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Phytochemical Composition and Antioxidant Activity Screening of Chloroform Leaves Extract of Man-To-Man (*Peperomia pellucida)* Harvested from Umunomo Ihitteafoukwu in Imo State, South Eastern Nigeria

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ABSTRACT: *Peperomia pellucida* commonly called man-to-man or pepper elder is a plant of immense medicinal value and used in the treatment of different ailments such as abdominal pain, abscess, acne, boils colic rhematic joint pain and also consumed as vegetable. This study was conducted to investigate the preliminary and quantitative phytochemical properties of *Peperomia pellucida*. The results obtained revealed the presence of alkaloids (2.49±0.02), phenols (0.05±0.01), flavonoids (0.59±0.01), saponins (0.64 ± 0.02), tannins (0.08± 0.01) with alkaloid registering the highest presence. *Peperomia pellucida* leaves extract exhibited significant antioxidant activity at minimum and maximum concentrations of 2.0 and 12.0mg/ml respectively (compared to the ascorbic acid used as the free scavenger). The antibacterial result also showed that *P. pellucida* extract inhibited the growth of the test organisms, with the highest growth inhibition against *Escherichia Coli* and the lowest growth inhibition against *Bacillus Cereus*. The antimicrobial properties indicate the potential usefulness of this plant in the treatment of various pathogenic diseases which in future can be developed as a potential antimicrobial agent used in the treatment of infectious diseases.

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There is a worldwide increase in dangerous infections caused by pathogenic microorganisms. (Khan *et al*., 2010). The use of medicinal plants in the treatment and management of these diseases have increased significantly such that a greater number of people in Nigeria now rely on plantbased medicines as their source of primary healthcare especially in rural communities. Infact it was reported that about 80% of the world's population rely on plants, while about 70-95% of people in developing countries depend on plant medicine as their primary source of healthcare. (Karunamouthi *et al*., 2013). Irrespective of all the progress in synthetic chemistry and biotechnology, plants are still an important source of medicinal preparations, both for cure and prevention. The chemical properties of plants appeared to be responsible for these nutritional and healing properties. (Setchell and Cassidy, 1999). Before the advancement of science and technology, plants have been the basic source of medicine for man. (Schmelzer and Omino, 2003). Phytochemical compounds are an expansive class of molecules derived from numerous plant sources. (Igwe and Okwunodulu, 2013). *Peperomia pellucida* belongs to the family Piperaceae and it is an annual shallow rooted herb. (Ghani, 1998). Research conducted by Ragasa *et a*l., (1998); Khan and Omoloso (2002) revealed that *P. pellucida* is used in the treatment of renal disorders, colic, acne, boil, abdominal pain, rheumatic joint pain and abscesses. *P. pellucida* is reportedly used traditionally in several pantropical countries to treat a wide spectrum of ailments and diseases such as skin sores (Arigonna-Black *et al*., 2002), gastrointestinal disorders, dysentry, diarrhea, indigestion (Mollik *et al*., 2010), abscesses and injuries (Bojo *et al.*, 19940). The Igbo tribe of Southern Eastern Nigeria uses the leaves of *Peperomia pellucida* to treat athlete's foot and skin infections brought on by germs and fungi. (Igwe and Mgbemene, 2014). The whole plant is used to stop haemorrhages (Egwuche, 2011); it is mashed and mixed with water to form a mixture, then heated and given orally to arrest wound bleeding or

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hemorrhage.(Majumder, 2011). Recent research also reported that the whole plant is also used to cure measles in Ogun state, Nigeria. (Sonibare *et al*., 2009). In this present study, we examine and test the phytochemical composition and antioxidant activity screening of aqueous leaves of man-to-man (*peperomia pellucida)* harvested from Umunomon Ihitteafoukwu in Imo State, South Eastern Nigeria.

