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Nutritional and chemical properties of *Piliostigma reticulatum* pod powder and its healing potential

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Abstract

The use of plants for medicinal purposes has deep historical roots and remains a vital source of healthcare, particularly in underdeveloped regions. This study analyzed *Piliostigma reticulatum* powder, a leguminous plant traditionally used in tropical areas to treat various ailments. Despite its widespread use in traditional medicine, there is limited scientific information on its bioactive properties. This research aimed to bridge this gap by examining the vitamin, phytochemical, proximate, and mineral compositions of *P. reticulatum* powder. The results indicated that the powder contains vitamins A, C, and E, all potent antioxidants. Phytochemical analysis revealed the presence of bioactive compounds, including saponins, tannins, and flavonoids, which are known for their therapeutic properties, such as antibacterial and anti-inflammatory effects. The proximate analysis highlighted the high carbohydrate and protein content, underscoring the plant's nutritional value. Mineral analysis revealed significant levels of calcium and magnesium, which are essential for various physiological functions. *Piliostigma reticulatum* pod powder has moderate antioxidant activity, with an IC_{50} (median inhibitory concentration) of 127 mg/mL and 129 mg/mL against diphenyl 1-picrylhydrazyl (DPPH) and hydroxyl (HO) radicals, respectively. In conclusion, the comprehensive analysis of *P. reticulatum* powder supports its traditional medicinal use and suggests its potential as a valuable nutritional and therapeutic resource. The findings paved the way for further research into the plant's bioactive compounds and their potential applications in modern medicine.

1. Introduction

Long before agriculture and up to the present day, man has known how to take advantage of and still takes advantage of wild plants for all his needs in food, for medicine, and many others. Indeed, the forests have been called upon for the multiple products and benefits they offer (food, fuel and remedies) (Ickowitz *et al., 2*022). In terms of food, forest resources provide essential supplements for the population through the consumption of leaves, fruits, and nuts of particular species, thus contributing to food and nutritional security (Melo *et al.,* 2021; Duffy *et al.,* 2021).

Developing regions, particularly underdeveloped areas, rely heavily on plant-based remedies for healthcare and complementary medicine (Duguma, 2020). Traditional medicinal plants are a valuable therapeutic and food resource utilized by the inhabitants of the African continent, particularly for healthcare purposes and consumption, serving as fundamental components for pharmaceuticals (Dasofunjo *et al.,* 2015).

In a country like Niger, the population relies on 40% of the vegetation for livestock feed, medicinal purposes, and human food (Ali *et al.,* 2016). Food Woody Species (ELA) include all woody plants that provide leaves, flowers, fruits, seeds, or other parts used for human consumption (Borelli *et al.,* 2022). *Piliostigma reticulatum* is known in all localities of Niger, often referred to as Kalgo or Kossey in the local language. It is a shrub that colonizes a wide range of soils. Slow-

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growing, *P. reticulatum* has numerous uses (including human and animal food and traditional medicine), which explains its extensive exploitation by local populations. More abundant in lowlands, plains, and along certain roads or tracks, its growth rate is primarily influenced by rainfall, human pressures, and grazing by ruminants.

Despite its importance in the daily life of the population, planting or sowing the species is not a common practice (Yelemou *et al.,* 2007). These leaves, fruits, and roots are used in human food. The leaves are picked to acidulate the cereal dough, keeping it intact for at least three days. They are pressed, and their juice gives traditional vinegar (Garba, 2000). This heavily exploited species regenerates both naturally and with assistance. Assisted natural regeneration (RNA) is the best-known and most practiced regeneration method in rural areas (Ali *et al.,* 2016). RNA is the systematic regeneration and management of trees and shrubs from felled tree stumps, root systems, germinating seeds, or wooded thickets, given the renewed interest shown by local populations in this species, which appears to be adapted to the deterioration of climatic conditions. Despite its extensive use in traditional medicine, there is limited scientific information available on its chemical composition and bioactive properties. This work aimed to provide the audience with a comprehensive analysis of the vitamins, phytochemicals, and mineral content of *P. reticulatum* powder. Among other things, to highlight the dietary use and its potential health benefits.

