# STUDIES ON THE PHYTOCHEMICAL PROPERTIES AND PROXIMATE ANALYSIS OF *PIPER UMBELLATUM* (LINN) FROM NIGERIA

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## ABSTRACT

*Piper umbellatum* L. is a tropical shrub with many medicinal and nutritional values in different parts of Nigeria. The leaves of *P. umbellatum* were obtained from Amaku Igbodo in Etche local local government area of Rivers State, Nigeria. The leaves were processed and analyzed for phytochemical properties and proximate composition to ascertain its importance in medicinal and culinary purposes, using standard analytical procedures. The phytochemical results revealed a very high amount of steroid (more than 95%), little traces of tannin and alkaloid. Saponin and phenol were slightly above 10% each, and flavonoid (less that 10%). Proximate analysis demonstrated the presence of protein (20. 56%), ash (17%), high amount of fibre (55.6%), moisture (less than 10%) and small amounts of carbohydrate and lipid. The presence of these substances accounts for its local use in herbal medicine and nutritional purposes. The implications of these findings and the need to conserve this underutilized and endangered plant species are discussed.

**Key words:** *Piper umbellatum*, Phytochemical analysis, Proximate composition

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## INTRODUCTION

The Niger Delta in Nigeria in addition to its rich mineral resources (petroleum) is also home to diverse faunal and floral zones (terrestrial and aquatic) that have national and global ecological and economic importance (Niger Delta Wetlands Centre, 2006). Constant exploration activities have resulted in the loss of its biodiversity. According to a World Bank (1995) report, the full significance of the Niger Delta's biodiversity remains unknown because new ecological zones and species continue to be uncovered and major groups such as higher plants and birds remain unstudied in large areas. One of such plant species that grows in the region is the *Piper* plant.

The genus *Piper* comprises of about 1000 to 2000 species of shrubs, herbs, and lianas that has economic and ecological values, of which *Piper umbellatum* (Linn.) is among, (Dyer and Palmer, 2004). *P. umbellatum* belongs to the order *Piperalis* and a member of the family *Piperaceae*. Several common names are associated with this plant: cow-foot leaf, fula-pulaar, wild pepper, pepper plants, pepper vines and 'njaa-nja' the local name in the area from where the present collection was obtained. Most *Piper* species are either herbaceous and vines with some growing as shrubs or small trees. A few species, commonly called 'ant piper' like *Piper cenocladum* lives in mutualism with ants. The fruit of the piper plant, called pepper corn when it is round and pea sized, as is usual, is dispersed by birds usually, but a times by fruit-eating mammals. Significant pests of this plant is usually herbivores that have evolved the ability to withstand the chemical defenses released by the plant.

*P. umbellatum* originated from tropical America, but is now found in tropical rain forest in Africa, Japan and the Indian Ocean Islands (Nunez *et al.,* 2005). The plant tolerates light winter forest where they occur as the dominant vegetation whenever they are found (Dyer and Palmer, 2004), but becomes scarce during the dry season. *P. umbellatum* occurs as undergrowth in

evergreen rainforest, and in river banks but not much in damp environments (Nunez, *et al.,* 2005).

*Piper umbellatum* usually grows up to height of 4m, branching off vigorously near the base. The leaves are usually alternate, simple and entire and stipules are absent, with petioles 6.5 – 30cm long, with sheath blade semi circular and cordate. The flowers are minute, usually bisexual and without perianth. The stamens are two in number, with superior ovary and three single-celled stigmas. The inflorescence is axillary or leaf opposed spikes from 5.5 – 15cm long, usually in false umbels with the pedicels bearing the bracts. The fruit is usually three-angled fleshy drupe, brownish in colour and single seeded with minute globules. *P. umbellatum* can flower all year round, depending on the availability of water. Propagation is mostly by the seed which are usually broken by direct sunlight on maturity. The plant is cultivated occasionally in home gardens, around the walls of uncompleted buildings as well as in the wild, where they had been human settlements (Desmarchelier, *et al*., 1997).