MATERIALS AND METHODS

Sample preparation and extraction: Peperomia pellucida leaves were harvested from Umunomo Ihitteafokwu in Ahiazu Mbaise Local Government Area of Imo State, South Eastern Nigeria. The plant materials were identified by Mr. Ibe Ndukwe of Taxonomy section, Forestry Department, Michael Okpara University of Agriculture Umudike, Nigeria. The leaves were handpicked and air dried for 30 days at room temperature. The dried leaves were milled to fine powder using laboratory mill. Extraction of the sample was done using the Maceration method as described by Farooq (2013).

Determination of Plant Chemicals: Phytochemical Analysis: Alkaloids and Flavoniods were determined according to gravimeter method described by Harbone (1973) while tannin was determined by the Folis-Denis spectrophotometric method described by Pearson, (1976). Saponins was done by the double solvent extraction gravimetric method of Harbone (1973). Phenols was determined by the Folin-ciocalted spectrophotometer method (AOAC, 1990).

Vitamin Contents: Ascorbic acid was determined by the method of the association of vitamin chemist as described by Kirk and Sawywer (1998). Niacin was determined by the method given by Okwu and Ndu, (2006). The scaler analyzer spectrophotometric method by Okwu, (2004) was used in the determination of riboflavin and thiamine. The method of AOAC as described by John (2006) was used in the determination of β-carotene.

Mineral Contents: The mineral elements comprising of potassium, magnesium, sodium, calcium, phosphorus, nitrogen, iron, copper and zinc were determined according to the method of Shahidi *et al*., (1999).

Proximate Analysis: The method described by John, (1995) was used to determine protein, crude fibre, lipids, ash and moisture content.

Anti-oxidant Activity determination: The free radical scavenging activity of *P. pellucida* extracts was measured in terms of radical scavenging ability or hydrogen donating by using the stable1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH) described by Man-Zocco *et al.,* (1998). 1.0 g of DPPH, a stable radical was dissolved in 100 ml of methanol. 3.0 ml of different concentrations of the test samples were added to 3.0 ml of a 0.004% methanol solution of DPPH and incubated for 30 min at room temperature. The decrease in absorbance of the solution brought about by the test samples was measured at 517 nm using a spectrophotometer. Ascorbic acid, which is a known antioxidant, was used as a reference standard. The radical scavenging activity was calculated as the percentage inhibition of DPPH discoloration using the equation below.

$$\% \text{ IDPPHR} = \left[\frac{\text{A}_{\text{blanck}} - \text{A}_{\text{sample}}}{\text{A}_{\text{blanckl}}}\right] x\ 100$$

Where IDPPHR = inhibition of DPPH radical; A_{blank} = Absorbance control; A_{sample} = Absorbance of the test sample

Antibacterial Activity determination: The antibacterial activity of the leave extract of *Peperomia pellucida* was done for 24 hours culture of four selected bacteria. The bacteria organisms used were *Staphylococcus aureus, Escherichia coli, Salmonella typhi and Bacillus cereus*. These organisms were pure clinical isolates obtained from Imo State Teaching Hospital, orlu, Imo state, Nigeria. The isolates were confirmed using the following biochemical tests; catalase test, oxidase test, methyl red test, indole test, Voges Proskaur reaction and citrate test. The growth inhibition of bacteria by the extract was done using the Agar well diffusion method as described by Babatunde *et al* (2014). Sterile liquid Mueller Hinton Agar media (LAM, M, UK) was poured into sterile petri dishes and allowed to solidify. 2.0 g of the extract was weighed into 10ml of dimethyl sulfoxide (0.1%) to give 200 mg/ml. this was further diluted to 100 mg/ml, 50 mg/ml, 40 mg/ml, 20 mg/ml, and 10 mg/ml. Sterile swab stick dipped into appropriate test organism suspended in normal saline was used to uniformly seed the dried agar. Sterile cork borer of 5 mm in diameter was used to make wells of 2.5 cm apart. The wells were labeled with appropriate concentration of the extract. The various concentrations were dropped into each well to fullness using sterile pasture pipette. The solvent (DMSO) in which the extract was prepared was used as a control. The plates were incubated at 37 ℃ for 24 hours. At the end of the incubation, zones around the wells were measured using transparent plastic ruler in millimeter. The experiments were conducted in triplets and the mean diameter zone of inhibition was recorded. Tube dilution technique was used in the test. One gram of the extract was dissolved in 5 ml of DMSO (0.1%) to get 200 mg/ml of the stock solution. That was diluted using serial dilution by transferring 1 ml of the stock solution into 1 ml of sterile nutrient broth to get 100 mg/ml solution. That was further diluted to obtain other dilution: 50 mg/ml, 40 mg/ml, 20 mg/ml and 10 mg/ml. 0.5 ml of standardized test organisms were inoculated into 0.5 ml of the diluted extracts and incubated at 37℃ for 24

