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Research article

Phytonutrients, antioxidants and anti-inflammatory analysis of peperomia pellucida

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ABSTRACT

The effectiveness of fruits and vegetables in the management of various forms of ailments and disorders has been attributed to the presence of bioactive components. Most fruits and vegetables are only available for a season and perish easily. Peperomia pellucida is a seasonal vegetable that is usually available only during rainy season. The study investigated the nutritional contents, antioxidant and anti-inflammatory activities of Peperomia pellucida as well as the effects of drying on these activities. Methanolic extract of fresh and dried leaves and stem of Peperomia pellucida were subjected to phytochemical screening and nutritional analysis including estimation of soluble proteins, sugars and amino acids. Antioxidant analysis included vitamin C and vitamin E estimation, assay of DPPH-free radical scavenging activity and antioxidant reducing power while anti-inflammatory analyses included assay of membrane stability activity and albumin denaturation inhibition. All procedures were carried out using standard methods. Phytochemical analyses revealed the presence of a number of vital phytochemicals including alkaloids, flavonoids, cardiac glycosides, tanins, triterpenoids and xanthoproteins. Phytochemicals present in dried leaves of the plant were found to be higher than what obtained in the fresh leaves while fresh vegetables were found to have nutritional content (Vitamin E, Vitamin C, Protein, Soluble sugar and free amino acid) than the dried vegetable. Furthermore, both fresh and dired leaf extracts of P. pellucida exhibited a wide range of antioxidant and anti-inflammatory potentials which were found to be comparable to what obtained in standard drugs. Peperomia Pellucida is a vegetable of high nutritional value and has prospects of being used in the management of various diseases in which oxidative stress and inflammation are implicated. Furthermore, Processing (drying) of the vegetable elicits a great reduction in the nutritional contents. This could be due to dehydration and denaturation of the vital components of the vegetable.

Keywords: Nutritional analysis, anti-oxidant activity, anti-inflammatory activity, P. pellucida

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INTRODUCTION

Fruits and vegetables have been used in the prevention and treatment of various types of ailments and diseases since prehistoric time. This is because bioactive components contained in them (phytochemicals) which primarily serve as defense against environmental hazards such as pollution, stress, drought, UV exposure and attack by pests and herbivorous animals [(1)] are also of therapeutic benefits to humans as a result of their antioxidant, anti-microbial, cytotoxic , and anti-inflammatory activities [(2)] . However, the potency of such bioactive phytochemicals in fruits and vegetables are usually affected by several factors such as place and time of collection, method of extraction as well as processing methods [(3)]. Peperomia pellucida (L.) Kunth, also known as pepper elder, shiny bush, silver bush, rat-ear, slate pencil, clearweed and man to man which belongs to the family Piperaceae. It is a common annual weed native to tropical North and South America [(4)]. Locally called ′rinrin' in Yoruba, it is an annual or short-lived perennial, entirely delicate, fleshy and glabrous herb usually growing to a height of about 15 - 45 cm. The stem is translucent pale green, erect or ascending or decumbent, freely branched internodes usually 3-8 cm long and hairless [(4)]. Amongst the Akokos in the South-Western part of Ondo State of Nigeria, it is consumed raw or in soup for the treatment and management of fever and high blood pressure (HBP). Several studies have attempted to investigate the phytopharmaceutical potential of various parts of the plant [5, 6, 7, 8, 9]. In this study, the nutritional contents as well as the antioxidant and anti-inflammatory potentials of the leaf and stem of P. pellucida were analyzed. The effect of processing (drying) on the nutraceuticals was also evaluated.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh vegetables (stem and leaf) of P. pellucida were collected from the main Campus of Obafemi Awolowo University, Ile-Ife and Ajebandele Community, Ile Ife, Osun State, Nigeria. It was identified and authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile Ife, Nigeria where specimen identification number (IFE 17461) was obtained.

