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Full Length Research Article

# Phytochemical, Proximate Composition, Mineral, Antioxidant, and Radical Scavenging Capacity of *Picralima nitida* Fruit Pulp Aqueous Extract

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## ABSTRACT
*Picralima nitida* (Apocynaceae) is widely known for its efficacy in ameliorating the discomfort associated with different diseases. The objective of this research is to investigate the phytochemicals, proximate composition, antioxidant, and antioxidant capacity of unripe aqueous fruit pulp extract of *P. nitida.* The unripe fruit pulp of *P.nitida* was dried, ground to powder, and soaked in distilled water. The aqueous extract obtained was analyzed for phytochemicals and found to contain alkaloids, eugenols, flavonoids, phenolics, saponins, tannins, and terpenoids, according to phytochemical analysis. Proximate composition showed a low fiber (0.43%), moisture content (13.09%), fat (14.51%), protein (8.17%), ash content (10.30%), and moderate carbohydrate (53.50%). The antinutrients present in the aqueous extract are alkaloids (8.72 mg/g), Saponins (8.65 mg/g), oxalate (75.95 mg/g) tannins (89.23 mg/g) and phytate (140 mg/g). Mineral analyses showed the presence of Calcium (11.60 mg/g), sodium (21.00 mg/g), and potassium (161.84mg/g) having the highest concentrations. Total phenol, total flavonoid, and vitamin C contents of the aqueous extracts of *P.nitida* fruit pulp had values of 39.24 mgGAE/g, 24.39 mgQE/g, and 17.02 mgAAE/g, respectively. Increased concentration of the extract led to increased percentage inhibition of DPPH and hydroxyl radicals. These findings revealed the inherent properties of *P. nitida* fruit pulp and its potential to be utilized therapeutically.
**Keywords**: *P. nitida*, Fruit pulp, Antioxidants, Nutraceutical, Antinutrients

## INTRODUCTION
Medicinal plants have been used to manage and treat different types of diseases with positive outcomes (Yakubu *et al*., 2018). The acceptability of these plant products is due to their effectiveness, availability, and reduced cost. The presence of phytochemicals and other bioactive components which may be operating in concert to bring about the intended result without having any negative consequences, is what gives plant extracts their efficiency.

*P. nitida* is a shrub widely distributed in Nigeria. The different parts of the tree; seed, stem-bark, leaves, roots, and seeds have been employed by traditional medical practitioners to treat and cure various ailments (Teugwa *et al*., 2013). The fruit has a smooth, rounded oval shape and apex. It measures 8 to 10 cm in diameter and 11 to 20 cm in length (Iwu, 1993). When unripe, the fruit is glabrous and leafy green; however, when mature, it turns yellow to orange. The white, tender pulp contains the seeds (Bruce *et al*, 2016). The fruit pulp is used in ethno-medicine to treat diabetes, dysmenorrhoea, and malaria (Ezeamuzie *et al*.,1994). François *et al*. (1996), and Olumese *et al*. (2023); reported the extracts obtained from fruit pulp are more effective than those obtained from the stem barks. The most common method of utilizing fruit pulp in ethno-medicine is to obtain cold or hot aqueous or alcoholic crude extracts. However, there is limited information on the constituents of the unripe fruit pulp. The unripe *P. nitida* aqueous fruit pulp extract is evaluated for phytochemical, proximate composition, mineral, antioxidant, and radical scavenging abilities in this study.

## MATERIALS AND METHODS
### Plant Sample
(*P. nitida*) fruit was purchased from New Benin Market in Benin City, Edo state, Nigeria. The fruit was taken for identification in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, and voucher number $UBH_p424$ was obtained. The unripe fruits were rinsed with water and peeled. The pulp obtained weighed 20 kg.

### Preparation of the Crude Extracts.
To achieve a constant weight at room temperature, the pulp was dried for 56 days. The dried pulp was ground into a fine powder (5.01 kg), and continuously stirred for 72 hours while it soaked in distilled water (1:10 kg/L). The slurry was then filtered via filter paper, cotton wool, and muslin cloth. The filtrate was freeze-dried using a Biobase BK-FD10 Freeze Dryer (China). A total yield of 637.6g (12.6%) was obtained. The freeze-dried extract was stored in an airtight container in a refrigerator at 4$^{o}$C.

### Screening for Phytochemicals
Phytochemicals (alkaloids, eugenols, flavonoids, glycoside, phenolics, reducing sugar, saponins, steroids, tannins and terpenoids) were qualitatively examined in the aqueous extract using known standard procedures (Edeoga *et al*. 2005).