2. Materials and methods

2.1 Sample collection

Dried fruits of *P. reticulum* collected from Maradi, Niger, were cleaned, ground into powder, sieved, and used for subsequent analyses (Figure 1).

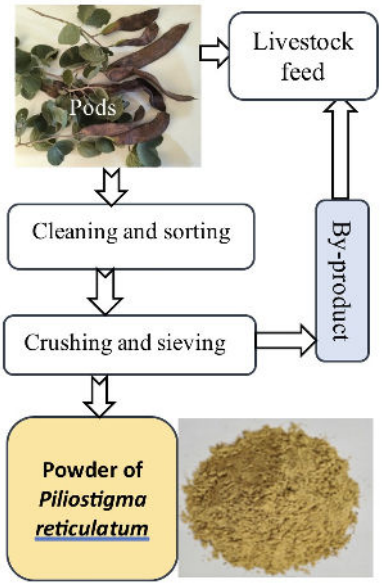

Figure 1. Flowchart of producing *Piliostigma reticulatum* pod powder.

2.2 Vitamins analysis

The *P. reticulum* dry fruit powder was extracted by dissolving it in a suitable solvent (ethanol or methanol) using a sonication or cold extraction method. The extract was then filtered to remove solid particles. Vitamin standards were prepared by dissolving known quantities of each vitamin in the solvent used for sample extraction. The concentration range of standards covered the expected vitamin content in the samples. The UV-Visible spectrophotometer was calibrated using a blank solution (solvent without the vitamin). Each sample was placed in a 1 cm cuvette, and absorbance was measured at the specific wavelengths for each vitamin. The absorbance values were recorded for each sample, and blank corrections were applied by subtracting the solvent absorbance (Sharma *et al.,* 2020).

The concentration of each vitamin in the samples was determined using Beer's Law:

$$A = \varepsilon.c.l$$

Where A is the absorbance, ε is the molar absorptivity, c is the vitamin concentration, and l is the path length of the cuvette (1 cm). A calibration curve was prepared using the absorbance of standard solutions to calculate the vitamin concentration in the samples. The results were expressed as the concentration of each vitamin (mg/L) in the samples.

2.3 Phytochemical screening

2.3.1 Saponins

The total saponin content was determined using the method described by Obadoni and Ochuko (2001). A 1 mL aliquot of the methanolic extract was mixed with 1 mL of 1% vanillin in methanol. Then, 5 mL of concentrated sulfuric acid (H_2SO_4) was added, and the mixture was heated in a water bath at 60°C for 10 min. After cooling, the absorbance was measured at 550 nm using a UV-visible spectrophotometer. Moreover, the saponin content was determined using a standard curve prepared with diosgenin as the standard. The results were expressed as milligrams of diosgenin equivalents per gram of the sample's dry weight.

2.3.2 Flavonoids

The flavonoid content was determined using the aluminum chloride colorimetric method (Chang *et al.,* 2002). A 1 mL aliquot of the methanolic extract was mixed with 1 mL of 2% aluminum chloride ($AlCl_3$) solution. The mixture was allowed to stand at room temperature for 10 min. Afterwards, the absorbance was measured at 430 nm using a UV-visible spectrophotometer. Then, the flavonoid content was

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quantified using a calibration curve prepared with quercetin as the standard. The results were expressed as milligrams of quercetin equivalents per gram of the sample's dry weight.

