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Nutritional composition, Phytochemical analysis and Antioxidant capacity of Ethanol extract of *Picralima nitida* fruit (bark and pulp)

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ABSTRACT: *Picralima nitida* (Apocynaceae) is an African pepper tree known for its medicinal benefits. This paper aims to investigate the nutritional composition, phytochemical analysis, and Antioxidant capacity of Ethanol extract of *Picralima nitida* fruit (bark and pulp) using standard methods. The proximate analysis of the dried powdered fruit (bark and pulp) had high carbohydrate, crude fiber content, moderate ash content, low moisture content, and low crude fat composition. The metal composition analysis showed the presence of various essential and non-essential elements, with sodium, potassium, and iron having the highest concentrations. The qualitative phytochemical screening showed the presence of saponins, alkaloids, tannins, terpenoids, phenols, flavonoids, glycosides, steroids, and phytosterols in varying concentrations in the ethanol extract. The quantitative phytochemical analysis revealed that the total phenol, total flavonoid, total tannins, proanthocyanidin, and total saponin contents had concentrations of 92.45 mg GAE/g extract, 331.64 mg QE/g extract, 64.89 mg TAE/g extract, 129.00 mg AAE/g extract, and 213.79 mg DE/g extract respectively. The ethanol extract of *P. nitida* fruits exhibited strong antioxidant activities, with the FRAP assay showing the highest activity, followed by DPPH radical scavenging activities. These findings suggest that *P. nitida* stem bark has significant potential as a natural source of antioxidants and essential minerals, which could be useful in developing functional foods and nutraceuticals.

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A medicinal plant is any plant that contains components that can be used for therapeutic purposes or that are precursors to the manufacturing of beneficial drugs. The use of plant-based remedies as a source of medicine for various human illnesses has increased due to factors like population expansion, lack of available medications, expensive treatment options, side effects, and drug resistance. The recent advancement of pharmaceutical and functional food items generated from medicinal plants and foods (mainly fruits and vegetables) has benefited all aspects of life, including physical disorder treatment, reduced use of synthetic antibiotics, and increased life span (Yessoufou *et al.*, 2013). These plants have been utilized for a very long time as safe, effective, and sustainable sources of phenolic compounds or phytochemicals, including phenolic acids, flavonoids, tannins, phytosterols, and anthocyanins, which function as free radical scavengers or antioxidants naturally. Alkaloids, tannins, saponins, flavonoids, phenols, steroids, and carotenoids, among others, are phytochemicals present in medicinal plants that serve various disease-prevention purposes (Barbosa *et al*., 2013). Significant anti-inflammatory, anti-diabetic, anti-aging, antibacterial, antiparasitic, antidepressant, anti-cancer, antioxidant, and wound-healing activities are present in these plant-derived chemicals (Bahramsoltani *et al*., 2014). These phytochemicals

are primarily believed to boost food and medicinal plants' antioxidant capacities, considerably assisting in the fight against various pathological conditions like cancer, diabetes, aging, cardiovascular disease, and other degenerative diseases (Yu *et al*., 2021). *Picralima nitida* is a medicinal plant in the genus Picralima and the plant family Apocynaceae. It is found in tropical African countries such as Ivory Coast, Nigeria, Uganda, and Gabon, and it is well known as Abeere among the Yoruba people in the Southwestern area of Nigeria (Osayemwenre *et al*., 2014). *P. nitida* is an understorey tree that grows up to 4-35 m tall, has a dense crown, and a trunk that is 5-60 m in diameter. Its cylindrical wood is pale yellow in colour, durable, elastic, and fine-grained, and it polishes up quite well. White, 3-cm-long flowers are produced by *P. nitida*, together with ovoid, maturely yellowish fruits. The leaves are rectangular (6–20 cm) and broad (3–10 cm), with 14–24 pairs of tough, small lateral nerves (Burkill, 1985). The plant is commonly used in traditional medicine to treat and manage malaria, abscesses, pneumonia, hepatitis, diabetes, and hypertension (Olajide *et al.*, 2014). In African ethnomedicine, several parts of the plant, including the leaves, fruits, and stem bark, have been used to treat a variety of illnesses, including fever, hypertension, jaundice, dysmenorrhea, malaria, and gastrointestinal disorders, among others (Osayemwenre *et al*., 2014). Many studies have previously demonstrated that various extracts of this plant are good sources of phytochemicals such as glycosides, alkaloids, triterpenes, flavonoids, polyphenols, saponins, and tannins (Teugwa *et al*., 2013). Several studies have reported on the phytochemical composition and antioxidant activity of extracts of parts of the *P. nitida* plant (De Campos *et al*., 2020; Osayemwenre *et al*., 2014; Ngaïssona *et al*., 2016). However, there is still limited information on the potential bioactive compounds and antioxidant properties of *P. nitida* fruits (bark and pulp) extracts. Therefore, the objective of this paper is to investigate the nutritional composition, phytochemical analysis, and Antioxidant capacity of Ethanol extract of *Picralima nitida* fruit (bark and pulp).

