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Nutritional, phytochemical and antimicrobial properties of two wild aromatic vegetables from Edo State

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ABSTRACT

The nutritional, phytochemical and antimicrobial properties of two underutilized leafy vegetables from Esanland in Edo State, Nigeria: Peperomia pellucida (L.) H.B.K; Piper umbellatum L. were investigated. Fresh leaves were shredded, sundried before milling into fine powder and subjected to qualitative and quantitative analysis. The proximate and mineral analysis revealed moderate quantities of protein, carbohydrate ascorbic acid and different mineral salts. The phytochemical screening recorded the presence of pharmacologically important substances such as tannins, flavonoids and cardiac glycosides in both plants. However, steroids, terpenoids and phlobatannins were present only in P. umbellatum. The powdered samples were extracted in three different solvents (water, ethanol and ether) and tested against five pathogenic organisms (Staphylococcus aureus, Klebsiella pneumoniae, Escheriachia coli, Streptococcus mutans and Candida albicans) isolated from patients admitted to the specialist Hospital, Irrua, Edo State, for various ailments. The extracts recorded appreciable zones of inhibitions (1-7mm), suggesting susceptibilities of the organisms. This indicates the potential usefulness of these plants in the treatment of various pathogenic diseases in this locality and hence can be developed as antimicrobial drugs/agents.

Key words: Phytochemicals, Antimicrobials, Nutritional, Peperomia pellucida, Piper umbellatum

INTRODUCTION

Vegetables are important sources of protective foods which are highly beneficial for the maintenance of good health and prevention of some diseases. The poor utilization of wild vegetables may be associated with lack of knowledge about how to access quantities that can satisfy daily human food requirements. These leafy vegetables that occur in the wild, account for about 10% of the world's higher plants often regarded as weeds. Many of them are resilient, adaptive and tolerant to adverse climatic conditions more than the exotic species which are cultivated [1].

Leafy vegetables contain water-soluble vitamins such as B and C, fat-soluble vitamins including A and D and also carbohydrates and minerals [2, 3]. Leaf concentrates made from fractioning freshly green leaves are one of the richest sources of iron. George [4] stated that though the bulk of the weight of wild leafy vegetables is water, they represent a veritable natural pharmacy of minerals, vitamin and phytochemicals. Phytochemicals are metabolites of which there are several classes including; alkaloids, flavonoids, glycosides, gums, polysaccharides, phenols, tannins, phlobatannins, terpens and terpenoids [5].

The evolution of bacteria resistance to currently available antibiotics has necessitated the search for novel and more effective antibacterial compounds [6]. Ethno-pharmacologist, botanist, micro biologist and natural product chemist are working hard to discover phytochemicals which could be developed for treatment of infectious diseases [7]. Efforts in this regard have been focused on plants. A good portion of the world's population rely on plants for the treatment of infectious and non-infectious diseases [8]. The two vegetables studied in this report are *Piper umbellatum* L. and *Peperomia pellucida* (L) H.B.K which belong to the family Piperaceae. Both vegetables are also used as spices. *P.umbellatum* is collected from the wild and sold in various markets in Esanland as leafy vegetables. *P.pellucida* is a common weed of moist environment which is not commonly eaten unless under special health conditions as vegetable in soups or as salad. *Peperomia pellucida* (shiny bush) is an annual, shallow rooted herb that belongs to the family Piperaceae commonly referred to as the pepper family. The plant is found in various shaded, damp habitats growing in clumps, thriving in loose humid soils, damp woods, niches in rocks and tropical and subtropical climate. It usually grows to a height of about 15 to 45cm and it is characterized by succulent stems, shiny, heart-shaped, fleshy leaves and tiny, dot-like seeds attached to several fruiting spikes. The plant has a mustardy odour [9]. The ethno-medicinal properties of *P. pellucida* is well known. It is used for treating abdominal pain, abscesses ,acne ,boils, colic, fatigue ,gout, renal disorders and rheumatic joint pain [10,11]. Anti-inflammatory, chemotherapeutic and analgesic properties have been found in crude extract of *P. pellucida*.