Preparation of Hydromethanolic Extract of P. pellucida

The methanol extract of P. pellucida was prepared according to a modified method [10]. Briefly, plant materials were divided into two equal parts. The first portion was cut into bits, crushed and squeezed to collect the juice. The juice was filtered, centrifuged and evaporated to thick slurry before finally dried to powder to produce fresh vegetable extract (FVE). The second portion was air-dried until completely dried and pulverized. The powdered plant material (280g) was soaked in 70% (v/v) methanol, in sealed plastic container at room temperature for seven days with occasional stirring. The suspensions were filtered using double-layered cotton material. The residues were re-extracted with same solvent 70% (v/v) methanol until the filtrate became colourless. The combined filtrate was evaporated to dryness at 40oC under reduced pressure in Rotary Evaporator (Buchi Rotavapor RII coupled to a Butchi Vacuum Pump V-700) to yield methanol extract of P. pellucida termed (PPME).

Phytochemical Screening of Methanol Extract of P. pellucida

The methanol extracts (FVE and PPME) were screened for the presence of secondary metabolites (flavonoids, alkaloids, saponins, tannins, anthraquinone and cardiac glycosides) using a procedure that was based on earlier reported methods (11, 12, 13).

(a) Flavonoids (ethylacetate/ammonia solution;0.5M ethanolic KOH); (b): alkaloids (Mayer's reagent; Wagner's reagent, and Dragendorff reagents); (c) saponin (frothing test, haemolytic test); (d) tannin (0.5 M $FeCl_3$ reagent); (e) anthraquinone (dilute H_2SO_4/benzene/ammonia solution); (f) cardiac glycosides (chloroform/ conc. H_2SO_4); (g) triterpenoids (chloroform/ conc. H_2SO_4); (h) steroids (conc. H_2SO_4); (i) xanthoproteins (dilute nitric acid/ ammonia solution); and (j) phlobatanins (heat + dilute HCl).

Quantification of Phytochemicals

i) Estimation of Total Phenolic Content in PPME

The total phenolic contents of both FVE and DVE using Folin-Ciocalteu's Phenol reagent reaction method according to the spectrophotometric method of Singleton et al [14] with tannic acid as standard. The concentration was expressed as µg/g extract tannic acid equivalent

ii) Estimation of Total Flavonoid Content of Methanol Extract

The estimation of the flavonoid content was carried out according to the spectrophotometric method described by Sun et al [15] based on the formation of aluminum-flavonoid yellow complex with quercetin as standard. The values are expressed as µg/g extract quercetin equivalent.

iii) Estimation of Tannin Contents of Methanol Extract

The estimation of tannin concentration was carried out according to the method described by Marker and Goodchild [16] with Folins- Ciocalteaus Phenol Reagent.

Nutritional Analyses of P. pellucida

Estimation of Soluble Proteins, soluble sugars and free amino acids from Fresh and Dry Leaves of P. pellucida

The soluble proteins of fresh and dry leaves of P. pellucida were estimated using bovine serum albumin (250µg/ml) as standard according to the spectrophotometric method described by Scacterle and Pollack [17]. Concentrations of soluble sugar in both fresh and dried P. pellucida was quantified according to phenol – sulphuric acid method [18] using α- D-glucose (250 µg/ml) a standard. The concentrations of free amino acids were quantified from P. pellucida using ninhydrin reaction according to the method described by Rosen [19] with L-glutamic acid as standard.

Estimation of Vitamin C and Vitamin E concentration

The concentrations of vitamin C of the fresh and dried leaf/stem extracts of P. pellucida were estimated according to Omaye et al [20] as modified and reported by Fajobi et al [21] while the extraction of vitamin E was carried out according to the method described by Santosh et al [22] and the content was estimated according to the method of Jargar et al [23] as reported by Fajobi et al [21] with slight modification. The concentration of the Vitamins was extrapolated from standard calibration curves and expressed as ug/g vegetable.

Evaluation of Antioxidant Activities of PPME

Assay of DPPH Free-Radical Scavenging Activity and Anti-oxidant reducing power of PPME

The radical scavenging ability of the vegetable was determined using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) according to the method of Blois [24] modified by Aina and Oyedapo [25]. The percentage radical scavenging inhibition by the extracts/standard was calculated as:

$$\text{Percentage DPPH Scavenging Activity} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

The reducing antioxidant power of PPME was carried out according to the method of Chu et al [26]. The assay depended on the ability of the extract to reduced Fe^{3+} to Fe^{2+}. Ascorbic acid of varying concentrations (0 – 350 µg/ml) was used as standard.