MATERIALS AND METHODS

Plant materials: Picralima nitida was obtained locally from New Benin market, Edo State in Oredo Local Government Area, Nigeria. A plant sample was authenticated in the Department of Plant Biology and Biotechnology, University of Benin, Nigeria. A herbarium or voucher number UBH-P424 was obtained and deposited in the herbarium.

Preparation of Extract: The plant sample (material) was harvested, gathered, and properly washed underneath clean tap water. It was then allowed to dry (air dry process), ground, and sieved. After pulverization, the plant was weighed, and the weight was 5100 g. Excursive extraction was done on the pulverized plant material by soaking it in ethanol reagent for 72 hours. To ensure proper extraction, the solution was occasionally stirred. At the end of the third day, the solution was carefully filtered through two layers of cheesecloth with thick cotton wool and a handkerchief placed on it. This process was repeated 4 times. Finally, the filtrate was allowed to pass through a filter paper to eliminate the residues. At the end of this process, the filtrate was then dried using a freeze-dryer, and the dried extract was weighed. A total yield of 147 g was obtained, and it was kept in a refrigerator in an airtight container until it was time to use it.

Chemicals and Reagents: Methanol, 1,1-Diphenyl 1-2-picrylhydrazyl (DPPH), Chloroform, Gallic acid, Folin Ciocalteu, phenol reagent and potassium acetate were products of Sigma- Aldrich Chemical Company Ltd (St. Louis, U.S.A) while ascorbic acid, aluminum chloride hydrate and ethanol were from JDH =, China.

Proximate Evaluation: Proximate analysis to determine moisture, protein, crude fat, crude fibre, and ash contents of dried powdered *P. nitida* fruits (bark and pulp) were carried out in triplicate according to the methods described by the Association of Official Analytical Chemists (AOAC, 2000).

Phytochemical Screening: Qualitative determination: The qualitative screening was carried out using established protocols as described by Sofowora (1993), and Trease and Evans (1983).

Quantitative Determination: Total Phenolic Content: The phenolic content of ethanol extract of *P. nitida* fruits (bark and pulp) was determined by the method described by Roy *et al.* (2018). The principle of the Folin-Ciocalteu assay is the reduction of Folin-Ciocalteu reagent (FCR) in the presence of phenolics resulting in the production of molybdenum-tungsten blue that is measured at 750nm, and the intensity of the blue colour increases with the increasing concentration of phenolics in the reaction medium. The phenolic content was estimated using the Folin-Ciocalteu reagent (FCR) method. In a test tube, 200 µl of extract/standard was added to 1.5 ml of 10% FCR reagent and incubated for 5 mins in the dark at room temperature, after which 1.5 ml of 5 % sodium carbonate was added and mixed correctly. This was again incubated for 2 hours in the dark, and the absorbance read at 750 nm. The standard curve was plotted using 1 mg/ml Gallic acid at different concentrations of 5, 10, 25, 50, 75, 100, 150, and 200

µg/ml. The phenolic content was expressed as milligrams of Gallic acid equivalent (GAE) per gram of dried extracts.