The use of plants as food and medicine dates back to the history of mankind (Nweze *et al,* 2004). As food, they have been used for more than 9,000 years as vegetables. The leaves of *P. umbelltum* are widely used as an emollient and in the treatment vulnerary and antiseptic disorders. They have been shown effective in the treatment of several ailments such as oedema, malaria, urinary and kidney problems, veneral infections, menstrual and stomach problems. It is also useful in the treatment of wounds and inflamed tumors. The root is diuretic, stimulant and promotes the flow of bile. According to Ropke *et al.,* (2006), numerous medicinal uses have been ascribed to *P. umbellatum* depending on the region. *P. umbellatum* is traditionally used in the treatment of premature babies, before the advent of modern medicine. This is done by putting the baby in a small basket lined and covered completely with *P. umbellatum* leaves. Then the

new-born with the mother is confined to a poorly ventilated room, till the baby matures. In those days, it is common to name such pre-mature babies 'nwa njaa-nja', meaning 'a child from *P. umbellatum* (Personal communication).

Some of the essential oils from the aerial parts of *P. umbellatum* have high content of β- pinene (27%), α-pinene (18%), E-nerodiol (12%) and β-caryophyllene (10%) (Perazzo *et al,* 2005). The roots and aerial parts contain 4-nerolidylcatechol, an antioxidant, which may explain its use in the treatment of skin cancer. It is also known to inhibit the effects of the mytotoxic phospholipase venoms of vipers (*Bothrops spp*.) (Nunez *et al*, 2005).

Plants have a limitless ability to manufacture aromatic substances mainly secondary metabolites, of which less than 10% have been synthesized. These metabolites are used by plants for different purposes like defense against predation by microorganisms, insects and herbivores (Akharaiyi and Boboye, 2010). Some of these metabolites may be involved in plant odour (terpenoides), pigmentation (tannins and quinines), and flavour (capsacin) (Mallikharjuna, *et al*., 2007). These defensive molecules give plants their medicinal value which is appreciated by human beings because of their great importance in health care of individuals and communities. *P. umbellatum* has for long being used for therapeutic purposes and food in different parts of Nigeria. However, little is known about its chemical and nutrient compositions to support its wild usage. Therefore, this study was undertaken to examine the phytochemical and proximate composition of *Piper umbellatum*, a tropical shrub with many medicinal and nutritional value, but is presently becoming extinct.

## MATERIALS METHOD

### Collection of plant materials and identification

Leaves of *Piper umbellatum* (Fig. 1) were collected from an uncompleted building in Amaku Igbodo, a rural community in Etche local government area, Rivers State, Nigeria.

**Fig. 1: *Piper umbellatum* L. growing in an uncompleted building**

The building was abandoned for years, allowing for the growth of vegetation. The plant was identified by Mr. C. Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria and authenticated by Dr. B. O. Green, a taxonomist in the Department of Applied and Environmental Biology, Rivers State University of Science and Technology (RSUST), were the voucher specimen of the materials was deposited. The leaves were separated from the stalk, washed in running water and air dried at room temperature for seven days. They were later ground into fine powder using a Kenitone

millennium quality electric blender. The powdered sample was stored in a dry, clean container and tightly closed for phytochemical and proximate analyses.

**Phytochemical screening**

This was carried out using different standard methods for saponin, steroids, flavonoid, tannin, alkaloid and phenol.

**Saponin**: 20g of the powdered leaf sample was boiled in 25ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth, indicating the presence of saponin. This was further confirmed by adding 5 drops of olive oil to 2ml aqueous extract in a test tube and shaken vigorously, then observed for the formation of emulsion (Tadhani and Subhash, 2006).

**Steroids:** 2ml of acetic anhydride was added to 0.5g ethanol extract of the sample with the addition of 2ml $H_2SO_4$. A dark green colour change indicates the presence of steroids.