Aziba *et al.* [12] reported that the analgesic properties of *P.pellucida* seem to be related to its effect on prostaglandin synthesis. It may have potential as a broad spectrum antibiotic as demonstrated in tests against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* [13]. Chloroform extracts from dried leaves of *P.pellucida* have been shown to exhibit anti-fungal activity against *Trichophyton mentagrophytes* [14]. The plant is used as an anti-convulsant, for the treatment of insomnia, as poultice for sores, wounds and poisonous bites. The whole plant is used for the treatment of diseases such as diarrhea dysentery, naso-pharyngeal infections; paralysis, epilepsy, convulsions, spasm; pulmonary troubles; skin, mucosae; tumors and cancers.

Piper umbellatum, also belongs to the family Piperaceae commonly called umbelled pepper or cowfoot vegetable. The plant is a perennial herb or scrambling shrub of up to 4m tall, much-branched from near the base; stems from a woody rootstock, succulent, ribbed and rooting at the nodes. The leaves are widely used as an emollient, vulnerary and antiseptic. They are employed in poultices on swellings, boils and burns; the juice is taken as an emmenagogue, galactagogue and diuretic or used as ear drops against earache. A decoction of the leaves or roots is taken to relief jaundice, malaria, urinary and kidney problems, syphilis and gonorrhoea, menstrual problems and stomach ache and it is also applied on wounds and inflamed tumors [15]. The leaves are also cooked and eaten as a vegetable. According to Atindehou *et al.* [15], methanol extracts of the leaves of *P.umbellatum* were active against chloroquine and pyrimethamine-resistant and also inhibits *P.falciparum in-vitro*.

Traditionally, infusions of leaves of *P.umbellatum* are used in the treatment of malaria, liver ailments and stomach ulcers. The leaves are eaten raw or cooked as a seasoning or as salad. It contains terpens and resins which is responsible for its biting taste [16]

The objectives of this study are to:

- (1) Determine the beneficial nutrients of *Piper umbellatum* and *Peperomia pellucida*
- (2) Make the people in this locality aware of their nutritional values and hence popularize their usage in diets.
- (3) Determine the phytochemical and antimicrobial properties of these wild aromatic vegetables.

MATERIALS AND METHODS

Collection and preparations of plant materials

The plant materials; *Peperomia pellucida* were collected from under shade within the campus and off campus located in the university town of Ekpoma, Edo State, *Piper umbellatum* was purchase from the main market in Ekpoma, Edo State. The plant samples (leaves) were hand-picked, washed in running tap water and air dried at room temperature. After 21 days of proper drying, they were grinded separately and sieved to obtain fine powder of each and stored in air tight containers for laboratory analysis.

Nutritional Analysis

The dry powder was subjected to nutritional analysis following the procedures recommended by [17] and adopted by [18] for ascorbic acid, proteins, fiber, carbohydrates and inorganic mineral salts.

Phytochemical analysis

Qualitative phytochemical screening of the powder of the plant samples was determined for; tannins, saponins, cardiac glycosides, steroids, alkaloids, flavonoids, phlobatannins and terpenoids. The chemical test was carried out using standard procedure as described by various author [19, 20, 21].

One gram of the powder was subjected to qualitative qualitative phytochemical tests for Alkaloid (Mayer reagent); Tannins (FeCl₃); Saponins (chloroform and H₂SO₄); Cardiac Glycoside (glacial acetic acid + FeCl₃ + H₂SO₄.); Steroid (chloroform. +acetic anhydride + Conc. H₂SO₄); Lignin (1% phloroglecitol + 75% ethanol) and Flavonoid (5ml of Ammonia solution + HO) Phlobatannins (1% aqueous hydrochloric acid) Terpenoids (Salkowshi Test)

Antimicrobial Activity**Aqueous Extraction**

One gram of powdered sample was weighed and placed in a sterile container and properly labeled. 10ml of distilled water was added. Petri-dishes containing prepared medium were streaked with the different test organisms (*Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans*) and properly labeled. Using a pipette, 0.1ml of the extract was inoculated using a pipette. The medium with the streaked organisms and inoculated solvent was placed in the incubator and allowed to grow for 24hrs. The zone of inhibition was then measured in mm.