Evaluation of Anti-inflammatory Activity of PPME

Assay of Membrane Stability Activity

The membrane stabilizing activity assay was based on methods developed in our laboratory [25, 27, 28, 29]. It measures the degree of hemolysis that occurs when a sample of red blood cells is subjected to oxidative stress by being placed in a hypotonic solution. Bovine blood samples were placed in anticoagulant bottles and centrifuges at 3,000rpm for 15 minutes. The supernatant was

removed and the erythrocytes re-suspended in fresh normal saline. This was mixed by inversion and centrifuges for 10 minutes. The process was repeated until clear supernatant was obtained. Erythrocyte suspension (2%) was then prepared by adding 98ml normal saline to 2ml of packed red blood cells. Typically, the reaction mixture contained 1ml hypo saline, 0.5ml of 0.1M phosphate buffer (pH 7.4) and varied concentrations of extracts/ ibuprofen, made up to 3ml with normal saline. Blood control and drug control were also prepared. The mixture was incubated at 56ºC for 30 minutes and absorbance was read at 560nm against reaction blank.

Percentage membrane stability was estimated using the expression below.

$$Percentage\ \ membrane\ stability = 100 - \frac{Abs\ test\ drug - Abs\ drug\ control}{Abs\ blood\ control} * 100$$

Assay of Albumin Denaturation Inhibition

Inhibition of albumin denaturation was carried out according to the procedure of Mizushima and Kobayashi [30] as modified and reported by Aina and Oyadapo [25]. Briefly, different concentrations of PPME (0 - 350 µg/ml) were pipetted into separate test tubes, followed by the addition of 0.5 mL albumin (1.5 mg/ml). The mixture was incubated at 37°C for 20 min and at 57°C for 3 min. 0.5 M phosphate buffer, pH 6.3 (2.5 ml) was added. The mixture (1 mL) was pipetted into a clean test tube and followed by the addition of copper-alkaline reagent (1 mL) and 1 ml of Folin-Ciocalteu's Phenol Reagent (1:10). The reaction mixtures were incubated at 55°C for 10 min. the tubes were cooled and the absorbance were read at 650 nm against reagent blank. The quantity of protein left and the percentage inhibition was calculated using the expressions below:

$$\text{Percentage Inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ Sample}{Absorbance\ of\ control} X100$$

RESULTS AND DISCUSSION

Percentage Yield of Methanol Extract of P. pellucida

Extraction of 2.5 kg of fresh P. pellucida yielded 17.76 g of methanol extract (0.72 % of the starting material) while that of 280 g dried leaf of P. pellucida yielded 32.27 g (11.52 % of the starting material).

Phytochemical Analysis of P. pellucida

Table 1 shows the summary of the phytochemical constituents of methanol extract of P. pellucida. The results revealed the presence of alkaloids, flavonoids, cardiac glycosides, tannins, triterpenoids, steroids, xanthoproteins, and phlobatannins while anthraquinone was found to be absent. This implies that the vegetable is highly rich in vital phytochemicals with potent and appreciable biological activities.

Nutritional Analyses of P. pellucida

The total phenolic content of fresh and dried leaf/stem of P. pellucida was 35.46 ± 0.17 mg TAE/g extract and 51.63 ± 0.25 mg TAE/g extract respectively. The flavonoid content of the extracts, the fresh and dried leaf/stem of P. pellucida was 627.45 ± 15.08 mg RE/g extract and 868 ± 26.12 mg RE/g extract respectively, implying that the phenolic and flavonoids contents of the methanol extract of dried leaves of P. pellucida was higher than that of the fresh leaves/stems of P. pellucida.

Table 2 shows that the nutritional contents of dried leaves/stem of P. pellucida are higher than fresh leaves/stems of P. pellucida. Soluble sugar, total protein concentration Vitamin C, vitamin E and free amino acid content of the dry leaves were all higher than what was obtained from fresh leaves

Table 1: Phytochemical Constituents of P. pellucida Methanol Extract

Phytochemicals	Presence/Absence
Alkaloids	+
Flavonoids	+
Saponin	+
Anthraquinones	-
Cardiac glycosides	+
Tannins	+
Triterpenes	+
Steroids	+
Xanthoprotein	+
Phlobatanins	+

