COMPARATIVE STUDIES OF PHYTOCHEMICALS IN *Lasianthera africana* AND *Piper umbellatum* LEAVES IN SOUTHERN, NIGERIA.

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Abstract: The quantitative assessment of phytochemicals in *Lasianthera africana* P.Beauv and *Piper umbellatum* L. leaves eaten mostly in southern Nigeria were carried out. The phytochemicals (hydrogen cyanide, oxalate, phytate, tannin, saponin, trypsin-inhibitor, alkaloid, flavonoids, and organic acids) were assessed following standard analytical procedures. The concentration of phytochemicals inherent in the leaves of *L. africana* and *P. umbellatum* varies: oxalate (7.766 ppm; 3.950 ppm), phytate (5.900 ppm; 7.426 ppm), saponin (6.800 ppm; 4.060 ppm), tannin (3.159 ppm; 1.837 ppm), trypsin-inhibitor (0.796 ppm; 0.863 ppm) and hydrogen cyanide (0.004 ppm; 0.002 ppm), total alkaloids (10.628 g/100 g; 6.460 g/100 g), total flavonoids (12.161 g/100 g; 6.158 g/100 g) and total organic acids (3.434 g/100 g; 7.035 g/100 g), respectively. The most abundant class of alkaloids and flavonoids in both species were benzypyrole and flavanol, respectively. The outcome of the study revealed that phytochemicals present in leaves varies from *L. africana* and *P. umbellatum*. The leaves of *L. africana* had higher concentration of hydrogen cyanide, oxalate, saponin, tannin, alkaloids and flavonoids compared to *P. umbellatum* leaves, which recorded high phytate, trypsin-inhibitor and organic acids. The presence of these bioactive components in the leaves of *L. africana* and *P. umbellatum* could be used for synthesizing useful medicine to reduce the risk associated with some life threatening diseases.

Keywords: *Comparative, Lasianthera africana, Piper umbellatum, phytochemicals, risk*

Introduction

Health promoting phytochemicals such as alkaloids, flavonoids, saponins and tannins which aids in the reduction of the risk of cancer and other degenerative diseases are contained in green leafy vegetables (Levander, 1990; Okwu, 2005). Some anti-nutritional phytochemicals such as tannins and phytate exhibit protective effect on human body, thereby making them to serve a dual purpose of reducing some essential nutrients and protecting the body against a number of biochemical, physiological and metabolic disorders (Aletor and Adeogun, 1995). The presence of these wide range of bioactive phytochemicals and secondary metabolites has made plants (vegetables) promising source of modern synthetic drugs for management of several diseases (Balogun *et al*., 2016). *Lasianthera africana* belongs to the family Icacinaceae. It is a glaborous shrub up to 4 m high with terete branchlets and white flowers in umbellate head like clusters found as understorey in secondary jungle and thickets in the rainforest of southern Nigeria, Western Cameroon extending to Zaire (Burkill, 1985). This plant is a delicious green leafy vegetable whose leaves are widely consumed as vegetable in soups mainly in the southern and eastern parts of Nigeria. In folk medicine, *L. africana* is used in the treatment of various diseases especially infections.

Its activities against pathogenic microorganisms like fungi, bacteria and protozoa have been reported (Andy *et al*., 2008). In addition, it is used as an anti-analgesic, laxative, anti-diabetic, antipyretic, and anti-malarial therapy (Okonkon *et al.,* 2009). *Piper umbellatum* Linn belongs to the family Piperaceae. According to Dyer and Palmer (2004), the genus *Piper* comprises of about 1000 – 2000 species of shrubs, herbs and lianas that has economic and ecological values. *Piper umbellatum* is a soft wooded, aromatic, evergreen shrub, often straggling. Isobe *et al*. (2002) discovered that *P. umbellatum* has antibacterial properties with specificity on *Heliobacter pylori.* The leaves of *P. umbellatum* are widely used as an emollient and in the treatment vulnerary and antiseptic disorders (Ropke *et al*., 2006). Phytochemical studies of *P. umbellatum* have demonstrated the presence of terpenes (mainly found in essential oil), alkaloids, flavonoids, sterols, and other classes of secondary metabolites (Carles and Roersch, 2010). *Piper umbellatum*, known as "nwa njaa-nja" in Etche Ethnic group in Nigeria, is traditionally used in the treatment of premature babies, before the advent of modern medicine (Nwauzoma *et al*., 2013).