Total Flavonoid Content: The flavonoid content of ethanol extract of *P. nitida* fruits (bark and pulp) was determined by the modified method described by Roy *et al.* (2018). The flavonoid content was determined using the aluminum chloride colorimetric method. To 1.0 ml of extract/standard, 0.3 ml of 5% sodium nitrite was added, and the solution was incubated for 5 minutes at room temp. After which, 0.3 ml of 10% aluminum chloride was added. This was again incubated for 5 mins at room temperature. Two milliliters of 1M NaOH was added and incubated for 10 minutes at room temperature. Quercetin (1 mg/ml) was used as standard, prepared at different concentrations of 10, 50, 75, 100, 150, 200, 250, and 300 µg/ml. The absorbance was read at 510 nm. The total flavonoid content obtained was expressed in terms of mg of Quercetin Equivalent per gram of extract.

Total Tannin Content: The tannin content of ethanol extract of *P. nitida* fruits (bark and pulp) was determined by the modified method described by Roy *et al.,* (2018). The principle of the F-C assay is the reduction of FCR in the presence of tannins from molybdate (VI) ion to Molybdate (V) ion to give a blue colour that is measured at 725 nm, and the intensity of the blue colour increases with the increasing concentration of tannins in the reaction medium. The tannin content was estimated by the Folin-Ciocalteu method. 1mg/ml gallic acid was prepared at different concentrations of 10, 50, 100, 200, 300, 400, 500, and 600 µg/ml. In a test tube, 100 µl (0.1 ml) of extract/standard was added to 7.5 ml distilled water, followed by 0.5 ml of the prepared 10% FCR and 1ml of the prepared 35% sodium carbonate solution. This was mixed well and incubated for 10 minutes at room temperature. The absorbance of the extracts and Gallic acid standards were read at 725 nm. The tannin content obtained was expressed as milligrams of Gallic acid equivalent (GAE) per gram of extract.

Total Saponins Content: The total saponin content was determined by the method described by Le *et al.* (2018). In a test tube with 0.25 ml of plant extract or standard (1 mg/ml Diosgenin) and 2.5 ml of 72% sulphuric acid, 0.25 ml of 8% vanillin (dissolved in ethanol) was added. This was incubated in a water bath for 15 minutes at 60 ^{o}C. After cooling, the extract' and the standard's absorbance were measured at 560 nm. Total saponins are calculated as milligrams of diosgenin equivalent per gram of extract (mgDE/g).

Proanthocyanidin Concentration: The determination of proanthocyanidins was based on the procedure defined by sun *et al*. (1998). A volume of 0.5 ml of 1mg/ml of extract was mixed with 1 ml of 4% valium methanol solution and 0.75 ml concentration of hydrochloric acid. The mixture was left undisturbed for 15 mins after which the absorbance was read at 500 nm. The absorbance of ascorbic acid/ catechin was read under the same conditions (mg/g) and was expressed as ascorbic equivalent (AAE). 4% vanillin-4 g of vanillin in 100 ml methanol. The concentrations used for standard ascorbic acid were 10, 25, 30, 75, 100, and 150 µg/ml.

Antioxidant Screening: DPPH Radical Scavenging Assay: DPPH scavenging activity was determined by the modified method described by Roy *et al.* (2018). DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a molecule with free radicals. In the presence of an antioxidant that donates an electron to DPPH, the purple colour of the DPPH radical turns yellow. The hydrogen atom donating ability of the plant extract is determined by the decolorization of the DPPH solution from purple to yellow, and the change in absorbance is measured at 517 nm. The extract stock solution (1mg/ml) was prepared into different concentrations of 10, 25, 50, 100, 200, 300, 400, and 500 µg/ml. Ascorbic acid was used as standard, and 1 ml of the extract or standard was added at different concentrations to 2 ml of the prepared 0.3 mM DPPH solution. The solution was shaken vigorously and incubated for 30 mins in the dark. Absorbance was then read at 517 nm.

