significantly, findings indicated the following results for sun dried and grated *Piper guineense* leaves kept in opened container and stored in open laboratory: tannins (3.93±0.64mg/g), alkaloids (7.22±1.08mg/g), saponins (6.74±1.15mg/g) and cyanogenic glycosides (0.24±0.07mg/g). It was also deducible from findings that the corresponding values for the phytochemicals examined in samples stored in closed container and kept in the open laboratory were:- tannins (4.86±1.00mg/g), alkaloids (16.03±2.09mg/g), saponins (14.93±2.18mg/g), and cyanogenic glycosides (0.49±0.06mg/g). What is evident here is that the storage of sun dried and grated *Piper guineense* leaves in closed container, compared to opened container, led to greater storage increases in the levels of the above mentioned secondary metabolites examined in stored *Piper guineense* leaves. Presumably, degradation and synthesis reactions with respect to the phytochemicals examined occurred simultaneously in sun dried and grated *Piper guineense* leaves stored in the open laboratory under ambient conditions. Then, the conditions in the closed containers it would appear, favoured synthesis reactions better than the conditions in the closed containers. It could also mean that in the closed container, less of degradation reactions involving the phytochemicals examined occurred when compared to the degree of degradation reactions that probably took place in the sun dried, and grated *Piper guineense* leaves stored in the opened container. Significantly, if the degradations reactions are favoured by sunlight, then more of it should take place in the samples stored in the opened container. Interestingly, also, it was deducible from results that at the end of the study period, the pattern of the final concentrations of the phytochemicals examined in the sun dried and grated *Piper guineense* leaves stored in the open laboratory whether in closed or opened containers, was related to that of their initial concentrations in the pre-stored samples. Significantly, the results indicated that the higher the initial concentration of the phytochemical examined in the pre-stored samples, the higher its value at the end of the two months storage period. Thus, abundance seemed to be relevant in the final concentrations of the phytochemicals examined in stored sun dried and grated *Piper guineense* leaves kept in the open laboratory,

The water activity studies with tannins, alkaloids, saponins and cyanogenic glycosides in sun dried, grated and stored *Piper guineense* leaves indicated that water activity fostered the biosynthesis of the aforementioned phytochemicals in the stored samples. It was particularly observed that the values of tannins, alkaloids, saponins and cyanogenic glycosides in sun dried, grated and stored *Piper guineense* leaves at the end of the storage period, were highest in the samples stored at a_w of 0.23. Remarkably, the results obtained for the phytochemicals examined in the samples stored at a_w 0.23, were tannins (8.46±1.37mg/g), alkaloids (26.35±2.41mg/g), saponins (24.60±1.49mg/g) and cyanogenic glycosides (0.86±0.21mg/g). On the other hand, the results obtained for the phytochemicals examined in the samples stored at

the highest storage a_w of 0.97 used in this study at the end of the storage time were tannins (4.52±0.93mg/g), alkaloids (14.15±2.63mg/g), saponins (13.21±2.14mg/100g) and cyanogenic glycosides (0.46±0.11mg/g).It was noted that the corresponding values of these secondary metabolites examined in sun dried and grated *Piper guineense* leaves stored at a_w 0.52 were lower than those of samples stored at a_w 0.23, but higher than those of samples stored at a_w 0.97. The implication of this is that the reactions that enhanced the values of the phytochemicals examined in the samples stored at different a_w, were favoured more at the low storage a_w of 0.23, wherein it would appear that the highest reduction in degradation reactions occurred, in comparison with the higher water activities used in this study. With respect to the storage conditions studied in this work, the reported storage increases were statistically significant ($P<0.05$). The noted increases in the phytochemicals examined after the sun drying of *Piper guineense* leaves were however not statistically significant ($P<0.05$).

It was posited by Sofowara (2008) that the age of plant and the season of harvest determine the amount of bioactive ingredients in them. It could also mean that particularly in the raw *Piper guineense* leaves samples studied in this work, in addition to the effects of postharvest handling on the concentrations of the phytochemicals examined, the factors mentioned by Sofowara (2008) contributed to the levels obtained for the different phytochemicals examined.

The relevance of occurrence of the phytochemicals examined, in *Piper guineense* leaves cannot be over emphasized. Significantly, some authors (Okwu and Okwu, 2004; Njoku and Akumefula, 2007, Dahuru *et al*., 2006; Parekh and Chinda, 2007; and Ekeanyamwu *et al*., 2010) noted that tannins which are reported in this work to be present in *Piper guineense* leaves, have anti-inflammatory, antioxidant, and antimicrobial properties; they are also relevant for healing wounds, regeneration and diuretics. However, tannins are anti-nutritional compounds (Solihah *et al*., 2012). They destroy thiamin, cause protein precipitation thereby inhibiting digestive enzymes; as well as reduce the bioavailability of iron (Omaye, 2004). The relevance of tannins in *Piper guineense* leaves especially in the processed and stored forms should be viewed from both the demerits and merits of tannins.

The occurrence of saponins in *Piper guineense* leaves especially in the stored forms as this study has revealed storage increases in saponins, will be nutritionally and therapeutically desirable to the extent that their threshold value is not exceeded, when they will becometoxicologically relevant. In particular, Fennema (1996) noted that saponins are inherent toxicants in plants, and they cause hemolysis of erythrocytes in-vitro. However, Okwu and Josiah (2006) considered the haemolytic activity of saponins therapeutically relevant. According to them, saponins have the property of precipitating and coagulating red blood cells (haemolytic activity), and are therefore used to stop bleeding; as well as in treating wounds. Also, Zwane *et al*. (2011) posited that saponins containing plants are important for their haemolytic, expectorative, anti-inflammatory and immune-stimulating activities. Evidently, the therapeutic values of saponins are overwhelming. The sun dried, grated and stored *Piper guineense* leaves as this work revealed, indicated good source of saponins. The nutritional and medicinal relevance of this plant with respect to its saponins content is enormous. The dose perhaps makes the difference between benefits and demerits of its saponins content.

Findings from this work also revealed slight increases in the levels of cyanogenic glycosides constituents of sun dried and grated *Piper guineense* leaves, when the samples were stored. It is imperative to mention that cyanogenic glycosides are precursors of hydrocyanic acid (HCN) and the latter is a toxicant. Some authors have indicated that long term consumption of cassava products containing low levels of HCN produces goitre and neuropathy (Maner and Gomez, 1973). Though HCN is thermally labile, the risk of cyanide poisoning exists when foods rich in cyanogenic glycosides are not properly processed, especially by heating before consumption. The increasing trend of consumption of raw vegetables by humans is worthy of note. Heat treatment of raw, grated and fermented cassava to produce *garri* helps to reduce the HCN content (Ukhun and Dibie, 1991). Thus, it could also mean that proper heat treatment of sun dried, grated and stored form of *Piper guineense* leaves, would help reduce any existing HCN in them..

CONCLUSION

Alkaloids, saponins, tannins and cyanogenic glycosides constituents of *Piper guineense* leaves were investigated both in the raw samples, as well as in the sun dried grated and stored samples. The findings indicated the presence of the examined phytochemicals in the studied samples. Additionally, increases in the levels of the phytochemicals examined were noted in the sun dried samples as well as in the sun dried and stored samples. The relevance of standardizing storage conditions for sun dried, grated and stored *Piper guineense* is thus obvious. In particular, while storage of sun dried and grated *Piper guineense* leaves especially at low a_w enhanced the availability of the studied secondary metabolites, there is need to strike a balance between the necessity for enhanced availability and toxicity, in the utilization of stored sun dried and grated *Piper guineense* in dietary and pharmaceutical preparations.

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