According to Ebana *et al*. (1996), the leaves of *L. africana* are rich in chemical compound of nutritional and medicinal importance. Etukudo (2003) also reported that aqueous extract of the leaf is usually taken orally or in enema form to treat indigestion, stomach discomfort and internal heat. The fruits are also used for the treatment of asthma, hypertension, skin diseases and wound healing (Isong and Idiong, 1997; Ajibesin *et al*., 2008; Jiofack *et al*., 2009). Some phytochemicals such as alkaloids, flavonoids, terpenes, anthraquinones, phlobatannins, cardiac glycosides, saponins and tannins occur in substantial levels in the leaf (Bassey *et al*., 2006). Andy *et al*. (2008) also reported that from time immemorial the plant has been exploited by traditional herbalists for the treatment of various ailments including typhoid fever, diarrhoea and candidacies amongst other ailments. However, according to Saidu and Okorocha (2013), plants are not necessarily required by humans for sustaining life, they also protect human against diseases. In our society today, the presence of health promoting and protecting compounds

such as alkaloids and flavonoids in the leaf can be used to manage diabetes mellitus that is common presently (Inyang *et al*., 2015). These plants, *L. africana* and *P. umbellatum* form part of the vegetables consumed mainly in southern Nigeria and considering the implications of some phytochemicals on human health, the study is aimed at evaluating some of the phytochemical constituents of *L. africana* and *P. umbellatum* leaves.

Materials and Methods

Source of plants and analysed parameters

The leaves of *L. africana* and *P. umbellatum* (Plates 1 and 2) were collected in a garden within the University of Port Harcourt (Lat. 4°54'15"N, Long. 6°54'35"E) and properly identified by a Taxonomist in the Department of Plant Science and Biotechnology Herbarium, University of Port Harcourt. The leaves were rinsed with distilled water to remove dirt and prepared differently to be used for respective analyses: hydrogen cyanide, oxalate, phytate, tannin, saponin, trypsin-inhibitor, alkaloid, flavonoids, and organic acids. The analyses were carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The hydrogen cyanide, oxalate, phytate, tannin, saponin, trypsin-inhibitor, flavonoid and alkaloid contents of the *L. africana* and *P. umbellatum* leaves were determined following method used by Okonwu *et al.* (2017a, 2017b) and Okonwu and Muonekwu (2019) while organic acids was determined according to method of Okonwu and Akonye (2019). Waters 616/626 liquid chromatography was the tool used in determining flavonoids, alkaloids, and organic acids content.

Plate 1: *Lasianthera africana* P. Beauv

Plate 2: *Piper umbellatum* L.

Results

Phytochemicals

The phytochemical constituents of *L. africana* and *P. umbellatum* leaves varied in their concentrations (Figure 1). These constituents had concentrations in the following order for *L. africana* and *P. umbellatum*, respectively: oxalate (7.766 ppm; 3.950 ppm), phytate (5.900 ppm; 7.426 ppm), saponin (6.800 ppm; 4.060 ppm), tannin (3.159 ppm; 1.837 ppm), trypsin-inhibitor (0.796 ppm; 0.863 ppm) and hydrogen cyanide (0.004 ppm; 0.002 ppm).