Nitric Oxide Radical Scavenging Assay: **N**itric oxide radical scavenging activity was determined by the slightly modified method described by Boora *et al.* (2014). The procedure is based on the principle that sodium nitroprusside in an aqueous solution generates nitric oxide, which reacts with oxygen to produce nitrite ions (radicals) that can be measured using Griess reagents. The antioxidants in the extracts then donate protons to the nitrite ions. Nevertheless, 1.0 mg/ml of the extract and standard quercetin were prepared and serially diluted with distilled water into different concentrations of 12.5, 25, 50, 100, 200, 400, 800, and 1000 μg/ml. Then 0.1% Griess reagent and 10 mM sodium nitroprusside were prepared in distilled water immediately before use. To 1.0 ml of the extract or standard at different concentrations was added 0.5 ml of the freshly prepared sodium nitroprusside. This was incubated for 2 hours in the dark at room temperature. After incubation, 1.5 ml of the freshly prepared Griess reagent was added and incubated for 30 min at room temperature. The absorbance was read at 546nm.

Ferric Reducing Antioxidant Power Assay: The assay is based on the ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) according to the method of Benzie and Strain (1996). Frap reagent was prepared by mixing 300 mM Sodium acetate buffer (pH 3.6), 10 mM TPTZ (Tripyridyl triazine) solution, and 20 mM $FeCl_3.6H_2O$ solution in a ratio 10:1:1. The TPTZ was dissolved in methanol, while $FeCl_3.6H_2O$ was dissolved in water. The extracts stock solution (1 mg/ml) and the standard (ascorbic acid) were serially diluted into 12.5, 25, 50, 100, 200, 400, 800 and 1000 µg/ml. To 1.0 ml of the serially diluted extracts and standard was added 3 ml of Frap reagent. The reaction mixture was incubated for 30 mins at 37ºC. The increase in absorbance was measured at 593nm.

Hydrogen peroxide Scavenging Activities: Hydrogen peroxide scavenging activities were determined by the method described by Fernando and Soysa (2015). The standard and extract (1 mg/ml) were serially diluted into 12.5, 25, 50, 100, 200, 400, 800, and 1000 µg/ml. To 350 µl of the standard and plant extracts was added 350 µl of 12 mM phenol solution, 100 µl of 0.5 mM 4-amino antipyrine, 160 µl of 0.7 mM hydrogen peroxide, and 350 µl of 1U/ml Horseradish peroxidase (HRP) prepared in sodium phosphate buffer (84 mM, pH 7). These were then incubated for 30 mins at 37ºC. The absorbances were measured at 504nm, and the percentage inhibition was calculated.

RESULTS AND DISCUSSION

Nutritional composition of P. nitida fruits (bark and pulp): The nutritional analysis of the dried powdered fruits of *P. nitida* (Table 1) revealed low moisture content of 6.05%, high fiber (23%) and carbohydrate (51.4%), moderate protein (7.87%) and ash content (9.5%) and very low-fat content (0.75%).

Table 1: Proximate composition of *P. nitida* fruits (bark and pulp)

Composition	% Amount
Moisture Content	6.05±0.05
Crude protein	7.87±0.02
Ash content	9.50±0.50
Crude fibre	23.00±1.00
Carbohydrate	51.39±0.07
Crude fat	0.75±0.05

The mineral analysis revealed the presence of microelements/trace elements, Fe, Zn, Cu, Cr, and macronutrients Na, K, Mg, Ca, and heavy metals in low detectable concentrations (Table 2).

The plant is a rich source of potassium, iron, magnesium, and zinc which suggests the plant may possess blood pressure-lowering properties, electrolyte balance, and hypoglycemic activities.