Alcohol Extraction

One gram lot of powered sample was weighed and placed in a sterile container and properly labeled. Ten milliliters of alcohol was added. Petri-dishes containing prepared medium was streaked with the different test organisms (*K. pneumonia*, *E. coli*, *Staph. aureus*, *Strept. mutans* and *C. albicans*) and properly labeled. Using a pipette, 0.1ml of the extract was inoculated using a pipette. The medium with the streaked organisms and inoculated solvent was placed in the incubator and allowed to grow for 24hrs. The zone of inhibitions was then measured in mm.

Ether Extraction

One gram of powered sample was weighed and placed in sterile container and properly labeled. 10ml of ether (diethyl ether) was added. Petri-dishes containing prepared medium were streaked with the different test organisms (*Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans*) and properly labeled. Using a pipette, 0.1 ml of the extract was inoculated into the prepared plates with test organisms. The plates were placed in the incubator for 24hrs to allow for growth of the organisms. The zone of inhibition was measured in mm.

RESULTS AND DISCUSSION

The phytochemical analysis of the two plants, *P.pellucida* and *P.umbellatum*, revealed the presence of bioactive constituents (Table 1). The study shows the presence of alkaloids, saponins, tannins, flavonoids and cardiac glycosides in both plants. However, steroids, terpenoids and phlobatannins were only present in *P. umbellatum* which is in line with previous studies of [18, 22]. The medicinal importance of tannins, saponins, flavonoids, alkaloids, steroids and cardiac glycoside which are components of traditional herbal preparation used in managing various ailments has been reported [23,24]. Alkaloids are reported to be most efficient therapeutically phytochemicals [25] It has been reported that pure isolated alkaloids and their derivatives are basic medicinal agent because of their analgesic, antispasmodic and antibacterial properties. Alkaloids are capable of reducing headache associated with hypertension. It has been reported that alkaloids can be used in the management of cold, fever and chronic catarrh [26].

Saponins are expectorants, cough suppressants and administered for hemolytic activities [19, 27]. Tannins are well known for their antioxidant and antimicrobial properties as well as for soothing relief, skin regeneration, as anti-inflammatory and diuresis [28].

Flavonoids are known for their antioxidant activity, and hence they help to protect the body against cancer and other degenerative disease such as Arthritis and Type II diabetes mellitus [29]. Cardiac glycoside acts on the heart muscles and increase renal flow (diuresis). Herbal preparation containing cardiac glycosides are used for the treatment of congestive heart failure and cardiac arrhythmia. The absence of tannins, saponins and alkaloids in the leaves of *P.pellucida* had previously been reported by [30], while [31] also reported the absence of flavonoids in leaves of this plant. In the present study, steroids, terpenoids and phlobatannins were observed in the leaves of *P.umbellatum*, but absent in *P.pellucida*. Steroids are administered mostly to athletes to increase muscles and bone synthesis. Steroids are also associated with hormonal control in women. This may be the reason why the leaves of *P. umbellatum* are used as vegetables for expectant and breast feeding mothers in this locality to ensure their hormonal balance since the steroidal structure could serve as the starting material in the synthesis of sex hormones [32]. It is reported that when taken in palm nut soup, it enhances lactation in newly born mothers. The most common steroid is cholesterol; steroids regulate carbohydrate and protein metabolism and poses anti-inflammatory properties. Terpenoids have antihepatotoxic properties, thus helping to prevent liver damage (cirrhosis), they equally have antimicrobial or antiseptic properties. Phlobatannins have astringent properties [33], and it had in an earlier report been recorded that the leaves of *P.pellucida* can be used as astringent as they contain phlobatannins. This is in agreement with the traditional medicine practices in this locality.