hours. Using solvent (DMSO) and test organisms without extract, a control was equally set up. The turbidity of the solution in each tube was observed after 24 hours, to find out if there was any growth. The lowest concentration of the extract dilution that showed no visible growth of the test organism was recorded as the MIC.

RESULTS AND DISCUSSION

The phytochemical composition of *P. pellucida* leave extracts as shown in Table 1. Table 2 gives the mineral element composition while the vitamin and proximate composition of P. pellucida leaves are shown in Table 3 and 4 respectively. Phytochemical composition of P. *pellucida* leaves (Table 1) shows that their concentration was in this order alkaloids > saponin > flavonoids > tannins > phenol). Alkaloids are important secondary metabolites originally defined as pharmacologically active compounds, primarily composed of nitrogen (*Croteau et al*., 2000; Ziegler & Facchini, 2008). Alkaloids are used as basic medicinal agent for their analgesic, antispasmodic and anti-bacterial effects (Frantisek and Hana, 1998; Uchegbu and Okwu 2012). Alkaloids are also known to improve immune functions, nutrition, and physical performance, being present in daily foods, beverages, and supplements. Some examples include the caffeine from coffee (or guaranine and mateine from other plants) with antioxidant, anti-inflammatory, and stimulatory properties; theobromine and paraxanthine from cocoa as antioxidants; and gingerol and shogaols (phenolic alkanones) present in ginger bearing antioxidant, anti-inflammatory, antimicrobial, and antitumoral properties (Senchina *et al*., 2014; Han *et al*., 2015). Saponins are known to make the bronchial secretion more liquid, reduce the congestion of the bronchi and ease coughing. They are effective in inhibitory tumor cell proliferation (Gauthier *et al*., 2011), lowering blood cholesterol and triacylglycerol (Megalli et al., 2006). Saponins as well exhibit antifungal, cytoxin, anti-platelet aggregation (Huang *et al*., 2007) and hemolytic activity (Gauthier *et al*., 2009). Flavonoids have miscellaneous favorable biochemical and antioxidant effects associated with various diseases such as cancer Alzheimer's disease (AD), atherosclerosis, etc. (Burak and Imey 1999). Flavonoids compliment the effects of ascorbic acid in the body. They protect the vascular system by strengthening, maintaining and repairing capillaries (Teyssier *et al.,* 2001). Tannins are astringents, bitter polyphenols that either bind and precipitate or shrink proteins. Tannins are organic substances of diverse composition with pronounced astringent properties that promote the healing of wounds and inflamed mucous membranes (Frantisk, 1998). The presence of tannin in plant has been associated with ulcer management, wound healing, control of bleeding and burns in herbal medicine (Okwu, 2005). Plants that contain phenols could be used as anti-inflammatory, immune enhancers and hormone modulators (Okwu and Omodamino 2005). Phenols are also known to have the ability to block specific enzymes that cause inflammation and to prevent diseases. (Uchegbu and Okwu, 2012).