Table 2: Estimation of the metal composition of *P. nitida* fruits (bark and pulp)

Metal	Concentration (ppm)
Iron, Fe	160.00
Zinc, Zn	34.00
Magnesium, Mg	49.00
Lead, Pb	18.00
Cadmium, Cd	Not detected
Calcium, Ca	30.00
Copper, Cu	10.00
Chromium, Cr	5.00
Potassium, K	3920.00
Sodium, Na	1506.10

Phytochemical composition of ethanol extract of P. nitida fruits (bark and Pulp): Table 3 shows the results of the phytochemical screening of the ethanol extract of *P. nidita* fruit (bark and pulp). The result revealed that the extract contains saponins, flavonoids, steroids, tannins, terpenoids, alkaloids, phenols, polysterols, glycosides, and proanthocyanins.

Table 3: Qualitative Screening of Phytochemical Constituents of ethanol extracts of *P. nitida fruit* (bark and pulp)

Phytochemicals	Methanol extract
Saponins	+++
Alkaloids	+++
Tannins	++
Terpenoids	++
Phenols	+++
Flavonoids	+
Glycosides	++
Steroids	+
Phytosterols	+
Anthocyanins	–

– Absent; + Present in low concentration; ++ Present in moderate concentration, +++ Present in very high concentrations.

Table 4: Quantitative Estimation of Phytochemical Constituents of Ethanol Extracts of *P. nitida fruits*

Phytochemical	Concentration
Total Phenol Content (mg GAE/g extract)	92.45±0.84
Total Flavonoid Content (mg QE/g extract)	331.64± 1.39
Total Tannin Content (mg TAE/g extract)	64.89±1.75
Total Saponin Content (mg DE/g extract)	213.79±2.50
Proanthocyanidin (mg AAE/g extract)	129.00±2.17

However, phytosterols were found to be in low concentration, while anthocyanins were not detected. Also, quantitative analysis of the secondary metabolites was evaluated in the plant extract (Table 4). The total phenol, total flavonoid, total tannins, proanthocyanidin, and total saponin contents had concentrations of 92.45 mg GAE/g extract, 331.64 mg QE/g extract, 64.89 mg TAE/g extract, 129.00 mg AAE/g extract, and 213.79 mg DE/g extract respectively.

Antioxidant Capacity: DPPH Radical Scavenging Activities: The DPPH radical scavenging activities of ethanol extract of *P. nitida* fruits (bark and pulp) is shown in Figure 1. The DPPH radical scavenging activities of ethanol extract of *P. nitida* fruits with

percentage inhibitions ranging from 8 - 91%; IC50 value of 66.01 µg/ml was lowered when compared to standard ascorbic acid with percentage inhibition 12 - 98%; IC50, 13.24 µg/ml). Although ascorbic acid has better radical scavenging (antioxidant) activities, ethanol extract of *P. nitida* fruit is also potent.

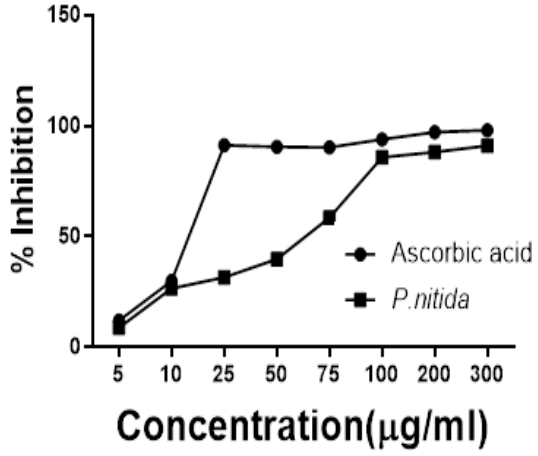

Fig 1. DPPH Radical Scavenging Activity of different concentrations of ascorbic acid and ethanol extract of *P. nitida* fruits. Values are expressed as mean ± SEM, n = 3/group

Reducing Potential of ethanol extract of P. nitida fruits (bark and pulp): The reducing potential of ethanol extract of *P. nidita* fruits is shown in Figure 2. The reducing power of ethanol extract of *P. nidita* fruits showed moderate reducing power when compared to ascorbic acid. The higher the absorbances, the greater the reducing power. The ethanol extract of *P. nitida* exhibited moderate reducing ability as revealed by the increased absorbance value ranging from 0.100±0.01–1.42±0.01 at 10-1000 µg/mL. However, it showed lower potential than ascorbic acid at the same concentrations.