2.3.3 Alkaloid

Alkaloids were extracted from *P. reticulum* samples using an acid-base method. A known weight (5 g) of this dried plant material was ground into a fine powder. Extraction was performed by shaking the sample with 100 mL of alcoholic solution (70% ethanol) for 1 h. After filtration, the resulting extract was acidified with 10 mL of 0.1 M hydrochloric acid (HCl) to solubilize the alkaloids in the aqueous phase. This aqueous phase was then extracted with 50 mL of chloroform for 30 min. After phase separation, the chloroform phase containing the alkaloids was concentrated by evaporating the solvent under vacuum at room temperature (Saptarini and Herawati, 2019). The extracted alkaloids were subjected to a colorimetric reaction using Dragendorff's reagent. Approximately 2 mL of Dragendorff's reagent was added to 1 mL of the concentrated extract. An orange precipitate, characteristic of the presence of alkaloids, was observed. The intensity of the precipitate was measured at 470 nm using a spectrophotometer. The quantity of alkaloids was determined by comparing the intensity of the precipitate with a standard curve obtained from a reference solution of the alkaloid. The results were expressed as milligrams of alkaloids per gram of dry matter.

2.3.4 Oxalate

The Volhard titration method, following the AOAC Official Method 974.24, was used to determine the oxalates.

2.3.5 Phytate

The phytate content of *P. reticulum* dry fruit powder was determined using a spectrophotometric method based on iron (III) complex formation. The procedure begins by extracting the phytates with a 2% hydrochloric acid (HCl) solution. The extract is then mixed with ferric chloride ($FeCl_3$), forming a colored complex with the phytate. The absorbance of this complex is measured at 500 nm using a spectrophotometer (Haug and Lantzsch, 1983). To quantify the phytates, a calibration curve is generated using a standard phytate solution.

2.3.6 Cyanogenic glycosides

The enzymatic method for determining cyanogenic glycosides in *P. reticulum* dry fruit powder was performed, with the extract obtained using methanol. Beta-glucosidase enzyme is added to the extract, which hydrolyzes the cyanogenic glycosides into cyanide. To detect cyanide, a colorimetric method, such as the picrate method, is employed, where cyanide reacts with picric acid to produce a yellow to red color. The absorbance of the solution is measured at 510 nm to quantify the cyanide concentration (Adindu *et al.,* 2024). Finally, a cyanide standard is used for calibration to calculate the cyanogenic glycoside content.

2.4 Proximate analysis

Proximate analysis was conducted to determine the nutritional content of organic *P. reticulum* dry fruit powder.

2.4.1 Moisture content

The moisture content was determined using the oven -drying method following the AOAC Official Method 925.10.

2.4.2 Ash content

The ash content, representing the total mineral content, was determined by incinerating the sample in a muffle furnace at 550°C until a white ash was obtained. The ash content was expressed as a percentage of the initial sample weight. According to recent reports, the ash content in *P. reticulum* powder indicates its potential as a good source of essential minerals (Mohammed *et al.,* 2023).

2.4.3 Crude protein

The Kjeldahl method, following the AOAC Official Method 979.09 was used to evaluate the crude protein content.

2.4.4 Crude fat

The Soxhlet extraction method, following the AOAC Official Method 920.39 was used to determine the crude fat.

2.4.5 Crude fiber

The crude fiber content of the sample was determined using the method AOAC Official Method 962.09.

2.4.6 Carbohydrate content

The carbohydrate content was calculated by difference, subtracting the sum of moisture, ash, protein, fat, and fiber contents from 100%.

2.4.7 Minerals

A known weight (1–2 g) of the sample was accurately weighed and placed into a crucible. The

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sample was ashed in a muffle furnace at 550°C for 4 h to remove organic matter. After cooling, the ash was dissolved in 10 mL of 5% hydrochloric acid (HCl) and filtered to remove any insoluble residues. The filtrate was then diluted to a final volume of 25 mL with deionized water. The mineral content was determined using a PerkinElmer AAnalyst 400 Atomic Absorption Spectrometer. The sample solution was aspirated into the flame, where the minerals were atomized and absorbed the specific wavelength light emitted by the element; for each mineral, the optimal wavelength was selected. A standard curve for each mineral was prepared by using a series of standard solutions with known concentrations of the respective minerals. The calibration standards covered the expected range of concentrations in the samples. The results were expressed as milligrams of the mineral per gram of sample (mg/g) (da Silva Medeiros *et al.,* 2020).