Table 1: Phytochemical composition of leaves of *Peperomia pellucida*

Phytochemicals	Composition (%)
Alkaloids	2.49±0.02
Flavonoids	0.59±0.02
Tannins	0.08±0.01
Saponins	0.64 ±0.01
Phenols	0.05±0.01

Values are means ± standard deviation of three determination

It is clear from Table 2's mineral element results that *P. pellucida* may be a rich source of minerals. With a high level of nitrogen, calcium, and magnesium, the results indicate the existence of nitrogen, potassium, phosphorus, calcium, magnesium, and sodium. Nitrogen is one of the primary elements in the body, it is necessary for the formation of various nitrogenous molecules, including hormones, neurotransmitters, and antioxidant defense components (Jerzy, 2019).. About 1.2% of the body weight of an adult person is calcium, which is a very significant mineral in human metabolism. In addition to its well-known function in bone building, calcium also aids in the regulation of muscle and nerve activity and the maintenance of the acid/base balance in our blood. Also crucial for the body's enzyme systems, neurotransmission, and muscle contraction is calcium (Ihekoronye and Ngoddy, 1985; Okaka *et al*, 1992). Magnesium is involved in the process that actively moves calcium and potassium ions across cell membranes, which is necessary for healthy nerve impulses, healthy muscular contraction, and healthy heart rhythm (Rude *et al*., 2012). Due to its significance, diets high in magnesium are linked to a much lower risk of developing diabetes (Larsson and Wolk 2007). The mineral phosphorus is necessary for the body to carry out a number of vital processes. Phosphorus is a component of bones, teeth, DNA, and RNA, according to Heaney (2012). Additionally, phosphorus aids in tissue healing and water removal. Phosphorus is a key component required for numerous biological activities, including the creation of DNA, ATP, and protein phosphorylation (International Review of Cell and Molecular Biology, 2013). All bodily tissues contain potassium, which is necessary for healthy cell function due to its role in preserving the volume of intracellular fluids and transmembrane electrochemical gradients (Stone *et al*., 2016). Symptoms of a potassium deficiency include paralysis and weakened muscles (Okaka *et al*., 1992). In order for nerve and muscle activity to be normal, sodium is essential. Sodium is necessary for maintaining cellular homeostasis and fluid equilibrium. Sodium controls muscle and neuron irritability, fluid balance, acid-base balance, osmosis, and the absorption of glucose (Ihekeronye and Ngoddy, 1985). The low sodium concentration of *P. pellucida* leaves might be an

advantage because too much salt might result in hypertension, which can then lead to various health issues.

Table 2: Quantitative determination of mineral content of *Peperomia pellucida* leaves

Parameter	Quantity (mg/100g)
Nitrogen	3.14±0.03
Phosphorus	0.49±0.02
Potassium	0.63±0.01
Calcium	2.82±0
Magnesium	1.19±0.06
Sodium	0.38±0.05