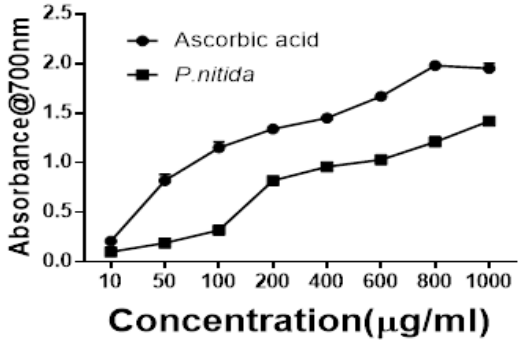

Fig 2. Reducing power of different concentrations of ascorbic acid and ethanol extract of *P. nitida* fruits. Values are expressed as mean ± SEM, n = 3/group

Nitric oxide inhibitory activities: The nitric oxide (NO) inhibitory activity was evaluated, as shown in Figure 3. The NO inhibitory ability of ethanol extract of *P. nitida* fruits was higher when compared to quercetin. The NO percentage inhibitions for the extract and quercetin were also the same at concentrations from 10 -100 µg/mL, but the extract had higher percentage inhibition ranging from 46.33 to 96.13% at concentrations from 200 -1000 µg/mL when compared to quercetin (Percentage inhibition; 41.45 to 99.09%)

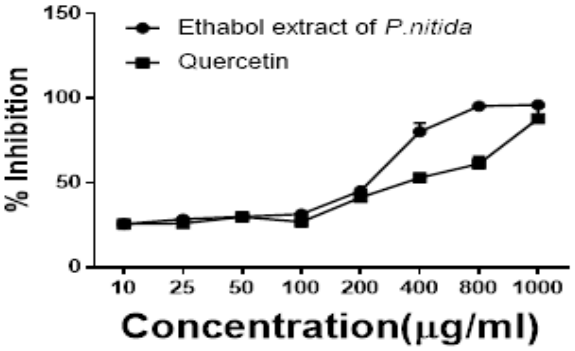

Fig 3: Nitric oxide inhibitory activities of different concentrations of quercetin and ethanol extract of *P. nitida* fruits. Values are expressed as mean ± SEM, n = 3/group

Ferri Reducing Antioxidant Potential: Ferric reducing antioxidant potential of *P. nitida* fruits (bark and pulp) is shown in Figure 5. Ferric reducing antioxidant potential of the plant extract (431±1.00 µM Fe (II)/g) shows higher potential when compared to ascorbic acid (239.5±5.04 µM Fe (II)/g).

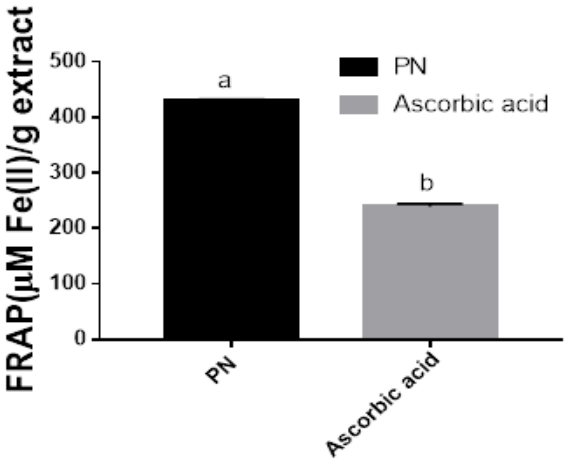

Fig 4: Ferric Reducing Antioxidant Potential of different concentrations of ascorbic acid and ethanol extracts of ***P. nitida*** **(PN)** fruits. Values are expressed as mean ± SEM, n = 3/group. Values with different alphabet indicate significant difference

Hydrogen Peroxide Radical Scavenging Activities: The ability of the extract to quench hydrogen peroxide radicals was measured using a standard colorimetric assay. As shown in Fig 5, it was observed that at concentrations from 10-1000 µg/ml, the percentage inhibition increases as the concentrations increase for both the extract and ascorbic acid. Interestingly the

extract scavenged hydrogen peroxide radicals better than ascorbic acid.