2.5 Fourier-transform infrared analysis

Fourier-transform infrared (FTIR) spectroscopy was employed to characterize the functional groups present in the *P. reticulum* dry fruit powder. The FTIR spectra were obtained using a (Schimadzu FTIR 8400S) spectrometer, with samples prepared as KBr pellets. The spectra were recorded in the range of 4000–400 cm^{-1}. Key absorption bands were identified, and corresponding functional groups were assigned based on standard FTIR spectral libraries (Sharma *et al*., 2020).

2.6 Antioxidant activities

2.6.1 Preparation of extract

An aqueous extract of the sample was obtained by weighing 10 g of the powdered sample and extracting it with 300 mL of 5% ethanol, followed by boiling for 15 min. After filtration, the filtrate was then evaporated to get the extract. The extract was stored in the refrigerator for further analysis.

2.6.2 DPPH radical scavenging activity

The free radical scavenging activity of *P. reticulatum* was measured using the diphenyl 1-picrylhydrazyl (DPPH) assay, as described by Bursal and Gulcin (2011). The stock solution was prepared by dissolving 24 mg DPPH in 100 mL of methanol and stored at 20°C until required. The working solution was obtained by diluting the DPPH solution with methanol to achieve an absorbance of approximately 0.98±0.02 at 517 nm using a Shimadzu UV-2550 spectrophotometer. A 3 mL aliquot of this solution was mixed with 1 mL of the extract at various concentrations (0.1, 0.5, 1, and 2 mg/mL) in three replicates. The reaction mixture was thoroughly shaken and incubated in the dark at room temperature for 15 min. The absorbance was then measured at 517 nm. The control was prepared as above without any sample. A lower absorbance of the reaction mixture indicates a higher free radical scavenging activity. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as follows:

$$\text{DPPH scavenged (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control reaction, and A_{test} is the absorbance in the presence of the sample of the extracts.

2.6.3 Reducing power

The reducing power of the extracts of *P. reticulatum* was determined by the method described by Oyaizu (1989). Aliquots of 1 mL of methanol extract of the sample (at 4 different concentrations: 0.1, 0.5, 1, and 2 mg/mL; three replicates) were mixed with 2.5 mL of 0.2 mM phosphate buffer solution at pH 6.6 and 2.5 mL of 1% potassium ferrocyanide. The mixture was incubated for 20 min at 50°C in a water bath. Then, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 min. After centrifugation, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. Absorbance was measured at 700 nm using a Shimadzu UV-2550 spectrophotometer. The control was prepared by substituting the same amount of diluted extract with methanol. The results were expressed in milligram equivalents of ascorbic acid per milligram of dry weight.

2.6.4 Hydroxyl radical scavenging activity

The scavenging activity of P. reticulatum extracts (1, 2, 3, and 4 mg/mL) on hydroxyl radical activity was measured using the method of Ilavarasan *et al.* (2005). The reaction mixture contained deoxyribose 1 mL (2.8 mM), KH_2PO_4 -NaOH buffer, pH 7.4 (0.05 M), 0.4 mL $FeCl_3$ (0.1 mM) and EDTA (0.1 mM), 0.2 mL H_2O_2 (1 mM), and different concentrations of the sample extracts in a final volume of 2 mL. The mixture was incubated at 37°C for 30 min, followed by the addition of 2 mL of trichloroacetic acid (2.8% w/v) and thiobarbituric acid. Thereafter, it was kept for 30 min in a boiling water bath and cooled. The absorbance was recorded at 532 nm in a UV–VIS spectrophotometer. The hydroxyl radical scavenging activity of the sample extracts was evaluated:

$$\text{Hydroxyl radical scavenging (\%)} = \frac{1 - A_{test}}{A_{control}} \times 100$$

Where, $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

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2.7 Statistical analysis

Data are presented as a mean ± standard deviation (SD). Statistical analyses were carried out using SPSS 21.0 software. A one-way analysis of variance (ANOVA) was performed on the antioxidant data, and the significance of differences between mean values was determined using the least significant difference (LSD) test at $\alpha = 0.05$.