**Flavonoid:** 10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The mixture was filtered using Whatman filter paper No 42 (125mm). Then, 4ml of the filtrate was mixed with 1ml of dilute ammonia solution and shaken vigorously. A yellow colouration indicates the presence of flavonoids (Harborne, 1973).

**Tannins:** This was carried out using the method of Trease and Evans (1983). 0.5g of the sample was weighed into a clean test tube. 20ml of water was added and boiled for 1hr. The sample was filtered and few drops of 0.1% ferric chloride was added and allowed to stand for 20 minutes for proper colour development. A brownish green or blue black colour indicates the presence of tannins.

**Alkaloid:** 5g of the plant sample was weighed into 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4hrs. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium

hydroxide was added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed, leaving the alkaloid. (Harborne, 1973).

**Phenol:** The amount of total phenol in the plant sample was determined using the spectrophotometric method, as described by Akharaiyi and Boboye (2010) with minor modifications. 5g of the sample was defatted with 2ml of diethyl ether using a soxhlet apparatus for 2 hrs. The fat free sample was boiled with 1000ml of ether for 15 minutes to extract the phenolic components. Then, 5ml of the extract was pipetted into 5ml flask and then 10ml distilled water was added. 4ml of ammonium hydroxide solution and 10ml of concentrated amyl alcohol were also added and left to react for 30 min. A blank that contained aqueous methanol instead of plant extracts was also prepared. The test mixtures were incubated for 40min at room temperature and the absorbance read at 725 nm using a UV–vis spectrophotometer.

**Proximate composition:**

This was done following the methods recognized by the Association of Official Analytical Chemists (AOAC, 1999) to test for the presence of ash, carbohydrates, fibre, lipids, moisture and protein.

**Ash**: The percentage composition of ash was determined by the furnace method. 20g of the sample was weighed into a preheated and weighed porcelain crucible. The crucible was inserted into a furnace and regulated to a temperature of 630°C and heated for 3hrs. The set up was then allowed to cool to room temperature and weighed again. Percentage composition of ash was then obtained as follows;

%Ash = (weight of crucible + ash –weight of crucible)/weight of sample x100/1

**Carbohydrates**: The percentage composition of carbohydrates was obtained by weighing 0.1g of the sample into 25ml volumetric flask. 1.3ml 62% perchloric acid was added and the mixture shaken for 20 min to allow for complete homogenization. The mixture was made up to 25ml with distilled water. The resulting solution was filtered through a glass filter paper and 1ml of the filtrate was transferred into a 10ml flask and diluted with distilled water. 1ml of the working solution was pipetted into a clean test tube and 5ml Anthoine Reagent was added and mixed thoroghly. The whole mixture was read at 630nm wavelength using the distilled water as blank. A standard glucose of 0.1mg/ml was also prepared and treated as the sample with the Anthoine reagent. Absorbance of the standard glucose was read and the value of carbohydrate (CHO) calculated as follows:

%CHO as glucose = 25 x Abs of sample/Abs of std glucose x100/1

**Lipids:** This was determined by soxhlet machine. 20g of sample was put into filter paper and was placed into a soxhlet extractor. The extractor was placed into a pre-weighed dried distillation flask and acetone was introduced into the distillation flask via the condenser end attached to the solvent extractor. The set up was held in place with a retort stand clamp. Cold water jet was allowed to flow into the condenser and the heated solvent was extracted in the process of continuous refluxing. After the lipid was extracted, the condenser and extractor were disconnected and the solvent evaporated to concentrate the lipid. The lipid in flask was then oven-dried to constant weight and re-weighed to obtain the weight of the liquid. Lipid content was calculated as:

% lipid = (weight of flask + extract) – (weight of flask)/weight of sample x 100/1.