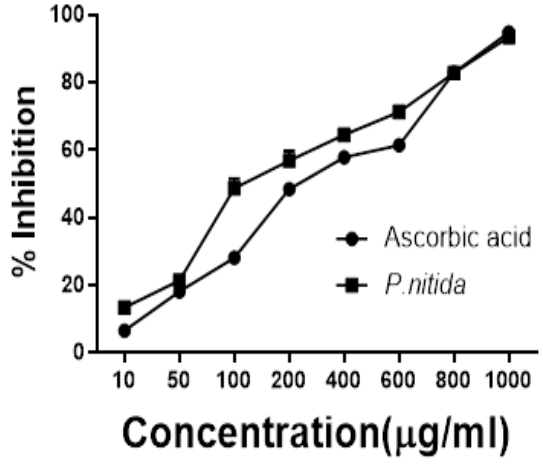

Fig 5: Hydrogen peroxidase scavenging activities of different concentrations of ascorbic acid and ethanol extract of *P. nitida* fruits. Values are expressed as mean ± SEM, n = 3/group

Recently, the interest in natural products has increased due to their potential health benefits. Among these are plants with medicinal properties that various cultures worldwide have traditionally used. *Picralima nitida* is a plant known for its diverse applications in traditional medicine. The proximate analysis showed that *P. nitida* fruit (bark and pulp) contained Moisture-6.05±0.05%, Crude protein-7.87±0.02%, Ash-9.50±0.50%, Crude fibre-23.00±1.00%, Carbohydrate-51.39±0.07%, and Crude fat-0.75±0.05%. These crucial nutrients, like carbohydrates, low crude fat, and crude protein, indicate the potential use of *the P. nitida* plant as a nutritionally valuable and healthy ingredient to improve health. The low moisture content observed in this study suggests that the fruit is less susceptible to microbial spoilage, with a longer storage period. *P. nitida* fruit has moderate protein content, slightly below the acceptable dietary protein for the diet formulation. However, the fruit could be used as a source of protein. Similarly, as observed by this study, the fruit is a good source of inorganic minerals with moderate ash content. The rich fiber of the fruit of *P. nitida* suggests its role in digestion, cholesterol-lowering, sugar-lowering, and gastric emptying (Chaney, 2006). The fruit is rich in carbohydrates, indicating that it may be an energy-rich food when consumed. Also, fruits are not very good sources of fats and are usually recommended as part of weight-reducing diets (Sheila, 1978). The results of the mineral composition of the ethanol extract showed a significant variation of minerals on the bark of *P. nitida*, which include: Iron-160.00 ppm, Zinc-34.00 ppm, Magnesium-49.00 ppm, Lead-18.00 ppm, Calcium-30.00 ppm, Copper-10.00 ppm, Chromium-5.00 ppm, Potassium-3920 ppm, and Sodium-1506.10 ppm. Minerals are required for normal growth, activities of muscles and skeletal development (such as calcium), cellular activity and oxygen transport (copper and iron), chemical reaction in the body, intestinal absorption (magnesium), fluid balance, and nerve transmission (sodium and potassium), as well as the regulation of acid-base balance (phosphorus). Iron is useful in preventing anemia and other related diseases (Oluyemi *et al*., 2006). Zinc is useful for protein synthesis, normal body development, and recovery from illness (Muhammad *et al*., 2011). The ethanol extract of *Picralima nitida* fruit (bark and pulp) was investigated to determine its phytochemical composition and antioxidant activities. The qualitative screening of the phytochemical composition of the ethanol extract of the stem bark of *Picralima nitida* reported in Table 1 revealed the presence of the phytochemicals- saponins, alkaloids, tannins, terpenoids, phenols, flavonoids, glycosides, and steroids in varying concentrations in the extract while anthocyanins were absent. The quantitative phytochemical screening showed a significant concentration of total phenolic, flavonoid, tannin, and proanthocyanidin content in the ethanol extract of the bark and pulp fruit of *Picralima nitida* -Total Phenol Content-92.45±0.84 (mg GAE/g extract), Total Flavonoid Content-331.64± 1.39 (mg QE/g extract), Total Tannin Content- 64.89±1.75 (mg TAE/g extract), Proanthocyanidin-129.00±2.17 (mg AAE/g extract), and Total Saponin Content-213.79±2.50 (mg DE/g extract). It has been discovered that a plant is functional property depends on its secondary metabolites, such as phenolics, terpenoids, or alkaloids (Murugan and Parimelazhagan, 2014). Phenols, flavonoids, and flavonols are polyphenolic chemicals found in plants that have significant antioxidant activity as well as a variety of biological functions such as anti-helminthic, analgesic, anti-inflammatory, anti-microbial, and anti-allergic activities (Oyedemi *et al*., 2012). Flavonoids have been shown to be more critical than other phytochemicals separated from the plant due to their capacity to help the body fight illnesses. Flavonoids are potent antioxidants, but their effectiveness depends on the molecule's shape and the hydroxyl group in the chemical structure (Iqbal *et al*., 2015).