### Quantitative determination of antinutrients.
### Determination of Total Tannins Content
Methanol (20 mL of 50%) and 0.20 mL of sample were combined, then they were stirred at 77 to 80 $^{o}$C for an hour. The extract was mixed with 20 mL of distilled water, 2.5 mL of Folin-Denis reagent, and 10 mL of 17% Sodium carbonate before being quantitatively filtered using a double-layered Whatman No. 1 filter paper. Twenty minutes were given for color development. A series of

standard tannic acid solutions were made in methanol, and their absorbance as well as samples were read on a UV/ Visible spectrophotometer (721S, China). The total tannin content was evaluated using the calibration curve.

**Determination of Total Saponins Content**

The study used a modified vanillin-sulfuric acid colorimetric procedure, the method described by Makkar *et al*. (2007) to estimate total saponins content. 50 mL of plant extract was combined with 250 mL of distilled water. This was then mixed with about 250ml of vanillin reagent. Then 2.5 mL of sulfuric acid at a 72% concentration was added and well mixed. For ten minutes, this solution was kept in a water bath at 60 °C. A UV/Visible spectrophotometer (721S, China) was used to measure the absorbance at 570 nm after it had been refrigerated in ice-cold water for ten minutes. Standard saponin solutions with concentrations between 0 and 25 ppm. The values were expressed using PPM.

**Determination of Total Alkaloids Content**

The total alkaloid was calculated using Harborne's method (1998). To a 250 mL beaker, 100 mL of 20% acetic acid in ethanol and 5g of the extract was added. The beaker was then capped and left for 2 hours. The extract was concentrated in a water bath to a quarter of its original volume. Concentrated ammonium hydroxide was added to the extract. The precipitate was extracted by filtering, rinsed with 1% ammonia solution, dried, and weighed after allowing the entire solution to settle. Duplicate analyses of each sample were performed.

**Determination of Total Phytate Content**

A modified version of the colorimetric methods of Raboy *et al*. (2000) and Vaintraub and Lapteva (1988) were used to measure the relative content of phytate in the sample. 100 mg (±0.2 mg) of freeze-dried sample and 2 mL of 0.65M HCl were placed in a sample bottle. The samples were centrifuged at 3000 rpm for 20 minutes using a Centrifuge (90-2 electric low-speed centrifuge, China) and shaken at room temperature for 12 hours. The supernatant extract was analyzed alongside phytate standard stock solution (10 mg were dissolved in 1 mL of 0.65 M HCl). 2m of Wade reagent, was added to the solution and was then allowed to react at room temperature for 15 minutes before being measured for absorbance at 430 nm with a UV/Visible spectrophotometer (721S, China).

**Determination of Total Oxalate Content**

A sample solution was prepared using distilled water and a dilution of 0.01 M KMnO4 in distilled water. The mixture included 0.1 to 1 mg of oxalic acid, 2 N H2SO4, and 0.003 M KMnO4. The absorbance was measured on a Shimadzu UV 1900 spectrophotometer (Japan), and a reagent blank was created. The calibration curve was linear.

**Proximate Analyses**

The proximate composition (Total ash, crude protein, fat, fiber) of the freeze-dried *P. nitida* fruit pulp was analysed using standard methods (AOAC, 2005).

**Mineral Analyses**

Minerals were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (Chinma *et al*., 2021). One gram (1 g) of each sample was mineralized by mixing with nitric acid and microwave-digested (CEM One Touch TM Technology, CEM Technologies, USA). The resulting solution was diluted with Milli-Q water (Millipore, Bedford, MA). Stock and working standard solutions were prepared by using NIST traceable CRMs of the test minerals. Extracts were analysed on an ICP-OES equipment (Spectro ARCOS, Spectro Analytical Instruments, Kleve, Germany). The results were expressed as mg/100g.

**Antioxidant and Radical Scavenging Capacity**
**Determination of Total Phenol Content**

The 100 µL aqueous extracts were oxidized with 2.5 mL of 10% Folin-Ciocalteau's reagent (v/v), and 2.0 mL of 7.5% sodium carbonate was used to neutralize the reaction. The reaction mixture was incubated for 40 minutes at 45°C and the absorbance was measured at 765 nm, Using a Lab-Tech digital colorimeter (1312, Indian). (Singleton *et al*., 1999). The total phenol concentration was then determined as the equivalent of 10 mg of gallic acid per 100 mL.

**Determination of Total Flavonoid Content**

Total flavonoid was determined as described by Meda *et al*. (2005). Aqueous extract (100ul) was combined with 0.5 mL of methanol, 50 µL of 10% $AlCl_3$, 50 µL of 1M potassium acetate, and 1.4 mL of water. The mixture was then let to sit at room temperature for 30 minutes. The reaction mixture's absorbance was then assessed at 415 nm using a Lab-Tech digital colorimeter (1312, Indian). Quercetin (10 mg/100 ml) was utilized as the reference.