Values are means ± standard deviation of three determination

The presence of ascorbic acid, riboflavin, thiamine, niacin, and alpha-carotene in *P. pellucida's* leaves suggests that it is a vitamin-rich plant. Compared to other vitamins, ascorbic acid has the highest composition (8.74%). Ascorbic acid is a crucial dietary antioxidant that significantly reduces the harmful effects of reactive oxygen and nitrogen species, which can cause oxidative damage to macromolecules like lipids, DNA, and proteins and are linked to chronic diseases like cardiovascular disease, stroke, cancer, neurodegenerative disease, and cataractogenesis (Halliwel and Gutteridge, 1999). Ascorbic acid is necessary for the formation and stability of collagen and is necessary for wound healing (Finglas *et al*., 1993). Naicin (also known as vitamin B3) is one of the water-soluble B vitamins, it is the generic name for nicotinic acid (pyridine-3-carboxylic acid), nicotinamide (niacinamide or pyridine-3-carboxamide), and related derivatives, such as nicotinamide riboside (Kirkland, 2014). Niacin is used by the body in a variety of ways, supporting processes in the neurological system, skin, and digestive system. In addition to serving as an antioxidant, niacin is involved in DNA synthesis and replacement as well as cell signaling. Pellagra, a disease marked by a pigmented rash or brown discoloration on skin exposed to sunlight and a roughened, sunburn-like look, is brought on by severe niacin insufficiency (Savvidou, 2014). Like all carotenoids, beta-carotene is an antioxidant. Carotene can quench a single oxygen atom, making it a useful antioxidant. Additionally, B-carotinoids have been linked to a number of positive health outcomes, including a reduced risk of several malignancies, cataracts, muscle degeneration, and cardiovascular events (Olson, 1999). According to epidemiological research, eating more foods high in beta-carotene and having greater beta-carotene levels in the blood are linked to a lower chance of developing lung cancer (Ziglar *et al*., 1996). Riboflavin and Thiamin were also found *in P. pellucida* leaves as additional vitamins. Flavin mononucleotide and riboflavin are two of the most important coenzymes (FAD). These coenzymes are essential for cellular processes, growth and development, energy production, and the metabolism of lipids, medicines, and steroids (Rivlin, 2010). Skin disorders, hyperemia (excess blood), edema of the mouth and neck, angular stomatitis, cheilosis (swollen cracked lips), hair loss, reproductive issues, sore throat, itchy and red eyes, and liver and nervous system deterioration are all indications of riboflavin deficiency (McCormick, 2010). Thiamine is essential for cell growth, development, and function since it is involved in energy metabolism. For the oxidation of glucose to occur, thiamine is required. Dietary thiamine deficiency results in only a partial oxidation of glucose. The most prevalent thiamine deficiency symptom is beriberi, which is primarily characterized by wasting and peripheral neuropathy (Berneur *et al*., 2014).

Table 3. Quantitative determination of Vitamin contents of *P. pellucida* leaves.

Parameter	**Quantity (mg/100g)**
Ascorbic acid	8.74±0.12
Riboflavin	0.34±0.01
Thiamine	0.21±0.03
Niacin	0.57±0.01
B-carotene	2.33±0.15

Results are expressed as MEAN of three determinations ± SD

Table 4. Proximate composition of *P. pellucida* leaves

Parameter	**Quantity (mg/100g)**
Ash Content	9.71 ±0.34
Crude Protein	19.58±0.22
Crude Fibre	11.45±1.7
Carbohydrate	53.43±1.52
Lipids (Fats)	5.83±0.74
Moisture Content	7.90±1.94

Results are expressed as MEAN of three determinations ± SD

Proximate composition of P. pellucida leaves (Table 4) shows that their concentration was in this order < carbohydrate > protein > fibre > ash > fats).

Proteins are essential for the body's defense mechanism, energy production, and amino acid synthesis (Eddy and Udoh, 2005). Proteins are vital nutrients for the body of an individual. They can be used as fuel and are one of the building components of body tissue. One of the three macronutrients in our diet, carbohydrates serve as the body's primary source of energy. Among the macronutrients, carbohydrates are the most significant source of food energy, making up between 40 and 80 percent of daily caloric consumption. Carbohydrates play an important role in the human body. They provide energy, contribute in the metabolism of cholesterol and triglycerides, regulate the metabolism of blood sugar and insulin, and aid in fermentation. Although there are several categories of carbs, the human diet mostly benefits from one particular subset (Mills *et al*., 2019). Dental carriers are linked to carbohydrates. It is well recognized that eating a lot of sugary foods causes plaque buildup, tooth damage, and cavities. Sucrose is the worst carbohydrate for dental decay. On the other hand, fructose serves as an energy source for oral cavity bacteria (Uhrig *et al*., 2018). Dietary fiber is the fraction of plant-based foods that human digestive enzymes are unable to completely break down (Lockyer and Nugent 2017). Dietary fiber and whole grains have health benefits that may include a lower