Alkaloids

A total of forty-one (41) alkaloids, which are grouped into nine (9) classes were present in the leaves of *L. africana* and *P. umbellatum* with total alkaloids of 10.628 g/100 g and 6.460 g/100 g, respectively (Figure 2). These classes of alkaloids with number of individual alkaloids were: purine (4), proto-alkaloid (4), piperidine (3), pyridine (3), isoquninoline (10), acridine (1), benzypyrole (8), tropane (3), and quinoline (6). Benzypyrole alkaloid was the most abundant class of alkaloid followed by quinoline, isoquinoline while acridine alkaloid was the least present in the plants. The percentage of these classes of alkaloids with respect to total alkaloid are benzypyrole (55.67%, 44.10%), quinoline (21.77%, 31.64%), isoquinoline (16.25%, 17.55%) and acridine (0.15%, 0.22%) for *L. africana* and *P. umbellatum*, respectively. This showed that the concentration of class of alkaloid may varied with the percentage occurrence from one plant to another. The leaves of *L. africana* had more alkaloid content compared to *P. umbellatum* leaves.

Flavonoids

A total of thirty-six (36) flavonoids, which are in six (6) classes were present in the leaves of *L. africana* and *P. umbellatum* with total flavonoids of 12.161 g/100 g and 6.158 g/100 g, respectively (Figure 3). These classes of flavonoids with number of individual flavonoids were: flavones (8), flavanols (8), isoflavones (3), flavonols (4), flavanone (12), and anthocyanin (1). The flavanols was the most abundant class of flavonoids followed by isoflavones while anthocyanin was the least present in the plants. The percentage of these classes of flavonoids with respect to total flavonoids are flavanols (59.01%, 61.22%), Isoflavones (36.31%, 31.20%), and anthocyanin (0.68%, 1.40%) for *L. africana* and *P. umbellatum*, respectively.

This showed that the concentration of class of flavonoids may varied with the percentage occurrence from one plant to another. The leaves of *L. africana* had more flavonoids content compared to *P. umbellatum* leaves.

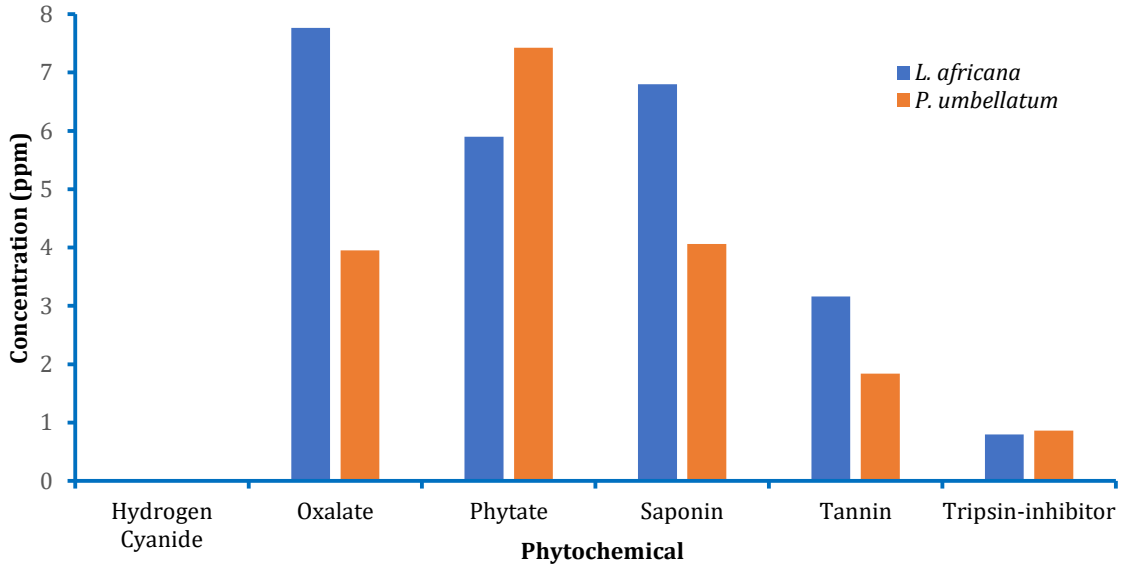

Figure 1: Phytochemical constituents of *Lasianthera africana* and *Piper umbellatum* leaves