Where (+) represent present and (-) represent absent

Table 2: Summary of phytochemical / Nutritional Analysis of Pepperomia pellucida/g Sample

Phytoconstituent	Fresh Vegetable (mg/g)	Dry Vegetable (mg/g)
Total Phenolics	35.54 ± 0.09	627.45 ± 26.12**
Total Flavonoids	51.62 ± 0.25	868.78 ± 45.25**
Total Tanins	2.47 ± 0.04	13.06± 0.19[a]
Vitamin E	0.14 ± 0.01	0.66 ± 0.01[a]
Vitamin C	0.17 ± 0.01	0.81 ± 0.01[a]
Protein	35.46 ± 0.17	51.63 ± 0.25a
Soluble sugar	9.04 ± 0.19	61.96 ± 1.95[a]
Free amino acid	0.49 ± 0.01	4.31 ± 0.05[a]
	2.47 ± 0.04	

**Each value represents the Mean ± SEMa of n=3 readings, $p<0.05$ was considered statistically significant.

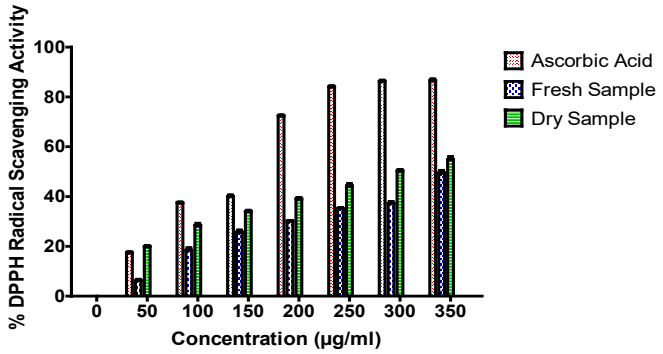
Preliminary results revealed that 1 g dry vegetable was equivalent to 14.1 g fresh vegetable. This means that overall, the nutritional content (Vitamin E, Vitamin C, Protein, Soluble sugar and free amino acid) of fresh leaves is much higher than that of dry leaves.

1, 1- Diphenyl-2- Picrylhydrazyl (DPPH) Radical Scavenging Activity of PPME

The results revealed that the methanol extract of fresh leaves of P. pellucida scavenged DPPH radical maximally at 350 µg/ml with a percentage scavenging of 49.46 ± 0.89 % while the dried leaves of P. pellucida scavenged DPPH radical maximally at 350 µg/ml with a percentage scavenging of 54.85 ± 1.04 %. The ascorbic acid used as positive control scavenged DPPH radical maximally at 350 µg/ml with a percentage scavenging of 86.52 ±

0.51 %. The DPPH activity of the standard (ascorbic acid) and the extracts (Fresh and dried leaves) was concentration dependent (Figure 1).

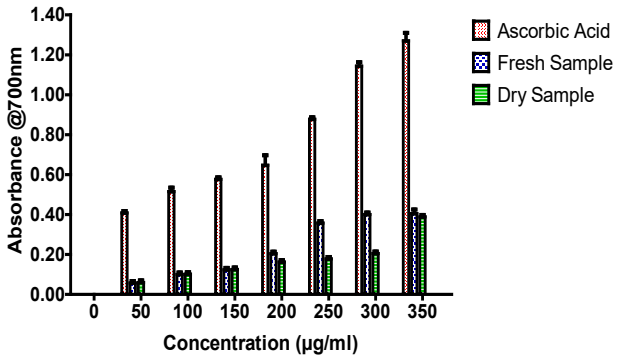
*Figure 1: Percentage DPPH Radical Scavenging Activities of the Ascorbic Acid, Fresh and Dry Vegetable.



Reducing Antioxidant Power

The reducing power of the extracts (PPME) is depicted in Figure 2. It showed that the reducing power of the extracts increased and correlated well with increase in concentration i.e. it is concentration dependent. Furthermore, the standard (ascorbic acid) had a higher reducing power than the extract. The percentage reducing power of the fresh and dry extracts were estimated to be 31 % and 29 % respectively.