**Determination of Vitamin C**

Vitamin C content of the aqueous extract was determined using the method of Benderitter *et al*. (1998).75µl of the extracts were added to DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea, and 270 mg CuSO4.5H2O in 100 ml of 5 M H2SO4), followed by 100 µL of 13.3% trichloroacetic acid (TCA) and water. After 3-hour incubation period at 37 $^0$C, the reaction mixture was added to the medium with 0.5 ml of 65% sulphuric acid (v/v), and the Lab-Tech digital colorimeter (1312, Indian) was used to detect the absorbance at 520 nm. Ascorbic acid as the reference (10 mg/100 mL) as the standard.

**DPPH Radical Scavenging**

DPPH scavenging activity was determined by the modified method described by Roy *et al*. (2018). DPPH (0.039 g) is dissolved in 80% methanol and made up to 250 mL with 50% methanol. The samples were mixed with distilled water, which served as the blank solution, and 0.6ml of DPPH. Duplicate tests were run on each

test. The samples are incubated for 15 minutes at room temperature in the dark and are then read using a UV/Visible spectrophotometer (721S, China) at a wavelength of 516 nm.

**Fenton Reaction**

This test uses a spectrophotometer to measure the antioxidant's capacity to scavenge OH radicals. After the samples were incubated at 37 °C for 30 minutes, 500 µL of TCA (2.8%) was added to each test tube and the incubation time was increased to 20 minutes. The test is read using a UV/Visible spectrophotometer (721S, China) at a wavelength of 532 nm.

**Statistical Analysis**

Analyses were performed in triplicate. The mean ± standard error of the mean is used to express results. Pearson's correlation coefficient was calculated from the results using Microsoft Excel 2003. The results were statistically analyzed by Analysis of variance (ANOVA), and Duncan's new multiple range tests. Statistical significance was accepted at P≤ 0.05.

## RESULTS AND DISCUSSIONS

## RESULTS

**Phytochemical Composition of the Aqueous Extract of *P. nitida* Fruit Pulp**

The freeze-dried samples were screened for the presence of phytochemicals. The result of the qualitative phytochemical analysis of *P. nitida* fruit is shown in Table 1. The phytochemical screening of the pulp of *P. nitida* fruits showed that glycosides, saponins, phenolic compounds, terpenoids, eugenols, alkaloids, flavonoids, tannins, and reducing sugars were present. Saponin was moderately present while others were present in low concentration. Steroids were not detected.

**Table 1:** Qualitative screening of phytochemical constituents of aqueous extracts of *P. nitida* fruit pulp.

| PHYTOCHEMICALS | AQUEOUS EXTRACT |
|---|---|
| **Alkaloids** | + |
| **Eugenols** | + |
| **Flavonoids** | + |
| **Glycoside** | + |
| **Phenolics** | + |
| **Reducing sugar** | + |
| **Saponin** | ++ |
| **Steroids** | – |
| **Tannins** | + |
| **Terpenoids** | + |

= Absent, + = trace amount; ++ = moderate amount

**Proximate Composition of *P. nitida* fruit pulp**

The proximate analyses of the freeze-dried fruit pulp of *P. nitida* (Table 2) showed a low fiber (0.43%), moisture content (13.09%), fat (14.51%), protein (8.17%), ash content (10.30%) and moderate carbohydrate (53.50%) of 100g of the total sample analyzed.

**Table 2:** Proximate composition of the aqueous extracts of *P. nitida* fruit pulp.

| COMPOSITION | AMOUNT (%) |
|---|---|
| **Moisture Content** | 13.09±0.18 |
| **Ash Content** | 10.30±0.04 |
| **Crude Fat** | 14.51±0.35 |
| **Crude Fibre** | 0.47±0.03 |
| **Crude Protein** | 8.17±0.58 |
| **Carbohydrate** | 53.46 ±0.73 |

Values are mean ± SD of three determinations.

**Mineral Composition**

The mineral analysis showed the presence of microelements/trace elements and macronutrients; namely, calcium, magnesium, potassium, sodium, zinc, manganese, copper, and Iron in the fruit pulp of *p. nitida*.