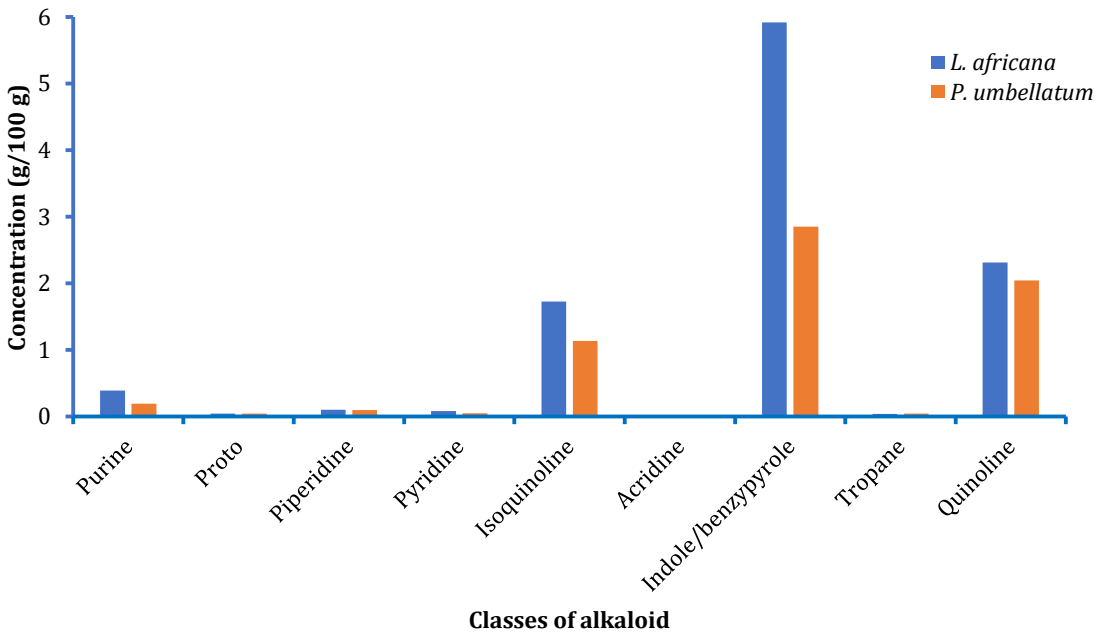

Figure 2: Alkaloid concentration in *Lasianthera africana* and *Piper umbellatum* leaves