**Moisture:** This was determined by the Ignition method. 1g of sample was weighed into a clean dried porcelain evaporating dish. This was placed on an oven and the temperature maintained at 105°C for 6 hrs. The evaporating dish was cooled in a desiccator to room temperature then reweighed and recorded. Weight of moisture was calculated by subtracting the weight of dried sample from the fresh as follows:

%moisture = fresh weight-dried weight/weight of fresh sample x 100/1.

**Protein:** Kjedahl (Digestion) method was employed to test the protein presence. 0.1g of sample was weighed and added to a clean 250ml conical flask. 3g digestion catalyst was added into the conical flask and 20ml concentrated $H_2SO_4$ added. The set up was then allowed to cool to room temperature with distilled water. Then, 20 ml of the digest was measured into a distillation flask held in place on an electro- thermal heater. The distillation flask was attached to Liebig condenser connected to a 10ml 2% Boric acid as indicator. 40% NaOH was injected into the digest with a syringe to make the digest alkaline. The mixture was heated to boil and the ammonium gas was distilled off through the condenser to the receiver beaker. The boric acid changed colour from purple to greenish as ammonia distillate was introduced. The distillate was titrated with 0.1M hydrochloric acid back to purple from green. The volume of hydrochloric acid added to effect the change was recorded as titre value and calculations made to obtain percentage nitrogen as an indicator to the presence of protein.

## RESULTS AND DISCUSSION

### Phytochemical screening

The phytochemical screening revealed that leaves of *P. umbellatum* contains a very high amount of steroid ( more than 95%), little traces of tannin and alkaloid, while saponin and phenol were

slightly 10% each. Flavonoid has less that 10% occurrence (Fig. 2). Flavonoids are beneficial to human health as anti-oxidants (Jouad *et al.,* 2001).The reasonable amount of phenols and saponins present in *P.umbellatum* is quite interesting. Phenolic compounds possess important pharmacological values, some having anti-inflammatory properties (Bruneton, 1995). The presence of saponin is well reported in plants (Edeoga *et al.* 2006; Belewu *et al*., 2009; Akundu 1984), where they serve as expectorants and emulsifying agents. Saponins are glycosides with distinctive foaming characteristics, and like phenols have anti-inflammatory properties. The anti-inflammatory activities of some saponin derivatives such as triterpenoids have been reported (Sahu and Mahato, 1994). According to Sparg *et al*. (2004), many saponins extracted from plant sources produce an inhibition of inflammation in the mouse carrageenin-induced oedema assay. Also Okwu (2003), reported on the use of saponin in the manufacture of insecticides, drugs and synthesis of steroid hormones. *P.umbellatum* contains a very high amount of steroids (Fig. 2), which acts as a natural preventive dietary product (Piirunon *et al*., 2000). They also have anti-protozoan and antibacterial activities (Soforowa 1993). Our finding reveals that *P.umbellatum* contains small amount of tannin, confirming an earlier report by Ihekoronye and Ngoddy (1985) that tannin is hardly present in food products of plant vegetable origin. A major use of *P. umbellatum* in the area from where it was collected is as vegetable source. Tannins have oxidation inhibiting activity and confer good flavour on leaves. The leaves of *P. umbellatum* have good aroma, which accounts for the use in culinary purposes.