*Figure 2: Reducing Antioxidant Power



Percentage Membrane Stabilizing Activity of P. pellucida

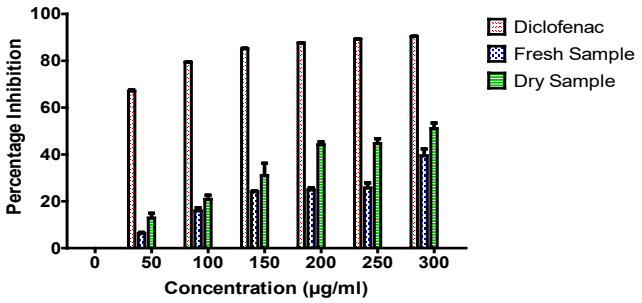
Results shows that the extracts (fresh and dry) as well as the standard drug (Ibuprofen) protected the red blood cells (RBC) against both heat and hypotonic induced lyses at varied concentrations ranging between 50 µg/ml and 300 µg/ml with a monophasic mode of protection. The mean maximum percentage stability of the extract was 87.20 ± 3.18% for methanol extract of fresh leaf/stem, 87.80 ± 6.25 % for methanol extract of dried leaf/stem of P. pellucida and 96.47 ± 0.99 % for the standard (Figure 3). The stabilizing activities of both extracts and standard were found to be concentration dependent.

*Figure 3: Percentage Membrane Stability Activities of the Extracts and standard drug (Ibuprofen)

Inhibition of Albumin Denaturation

Figure 4 shows that denaturation of albumin was inhibited at various concentrations by PMME as well as the standard drug.

Figure 4: Percentage Inhibition of Albumin Denaturation



*Each value represents Mean ± SEM of n = 3, values of $P < 0.05$ were considered statistically significant.

DISCUSSION

Phytochemicals are bioactive compounds found in parts of plants, fruits and vegetables. They contribute to the color, aroma, and flavor and protect plants from environmental hazards such as pollution, stress, drought, ultraviolent (UV) exposure and pathogenic attack 31. More importantly, it is believed that they are responsible for the protective, ameliorative and therapeutic effects associated with consumption of plant products. This is because many of them possess antioxidant, anti-inflammatory and anti-microbial activities. Phytochemical screening of methanol extract of P. pellucida revealed the presence of alkaloids, flavonoids, saponin, cardiac glycosides, tannins, triterpenoids, steroids, xanthoproteins, phlobatannins but anthraquinone was found to be absent. This is in agreement with earlier observations of Egwuche et al. 5 and Gini and Jothi, (2013) 6 who also observed the presence of all the above phytoconstituents except anthraquinone from the extracts of P. pellucida. The presence or absence of a phytoconstituent as well as its quantity depend on a number of factors such as place of collection, time of collection, period of collection as well as extraction methods and solvent employed in the extraction. Moreover, studies have shown that hydroalcoholic tends to extract more active biomolecules than aqueous or other solvents.

Quantification of antioxidants potentials (phenolics, total flavonoids, vitamin C and vitamin E) revealed that the vegetable (P. pellucida) is rich in vital phytoconstituents that possess and exhibit potent and appreciable activities. The analysis of the results shows that the phenolic and flavonoids contents of the methanol extract of dried leaves of P. pellucida was higher than the methanol extracts of the fresh leaf of P. pellucida. This might be due to the presence of high water in the fresh vegetable as the of 1g of dried leaf was found to be equivalent to 14.1g of fresh leaf (93 % water) during preliminary study. The phenolic content of the vegetable was evaluated by the Folin Ciocalteu's Phenol reagent reaction method14. The phenolics content of both fresh and dry vegetables were very

high in reference to standard tannic acid indicating that the methanol extract of the vegetable contained mostly polyphenol. Analyses of the extracts of both fresh and dry vegetables revealed high flavonoid and phenolic contents. Dare et al 32 and Mathias et al. 33 reported higher flavonoid contents in the acetone extracts of fermented and unfermented Theobroma cacao seeds.

The nutritional contents of fresh and dried leaf of P. pellucida revealed that the dried leaves of P. pellucida had a lower nutritional content than the fresh leaves/stems of P. pellucida. This agrees with the report of Sheetal et al 34 who reported the retention of nutrients in green leafy vegetables on dehydration where the nutritional content of fresh and dried vegetable per 100g was investigated and the results showed that the fresh vegetable had higher nutrient contents. This present study observed a lower nutritional content in 1g of dried leaf of P. pellucida when compared with 1g of fresh leaf of P. pellucida; this could be as a result of the moisture content of the fresh P. pellucida. More so, higher physiochemical content was observed in 1g of dried leaf of P. pellucida when compared with 1g of fresh leaf of P. pellucida, indicating that drying has a positive effect on phytochemical content.