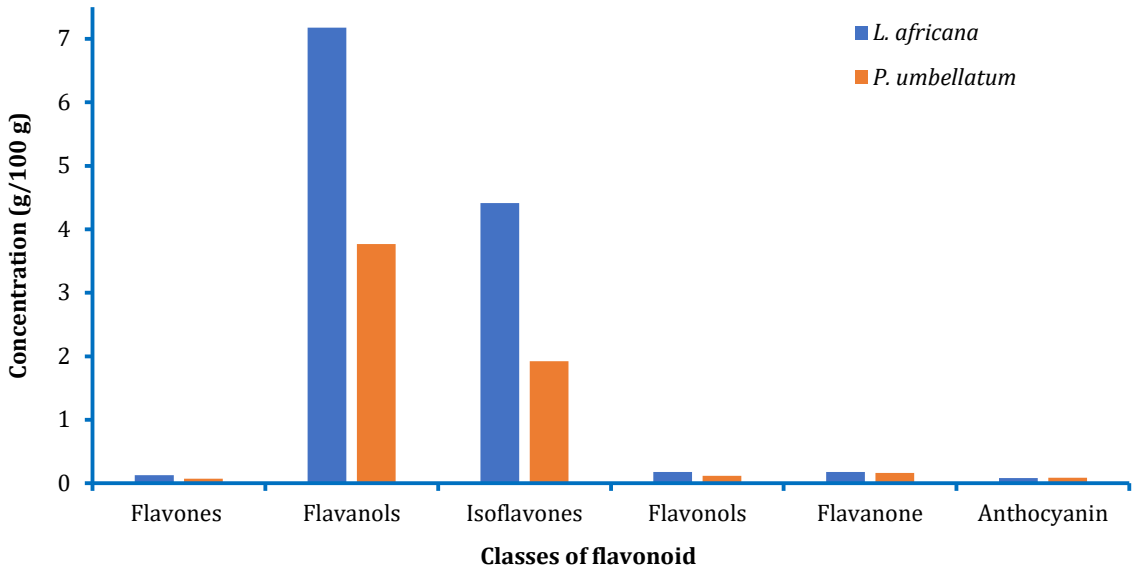

Figure 3: Flavonoid concentration in *Lasianthera africana* and *Piper umbellatum* leaves

Organic acids

The *P. umbellatum* leaves (7.035 g/100 g) had a higher total organic acid than the *L. africana* leaves (3.434 g/100 g) (Table 1). The most abundant organic acids in *P. umbellatum* were hydrochlorathiazide acid and captopril acid with 28.47% and 16.36% of the total organic acid while the least is E-strophanthin acid (0.03%). For *L. africana*, shikimic acid is most abundant organic acid followed by hydrochlorathiazide acid and glucamic acid with 18.67%, 12.70% and 10.86%, respectively while the least is glycyrhizic acid (0.03%).

Table 1: Organic acids content of *Lasianthera africana* and *Piper umbellatum* leaves

Organic acids (g/100 g)	*L. africana*	*P. umbellatum*
18-beta-glycyrrhetinic acid	0.002	0.009
Acetic acid	0.056	0.087
Ameodipine acid	0.007	0.028
Atenonol acid	0.031	0.141
Butyric acid	0.294	0.469
Captopril acid	0.250	1.151
Citric acid	0.057	0.054
Digitoxin acid	0.006	0.025
Digoxin acid	0.012	0.057
Enalapril acid	0.032	0.052
E-strophanthin acid	0.002	0.002
Furosemide acid	0.005	0.004
Galacturonic acid	0.227	0.172
Gallic acid	0.061	0.074
Glucamic acid	0.373	0.366
Glycyrhizic acid	0.001	0.005
Glycyrrhetinic acid	0.018	0.069
Hydrochlorathiazide acid	0.436	2.003
Lisinopril acid	0.022	0.019
Malic acid	0.294	0.469
Metoprolol acid	0.141	0.552
Nifedipine acid	0.008	0.037
OLeandrin acid	0.013	0.021
Propranolol acid	0.007	0.012
Pyvruvic acid	0.036	0.027
Shikimic acid	0.641	0.495
Succinic acid	0.233	0.438
Valeric acid	0.166	0.184
Varapamil acid	0.003	0.013
Total Organic acid	3.434	7.035

Discussion

Studies have shown that phytochemicals are sourced most from natural plant food sources (Schippers, 2000; Liu, 2004). Also, some phytochemicals have the ability to act as antioxidant (Tsao and Akhtar, 2005). Oxalates as found in both plants in this study are anti-nutritive in nature and can form non-absorbable insoluble salts with Ca^{2+}, Fe^{2+} and Mg^{2+} rendering these minerals unavailable (Philip and Owen, 2014). However, a diet with high oxalate content is prone to increase kidney stone formation and may lead to reduction of Ca absorption (Philip and Owen, 2014). It is estimated that 80% of kidney stones are formed from calcium oxalates (Coe *et al*., 2005). According to Inuwa *et al.* (2011), the lethal doses of phytate, tannin and oxalate are 50-60 mg/kg, 30 mg/kg and 2.5 g/kg, respectively. The phytate, tannin and oxalate of *L. africana* and *P. umbellatum* were very low compared to the lethal dose reported by Inuwa *et al.* (2011) and this suggest that the consumption of these plants may not have health implications. It has been reported that leaves containing tannins can be used for the treatment of intestinal disorder (Akindahunsi and Salawu, 2005). Basu *et al.* (2007) documented that the presence of tannin reduces plasma fat. The levels of oxalate in the human diet either as vegetables or fruits are important due to the associated health risks. The trypsin-inhibitors obtained in this study were lower to that reported by Kuku *et al.* (2014). They reported that trypsin inhibitor content for unprocessed and under-processed seeds of *Telfairia occidentalis* were 23.18 TIU/mg and 2.13 TIU/mg. Although the plant parts are different, this suggests that the leaves contain low trypsin-inhibitors than the seeds.