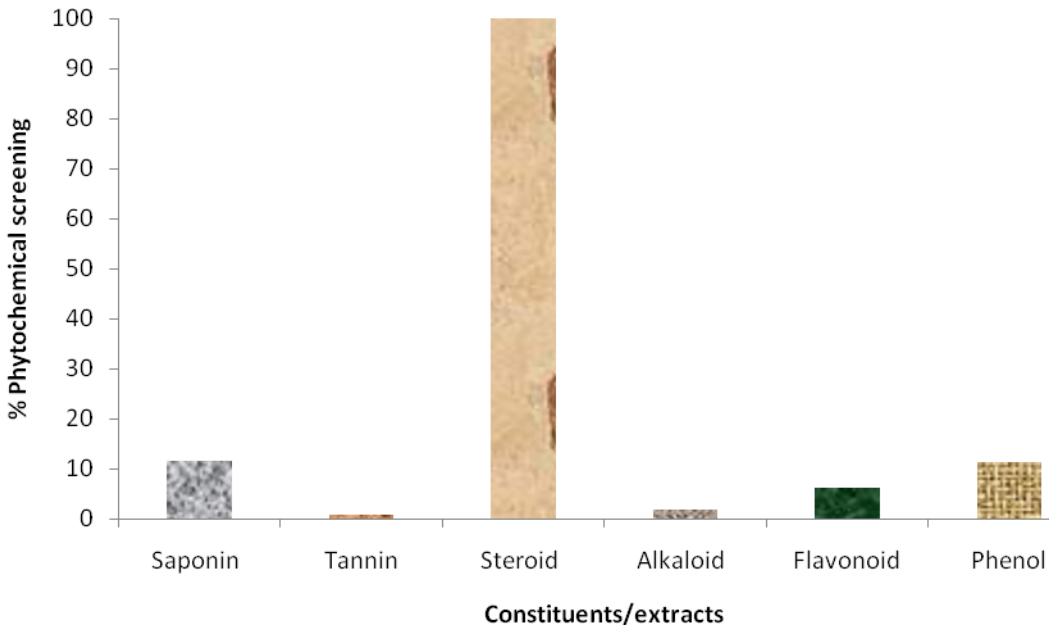


**Figure 2: Phytochemical composition of the leaf of *P. umbellatum***

**Proximate analysis**

Proximate analysis of the leaves of *P. umbellatum* demonstrated the presence of protein (20%), lipids, ash (17%), high amount of fibre (55.6%), moisture (less than 10%) and small amount of carbohydrate and lipid (Fig 3). The high fibre and protein content is a further confirmation of its use as vegetable. Fibre reduces tracolonic pressure which is beneficial in diverticular disease (Belewu *et al.* 2009). Plants with high fibre are adequate for better rumination and digestion in ruminant animals (NRC, 1978). The presence of ash is a reflection of the mineral content in the plant.

Phytochemical properties are the various bioactive chemical compounds found in plants, as antioxidants which are considered to be beneficial to human health. Our findings show that leaves of *P. umbellatum* is very rich in nutrients and chemical substances which offer great potential for food and pharmaceutical companies. Its conservation is therefore very important for further studies on its medicinal and other benefits.

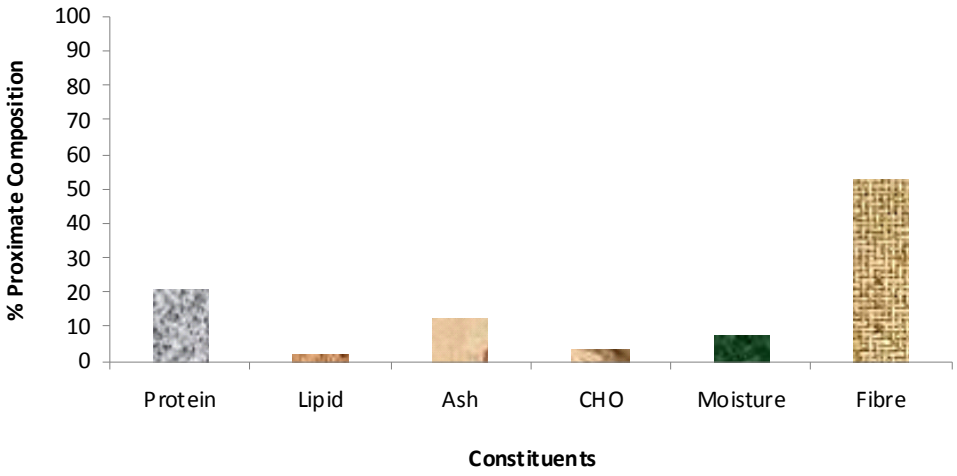


**Figure 3: Proximate composition of the leaf of *P. umbellatum***

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