risk of death and lower incidences of coronary heart disease, colon cancer, and type 2 diabetes (Morenga *et al*., 2019). A food's ash content is a gauge of its mineral content. Ash residue is typically used as a measurement of the materials' mineral composition (James, 1995; Onwuka 2005). Water is necessary for circulation, cellular homeostasis, temperature control, substrate transfer across membranes, and metabolism. Water is a necessary component of food because it transports nutrients effectively and participates actively in a number of metabolic processes. Every food processor places a high priority on food moisture content since it affects a variety of biochemical reactions and physiological changes in food (Onwuka, 2005). Foods high in fat have a high calorie density by nature. More calories are found in diets high in fat than in those heavy in protein or carbs. High-fat diets are therefore a practical source of energy. The majority of meals that contain lipids contain the fat-soluble vitamins. Lipids, particularly fats, make meals more palatable and are crucial emulsifiers for many medicinal preparations (Ononogbu, 2002).

Table 6: Antioxidant determination of *P. pellucida* leaves

Concentration (mg/ml)	**Ascorbic acid**	***P.pellucida* crude extract**
2.0	8.35± 0.02	5.02 ± 0.01
4,0	12.40 ± 1.08	8.7 9± 0.02
8.0	21.65 ± 1.32	14.20 ± 1.02
10.0	24.08 ± 0.02	17.50 ± 0.04
12.0	43.82 ± 2.1	17.50 ± 0.04

*MEAN of three determinations ± SD

The result revealed that the extract exhibited significant free radical activity at minimum and maximum concentration of 2.0 and 12.0mg/ml compared to the ascorbic acid used as a standard free scavenger. *P. pellucida* has DPPH scavenging and it was also observed that a dose-response relationship is found in DPPH radical scavenging activity for crude extract, as the concentration increased the activity also increased. Antioxidants show activities at different levels of protection (Cadena and Packer, 1996). They are of great importance as possible protective agents to help human body to reduce oxidative damage. As shown in Table 3 below, four organisms were used for the antibacterial activity of the leaf extract of Peperomia pellucida (*Staphylococcus aureus, Escherichia coli, Salmonella typhi and Bacillus cereus).* The antimicrobial assay at different concentration of methanol leaf extract of *P. pellucida* has shown antimicrobial activity against the test organisms. The microorganisms are human pathogens that have been involved in causing diseases and infections in man (Igwe and Echeme; 2013). Some of the diseases they can cause include skin infections, pneumonia, meningitis, bacteremia, sepsis, toxic shock syndrome, urinary tract infections, vomiting, diarrhoea, anaemia, typhoid fever, kidney infections, osteomyelitis, endocarditis, septicemia and wound infections (Kotiranta *et al* 2000).

Table 7: Zones of growth inhibition at varying concentrations of methanolic extract of *P. pellucida* leaves against the test organisms.

	Zone of Inhibition					
Test organisms	100mg/ml	50mg/ml	40mg/ml	20mg/ml	10mg/ml	DMSO (0.1%)
S. aureus	20.60±2.70	16.33±1.41	12.00±1.33	9.00±0.20	6.30±67	-
E. coli	16.33±1.41	12.40±1.08	8.00±0.12	6.00±0.00	-	-
S. typhi	14.03±0.50	13.33±0.33	11.93±0.09	9.75±0.50	-	-
B. cereus	14.00±0.25	10.00±0.18	7.02±0.5	-	-	-

*Results are expressed as MEAN of three determinations ± SD: Note: *S. aureus* = *Staphylococcus aureus, E. coli* = *Escherichia coli, S. typhi* = *Salmonella typhi, B. cereus* = *Bacillus cereus,* DMSO = Dimethyl sulfoxide, - = No zone of inhibition.

Conclusion: Peperomia pellucida is a good source of phytochemicals – alkaloids, tannins, flavonoids, saponin and phenols which could be responsible for the versatile medicinal properties of this plant. Vitamins, mineral elements and proximate compositions of the leaves of this plant signify that the plant provides nutritional benefits.

This work has also revealed the antibacterial activities of the leaves of this plant on known human pathogen, and it was also found to have high antioxidant activity. *P. pellucida* leaves appears to be suitable for developing drugs that can be used to treat several diseases.

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