Flavonoids from natural sources and their derivatives have been crucial bioactive molecules used in medicine (Torres-Piedra *et al*., 2010) and have health benefits (Brodowska, 2017). They have the protective capacity against biological impurities from microbes (Grace, 1994; Treutter, 2005). It has been reported that flavonoids obtained from food materials have the potential to inhibit tumour formation (Ren *et al.*, 2003; Aggarwal and Shishodia, 2006). Flavonoids have been reported to play similar roles as vitamins in the human system (Ostrowska and Skrzdlewska, 2005; Mitek and Gasik, 2009). The two plants in this study, *L. africana* and *P. umbellatum,* have flavonoids in them thus conferring beneficial values.

On the other hand, the malic acid concentration obtained in the *L. africana* and *P. umbellatum* leaves using HPLC was higher compared to that reported on several vegetables by others (Belitz *et al*., 2004; Ayaz *et al*., 2006; Arias-Carmona *et al.,* 2014). Belitz *et al*. (2004) observed distinctions in the malic acid content between vegetables on fresh weight basis: spinach (0.042 g/100 g) and rhubarb (0.91 g/100 g). Also, Ayaz *et al*. (2006), stated that *Brassica oleraceae* (Kale) leaf contained 0.151 g/100 g malic acid on dry mater. Turnip greens (0.089 g/100 g) and turnip tops (0.037 g/100 g) varied in their malic acid content (Arias-Carmona *et al.,* 2014). The higher concentration of malic acid in *L. africana* and *P. umbellatum* leaves may probably increase the acidity of the prepared food and thus giving more flavour. The citric acid concentration in *L. africana* and *P. umbellatum* leaves were lower and differ from the values reported by other researchers (Ayaz *et al*., 2006; Flores *et al.* 2012; Arias-Carmona *et al.,* 2014). Ayaz *et al.* (2006) reported a value of 2.213 g/100 g dry matter in kale leaf. Flores *et al.* (2012) indicated that fresh weight of leafy vegetables showed a high concentration of citric acid that varied from 0.077, 0.789, 1.064, 0.118 and 0.356 g/100 g in green pepper, red pepper, tomato, *L. sativa* and lamb's lettuce, respectively. The low citric acid concentration in *L. africana* and *P. umbellatum* leaves suggest that the plants need to preserved properly since citric acid occurs naturally in fruits and vegetables and serves as a natural preservative and flavouring in foods.

Conclusion

The study showed that the leaves of *L. africana* and *P. umbellatum* were found to contain a large number of important classes of bioactive compounds ranging from phytochemicals such as tannins, saponins, alkaloids, flavonoids, oxalate and organic acids. The presence of all these bioactive compounds in the leaves suggests that it is of high medicinal value and could frequently be used as starting materials for the synthesis of some useful drugs. The study also showed that phytochemicals present in leaves of the two plants varies from one plant to another. The leaves of *L. africana* had more hydrogen cyanide, oxalate, saponin, tannin, alkaloids and flavonoids compared to *P. umbellatum* leaves, which recorded high phytate, trypsin-inhibitor and organic acids.

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