The nutritional properties of both plants are summarized in Tables 2 and 3. It was observed that *P. umbellatum* contains higher amount of ascorbic acid (181mg/100g) compared to *P.pellucida* (165.8 mg/100g). The protein content of *P. pellucida* was 7.68%, the fiber content (22.3%) and carbohydrate content (42%) as against that of protein content of *P. umbellatum* (3.9%); fiber content (20.2%) and carbohydrate content (38%). The calcium components of *P. umbellatum* was (2.36%), potassium (4.1%), magnesium (0.88%), sodium (0.12%) and Iron (0.05%). The records for *P. pellucida* are calcium (1.85%), potassium (3.48%), magnesium (0.43%), sodium (2.79%) and iron (0.92%). *P. umbellatum* showed high calcium and iron contents compared to *P. pellucida*.

The chemical properties of plants have been shown to be responsible for the nutritional and healing properties [34, 35]. High crude fiber (22.3%) and carbohydrate (38.97%) were recorded in leaves of *P.pellucida*. Food fibers have been shown to aid absorption of dietary minerals as well as reduce absorption of cholesterol. The crude protein content (7.68%) observed in *P.pellucida* compared favourably with and in most cases surpassed those reported for most medicinal plants [36]. This signifies that *P.pellucida* contains healing properties as proteins are essential for the synthesis/repair of body tissues and as enzyme [37]. The high ascorbic acid content observed in *P.umbellatum* (181mg/100g) signifies that the plant contains antioxidant properties. High intake of ascorbic acid correlates with reduced gastric cancer [38].

The antimicrobial properties of the plants were also investigated and the results summarized Tables 4 and 5. The results showed that the aqueous extract of *P. pellucida* had no activity against the five test organisms used in the present study. However, the aqueous extracts of *P.umbellatum* showed inhibition to the growth of *Streptococcus* (1-4mm), *Klebsiella* (1-4mm) and *Candida* (1-4mm), but had no inhibition activity on *Staphylococcus* and *Escherichia*. The alcohol extract of *P. pellucida* inhibited the growth of *Staphylococcus* (1-6mm), *Streptococcus* (1-5mm), *Escherichia* (1-5mm) and *Klebsiella* (1-4mm). No aqueous extract of the two plants recorded activity against *Candidas*. Alcoholic extract of *P.umbellatum* was more active against *Streptococcus* (1-7mm) and to a lesser extent, *Staphylococcus* (1-6mm), *E.coli* (1-4mm) and *Klebsiella* (1-5mm). Again, no activity was recorded with alcohol extract of *P.umbellatum* against *Candida*. Furthermore no activity was recorded against any of the test organisms with ether extract of *P.pellucida*. Moderate activity was recorded with ether extract of *P. umbellatum* against *Staphylococcus* (1-4mm), *Streptococcus* (1-4mm), *E.coil* (1-4mm), *Klebsiella* (1-4mm) and *Candida* (1-4mm). Ether extract of *P.umbellatum* was observed to have growth inhibition activity against all test organisms used for this study. The poor inhibitory activities observed in the aqueous extract of *P. pellucida* indicate that the solvent may not be good for the extraction of bio-active components of the plant thus, the use of aqueous extract in medical preparations or its use as a salad does not confer any antibacterial properties on the consumer. From the present study, it was revealed that alcohol extract shows inhibitory effect against all test organisms; *P. pellucida* extract on *Staphylococcus* (1-6mm), *Streptococcus spp* (1-5mm), *E.coli* (1-5mm), *Klebsiella* (1-4mm), for *P.umbellatum*, alcohol solvents shows inhibitory activity against *Staphylococcus aureus* (1-6mm), *Staphylococcus* (1-7mm), *E.coli* (1-4mm), *Klebsiella* (1-5mm). *Staphylococcus* has been isolated from wound and eye infection [39]. This shows that the plants understudy can be use for eye treatment as well as applied on injury/cut for healing. All the extracts had the same concentration and based on the observed zones of inhibition for all extracts; it could be inferred that alcohol extract had the highest inhibitory activity (1-7mm) on *Streptococcus*, followed by ether (1-4mm) on all the