**Table 3:** Estimation of the metal composition of the aqueous extracts of *P. nitida* fruit pulp.

| METALS | CONCENTRATION (mg/100g) |
|---|---|
| **Copper, Cu** | 0.14 ± 0.11 |
| **Manganese, Mn** | 1.20 ± 0.12 |
| **Magnesium, Mg** | 4.40 ± 0.15 |
| **Iron, Fe** | 4.40 ± 0.10 |
| **Zinc, Zn** | 4.80 ± 0.11 |
| **Calcium, Ca** | 11.60 ± 0.10 |
| **Sodium, Na** | 21.00± 0.15 |
| **Potassium, K** | 161.84± 0.11 |

Values are mean ± SD of three determinations.

**Anti-Nutritional Composition of *P. nitida* Fruit Pulp**

The results of the antinutritional components observed in this study is shown in Table 4. Phytate (140±1.2) had the highest content, while the saponins (8.647±0.23) was the least in the assayed fruit pulp.

**Table 4:** Quantitative determination of antinutrients in *P. nitida* fruit pulp

| ANTINUTRIENTS | QUANTITY (mg/100 g) |
|---|---|
| **Alkaloid** | 8.72 ± 0.17 |
| **Oxalate** | 75.95 ±3.77 |
| **Saponin** | 8.64± 0.23 |
| **Tannin** | 89.23 ± 2.45 |
| **Phytate** | 140.00±1.2 |

The results obtained are presented as mean ± standard deviation of triplicate determination.

**Antioxidant and Antioxidant Capacity**

Total phenol, total flavonoid, vitamin C contents (antioxidants) and the percentage inhibition of DPPH by the aqueous extracts of *P. nitida* fruit pulp are represented in Table 5, 6 and 7.

**Table 5:** Quantitative Estimation of Antioxidants of the Aqueous extracts of *P. nitida* fruit pulp.

| PHYTOCHEMICAL | CONCENTRATION |
|---|---|
| **Total Phenol (mg GAE/g extract)** | 39.24 ± 0.45 |
| **Total Flavonoid (mg QE/g extract)** | 24.39 ± 0.74 |
| **Vitamin C (mg AAE/g extract)** | 17.02 ± 0.71 |

The results obtained are presented as mean ± standard deviation of triplicate determination.

**The Radical Scavenging Abilities of Aqueous Extracts of *P. nitida* Fruit Pulp**

The aqueous extracts of *P. nitida unripe* fruit pulp promoted an increased ability to scavenge DPPH and OH radicals with increasing extract concentrations (Tables 6 and 7).

**Table 6:** Percentage Inhibition of DPPH radicals bythe aqueous extract of *P. nitida* fruit pulp.

| EXTRACT CONC. (µg/mL) | % INHIBITION |
|---|---|
| **166** | 24 |
| **333** | 35 |
| **500** | 45 |
| **666** | 55 |

The values are mean ±SD of duplicate determinations.

**Table 7:** Percentage inhibition of hydroxyl radical by aqueous extracts of *P. nitida* fruit pulp.

| EXTRACT CONC. (µg/mL) | % INHIBITION |
|---|---|
| **71** | 26 |
| **142** | 40 |
| **214** | 61 |
| **285** | 73 |

Values are mean ±SD of triplicate determinations.

**DISCUSSIONS**

The result of the qualitative phytochemical analysis of *P. nitida* fruit reported in Table 1 is at par with previous reports by (Moja *et al.,* 2003) who studied flavonoids, tannins, and alkaloids in many plants and found that these chemical constituents were found in sufficient amounts in the studied plants. It has been reported that a plant's functional property depends on its secondary metabolites (Murugan and Parimelazhagan, 2014). Phenols, flavonoids, and flavonols are polyphenolic chemicals found in plants that have significant antioxidant activity (Oyedemi *et al*., 2012). The freeze-dried fruit pulp of *P. nitida*, had crude fiber (0.43%) as the lowest value, and carbohydrate content (53.50%) had the greatest value. The moisture content of the freeze-dried extract of *P. nitida* fruit pulp (13.09%) was higher than the value (10.67%) reported by Nwaogu (2016) on the dried powdered seeds. This could be because the pulps have higher water absorption capacity than the seeds. The moisture present in food is a good indicator to assess the sensitivity and stability of food to spoiling bacteria. Low moisture content tends to obstruct or prevent microbial contamination and chemical degradation (Hussain *et al*., 2009). Ash content amount is useful in defining the sample's purity and authenticity. The ash content value (10.30%). Foods with a high ash content have a high mineral content. Minerals play a role in human metabolism, bone health, and water balance. The percentage of crude fibre in the present study was 0.43%. Fibre is one of the essential body nutrients. Its presence in foods helps to lower risks of constipation, high blood pressure, diabetes, cardiovascular disease, cancer, and obesity. The crude fat content value was 14.51%. Fats provide storage and carry metabolic fuel, insulate subcutaneous tissues from heat and electricity, act as emulsifiers in the production of medications, and serve as structural components of biomembranes. The percentage crude protein value (8.17%) was slightly higher than the 7.87% reported by Olumese *et al*. (2023) in *P. nitida* fruits (bark and pulp). Crude protein is an index of food's calorific value (Uyo *et al.,* 2013). Protein is crucial for children's healthy growth and development, the preservation and repair of damaged tissues, the generation of immunoglobulin for the body's defence, the production of metabolic enzymes, and the growth of lean muscle mass.