Antioxidant activities of extracts cannot be evaluated using a single method due to the fact that different types of ROS and RNS are generated during normal metabolic activities. In this study, DPPH-radical scavenging activity and Ferric reducing antioxidant power were evaluated. The reducing power of P. pellucida was obtained by the method of ferric reducing antioxidant power (FRAP) using ascorbic acid as standard due to the presence of free hydroxyl group in its structure thus preventing the oxidation of free radicals. Ferric reducing antioxidant power (FRAP) measures the ability of antioxidants to reduce ferric 2, 4, 6- tripyridyl-s-triazine complex to intensively blue colored ferrous complex in acidic medium. Hence, any compound which is having redox potential lower than that of redox pair Fe (III)/Fe (II) can theoretically reduce Fe (III) to Fe (II). In the present study, the reducing power of the plant was concentration dependent, and agrees totally correlates with the report of Nilima and Hande [35].

Inflammation is a protective response involving immune cells, blood vessels, and molecular mediators of body tissues to harmful stimuli, such as pathogens and damaged cells [36]. It plays significant role in initiating tissue repair, eliminating the initial cause of cell injury, and clearing out damaged tissues [34]. Various methods such as inhibition of albumin denaturation, platelets aggregation, lysosomal membrane stabilization and stabilization of erythrocyte membranes has been used to screen anti-inflammatory drugs or agents 34. This study employed the stabilization of erythrocytes membrane and inhibition of albumin denaturation to investigate the anti-inflammatory activity of P. pellucida. The results show that the red blood cell membrane stabilizing activities of the extracts were concentration dependent and were as active as that of the standard anti-inflammatory drug (Ibuprofen), this could be as a result of P. pellucida been rich in flavonoids. Flavonoid-rich compounds have been shown to exhibit very high anti-inflammatory potentials. The mode of response of the erythrocyte was monophasic, in agreement with the earlier observations of Oyedapo et al 11 in the investigations of red blood cell membrane stabilizing potentials of extracts of Lantana camara and its fractions. The assay of albumin denaturation showed that there was inhibition of albumin denaturation at various concentration of the PPME and standard drug. The standard drug (diclofenac) showed the highest percentage inhibition when compared with PPME. This also was in line with the observations of Aina and Oyedapo25 who also reported the inhibition of albumin at various concentrations of the extracts and standard drug. It is therefore possible that the anti-inflammatory potential of PPME might be attributed to the phytochemicals present in it.

DPPH assay is a direct and reliable method for determining radical scavenging action of plant extracts, fractions and isolated compounds that is based on the ability of DPPH to decolorize in the presence of antioxidants due to their hydrogen donating ability. The present study revealed that the DPPH activity of the standard (ascorbic acid) and the extracts (Fresh and dried leaves) were concentration dependent. The methanol extract of the dried leaf/stem of P. pellucida was observed to exhibit a higher percentage scavenging DPPH radical when compared with the methanol extract of fresh leaf/stem. The ability of the vegetable to scavenge free radicals might be due to the presence of phenolics such as flavonoids, tannins, which act as antioxidants by the mechanism that involve adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. This result agrees with the report of Rajani et al 36 that scavenging of DPPH radical increased with increasing concentrations of the extracts and there was a significant correlation between phenolic content and the scavenging of DPPH radical in all the leafy vegetables.

CONCLUSION

The study reveals that P. pellucida has quite a number of phytochemicals, phytochemicals present in the dried leaves of P. pellucida was found to be higher than the fresh leaves indicating that drying elicit a positive effect on the quantity of the phytochemical while the nutritional contents of the dried leaves of P. pellucida was found to be lower than the fresh leaves, indicating that processing (drying) causes a reduction in the nutrient contents. More so, the vegetable was found to exhibit a wide range of antioxidants and anti-inflammatory potentials enabling it to be useful in the management of

several disease conditions.

Declaration of Conflict of Interest

Authors declare that there is no conflict of interest.

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