test organisms including *Candida* (which is a fungus). The aqueous extract was observed to show minimum inhibitory activity of 1-4mm on *Streptococcus*, *Klebisella* and *Candida albican* with *P.umbellatum* extract. It has been observed that natural products are more extractable using alcoholic solvents. This effect could be due to the fact that the active ingredients are more soluble in alcohol solvent than others [40, 41]. The antimicrobial effect of the plant leaves suggest that they may possess remarkable therapeutic action in the treatment of gastro-intestinal infections [41]. No antifungal activity was observed by *P.pellucida* with the fungus. *C.albicans*. *E.coli* and *C. albicans* are major causative organisms of diarrhoea in humans. This study shows that *P.umbellatum* extract which shows inhibitory effect on these organisms functions as a potential anti-diarrhoeal phytomedicine. Previous studies [6] confirm that alcohol is a better solvent for the extraction of antimicrobials from *P. pellucida* and *P. umbellatum* for the inhibition of growth of some pathogenic bacteria. Similarly studies [22], with aqueous extracts of *P. pellucida* on *Staphylococcus aureus* strain showed that water is not a good solvent for the extraction of solutes which have inhibitory activity.

Table 1: Phytochemical analysis

Name of vegetable	Alkaloids	Flavonoids	Saponins	Tannins	Cardiac glycosides	Steroids	Terpenoids	Phloba- tannins
<i>Piper umbellatum</i>	+	+	+	+	+	+	+	+
<i>Piperomia pellucid</i>	+	+	+	+	+	-	-	-

+ indicates present; - indicates absent

Table 2: Nutritional value of two wild aromatic vegetable from Ekpoma; Edo State, Nigeria.(%)

Name of vegetables	Ascorbic acid (mg/100g)	Carbohydrates	Protein	Moisture (Fresh samples)	Fiber
<i>Piper umbellatum</i>	181.0	38	3.9	71.6	20.2
<i>Peperomia pellucida</i>	165.8	42	7.7	78.9	22.3

Table 3: Mineral composition of two wild aromatic leafy Vegetables from Ekpoma, Edo State, Nigeria (%).

Vegetables	Calcium	Potassium	Magnesium	Sodium	Iron
<i>P. umbellatum</i>	2.36	4.1	0.88	0.12	0.05
<i>P. pellucida</i>	4.14	3.5	0.43	0.28	0.66

Table 4: Zones of inhibition (mm) for *P. pellucida*

Test Organisms	Extract (0.1g)		
	Aqueous	Alcohol	Ether
<i>Staph aureus</i>	-	6	-
<i>Sirep mutans</i>	-	5	-
<i>E. coli</i>	-	5	-
<i>K.pneumoniae</i>	-	4	-
<i>C. albicans</i>	-	-	-

Table 5: Zones of inhibition (mm) for *P. umbellatum*

Test Organisms	Extract (0.1g)		
	Aqueous	Alcohol	Ether
<i>Staph. aureus</i>	-	6	4
<i>Streptococcus spp</i>	4	7	4
<i>E coli</i>	-	4	4
<i>K.pneumoniae</i>	4	5	4
<i>C. albicans</i>	4	-	4

CONCLUSION

The proximate and mineral analysis revealed moderate quantities of protein, carbohydrate ascorbic acid and different inorganic salts. These are important nutrients required by the body to function properly. The phytochemical contents of the leafy vegetables serve as supplements for food and also have the potential to improve the health status of its users through their anti microbial properties. The phytochemical screening of the two plants under study show that they are rich in alkaloid, flavonoids, saponins, steroids, terpenoids and phlobtannins. It has been noted that the presence of phytochemical compounds in these plants are responsible for the observed biological activities including antibacterial, antiviral, and anti-diabetic properties of these plants. Results from this study are the affirmation of the use of these plants in the management of various ailments in this locality. Therefore, a study of these plants could provide the herbal solution for the treatment of disease cause by the test organisms (*E.coil*, *Staph. aureus*, *C. albicans*, *K. pneumonia*, and *Strept. mutans*).

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