The results of the mineral composition of the aqueous extract revealed a significant variation of minerals. Minerals are necessary for appropriate body development and maintenance (Haruna *et al*., 2015). They aid in the maintenance and improvement of muscle, heart, and brain functioning, as well as the formation and maintenance of healthy bones and teeth (Jéquier and Constant, 2010). To maintain osmotic equilibrium, magnesium is necessary in plasma and extracellular fluid. It also participates in a variety of biological events such as oxidative phosphorylation, glycolysis, and protein synthesis (Gröber *et al*., 2015). Potassium has the highest value in the samples. It is essential for optimal cell activity, muscular contraction, and blood pressure management. Calcium is necessary for bone and muscle growth, synaptic nerve signal transmission, and blood clotting (Ozcan and Akbulut, 2008). Calcium supplementation is widely advised, especially for youngsters and pregnant women (Insel *et al*., 2011). Zinc is essential for proper body development, protein synthesis, and wound healing. It is also a component of numerous enzymes in the human body (Afolayan and Jimoh, 2009). However, excessive zinc consumption is hazardous to human health (Ogundola *et al*., 2018). Sodium is important in the regulation of acid-base balance, normal cell activity, metabolite transport, nerve impulse transmission, and blood pressure regulation (Unuofin *et al*., 2017). Iron is required for the synthesis of hemoglobin, energy metabolism, and oxygen transport (Gaeta and Hider, 2005). Manganese is required for all biochemical activities as well as the preservation of nerve and muscle electrical potentials. It also aids in the delivery of oxygen from the lungs to the cells and the

activation of enzyme processes involved in glucose, lipid, and protein metabolism (Jacob *et al*., 2015). Copper is a trace mineral that is essential for various enzymatic activities, organ function, collagen production, energy generation, and hemoglobin development (DiNicolantonio *et al*., 2018). Saponins have been found to have hypocholesterolemic and anticarcinogenic properties (Koratkar and Rao, 1997). Plant alkaloids and their synthetic counterparts are utilized as fundamental therapeutic agents. The majority of plants used to treat ailments have varying quantities of alkaloids (Okwu, 2004). The presence of these significant antinutrients in *P. nitida* fruit pulp may have endowed it with a variety of medicinal qualities.

Antinutrients are potentially hazardous to human health because they reduce protein digestibility and mineral bioavailability. Saponin and alkaloid levels were low in the fruit pulp, but tannin and oxalate were high in quantity. However, the existence of moderate levels of these antinutrients should not be an issue if adequately processed, because processing reduces antinutrient levels to allowable limits (Ndidi *et al*., 2014). Phenolics are antioxidants that are beneficial to human health as they possess antioxidant capacities and are able to combat damage carried out by free radicals. Vitamin C content 17.02 ± 0.71 mg AAE/g extract was low, suggesting this vitamin is not present in appreciable amounts. Oxidative stress has been implicated in the pathogenesis of several diseases based on the roles of free radicals in the cascade of biochemical changes (Krishna-Kumar *et al.,* 2012). Antioxidants inhibit oxidation and chemical reactions that can produce free radicals**.** However, a negative shift in favour of free radicals may result in oxidative stress that catalyses the proliferation of diseases. Natural antioxidants can prevent, or reverse abnormal health effects associated with antioxidants and oxidative stress (Sakha *et al*., 2018). In this study, aqueous extract of *P. nitida* exhibited noteworthy scavenging effects of DPPH (2, 2 diphenyl-1-picrylhydrazyl) and OH radicals, hence offering scientific support for their application in various conventional medical conditions.

## CONCLUSION

The findings of this study suggest that the phenolic and flavonoid phytochemical components present in the unripe fruit pulp of *P. nitida* and the ability to scavenge free radicals may be responsible for its therapeutic capabilities to treat a variety of illnesses and diseases like cardiovascular disease, diabetes, inflammation, etc the plant exerts its curative effects through other phytochemical components like Saponins; which are hypocholesterolemic. The high concentration of K+ may help lower blood pressure, renal stones, osteoporosis, and stroke prevention.

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