The antioxidant activity of the extract was evaluated using various *in vitro* assays, including the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, reducing power assay, nitric oxide inhibitory activities, hydrogen peroxidase scavenging activities, and ferric reducing antioxidant power (FRAP) assay. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay result is shown in Figure 1. The IC_{50}

value measures the concentration of an antioxidant-containing substance required to scavenge 50% of the initial DPPH radicals. The lower the IC_{50} value, the more potent the substance is at scavenging DPPH, which implies a higher antioxidant activity (Olugbami *et al*., 2014). Figure 1 shows the percentage radical scavenging activities of the extract and the standard ascorbic acid. The extract exhibited an appreciable percentage of radical scavenging activities with the standard. The reducing power of a compound may give a better clue as to its reductive capacity (Oikeh *et al*., 2014). The reducing potential measures the anti-oxidative capacity of the extract by assessing its ability to donate electrons to electron-seeking free radicals. The extract has significantly lower ($p< 0.05$) reducing potential than the standard ascorbic acid at lower concentrations but showed significantly higher values at higher concentrations. Due to the reduction of the Fe^{3+} ferricyanide complex to the ferrous (Fe^{2+}) form in the presence of reductants such as antioxidant compounds in the sample, a compound's reducing capacity may be a significant predictor of its potential antioxidant activity (Rahman *et al*., 2015). Reducing power, in addition to the Ferric Reducing Antioxidant Potential (FRAP) assay, offers further information into the reductive capacity and, consequently, the antioxidant activity of a biological sample. This present study showed that the ethanol extract compares favorably with ascorbic acid as it has a higher FRAP value than ascorbic acid. Shiddhuraju *et al* (2002) suggested that Ferric reducing potential of bioactive compounds was associated with antioxidant activity. It measures the reductive ability of antioxidants and is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of sample extract (Huda-Faujan *et al*., 2009). This result confirms the presence of the antioxidant activity that was observed for the DPPH assay. The hydrogen peroxidase scavenging activities of different concentrations of the ethanol extract of *P. nitida* fruit (pulp and bark) was significantly higher than the standard ascorbic acid at lower concentrations but showed corresponding values with the standard ascorbic acid at higher concentrations.

Conclusion: The results of this study have revealed that ethanol extract of *P. nitida* (bark and pulp) possesses natural phytochemicals and antioxidants which may be attributed to its medicinal properties. However, more research is required to identify, describe, and assess the toxicity and pharmacological properties of the active substances that may be responsible for the reported antioxidant effects.

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