3. Results and discussion

The phytochemical screening and quantitative estimation of the percentage yield of the chemical constituents of *P. reticulum* powder showed that the powder contains tannin (2.55 mg/100 g), oxalate (0.70 mg/100 g), phytate (0.30 mg/100 g), saponin (3.60 mg/100 g), cyanogenic glycoside (3.86 mg/100 g), alkaloids (0.86 mg/100 g) and flavonoid (1.05 mg/100 g) as shown in Table 1. Phytochemicals exhibit bioactivity and are responsible for specific physiological effects on the human body through various plant components (Usin and Daramola, 2022; Gupta, 2023). Several of these biochemical constituents have been shown to exhibit various biological and pharmacological effects, including antibacterial, antihypertensive, and anti-inflammatory properties, among others (Petrocelli *et al.,* 2023). The presence of these chemicals in *P. reticulum* powder is, therefore, a strong indication that the powder possesses valuable medicinal properties that have yet to be explored.

In the examination of *P. reticulum* powder through proximate analysis, the carbohydrate component exhibited the highest percentage at 70.31%, whereas the crude fat content was identified as the lowest at 2.50% among the various nutrient compositions. This numerical data, in conjunction with an energy value of 403.52 kcal/100 g detailed in Table 1 above, underscores the significance of *P. reticulum* powder as a valuable and practical energy source. With a protein content of 11.30%, this powder has the potential to serve as a protein source for plants due to its elevated levels. Additionally, the ash, moisture, and crude fibre content were determined to be 5.98%, 3.62%, and 5.87%, respectively.

The powder of *P. reticulum* plant is rich in vitamins A, C, and E, as shown in Table 2. These nutrients are considered antioxidants, and their presence in relatively low levels compared to substances that are prone to oxidation can effectively inhibit or hinder the oxidation process of these substances (Sinbad *et al.,* 2019). A growing body of evidence suggests that natural antioxidants, such as vitamins A, C, and E, protect the body against various degenerative conditions, including cancer, aging, and atherosclerosis (Rahaman *et al.,* 2023). The significant quantity of these compounds present in the powder of *P. reticulum* signifies the importance of the plant in terms of nutrition and medicinal properties.

The mineral composition of *P. reticulum* powder exhibited elevated concentrations of calcium and magnesium (1670.30 and 263.47 mg/100 g) as delineated in Table 2. The substantial calcium levels are notably high. The findings suggested that these powders may hold considerable physiological significance, particularly in regions where conditions such as muscle weakness, heightened nervous system irritability, and spontaneous action potential initiation in neurons are prevalent (Ighodaro and Agunbiade, 2012). Furthermore, *P. reticulum* powder serves as a notable source of iron (36.25 mg/100 g), while the quantities of manganese (4.45 mg/100 g), copper (2.19 mg/100 g), Zinc (3.51 mg/100 g), and lead (0.8 mg/100 g) present are comparatively modest. The diminished presence of heavy metals in the powder is advantageous, given the potential toxicity associated with heavy metal build-up within the body (Musina *et al.,* 2018).

The FTIR analysis of *P. reticulatum* fruit powder reveals significant insights into its phytochemical composition by identifying characteristic functional groups (Figure 2 and Table 3). The absorption bands observed in the 597-700 cm^{-1} range, particularly at

Table 1. Proximate compositions and quantitative values of the phytochemicals present in *Piliostigma reticulatum* dry fruit powder.

Proximate compositions		Quantitative values of the phytochemicals	
Parameters	Content (mg/100 g)	Parameters	Content (mg/100 g)
Moisture	11.75±2.79	Tannin	2.55±0.20
Protein	6.70±3.62	Oxalate	0.70±0.10
Fat	0.05±0.02	Phytate	0.30±0.05
Ash	5.62±2.71	Saponin	3.60±0.30
Carbohydrate	71.20±4.70	Cyanogenic glycoside	3.86±0.40
Crude fiber	4.69±0.40	Alkaloids	0.86±0.10
Energy value	330.81±37.05kcal/g	Flavonoid	1.05±0.10

Values are means of triplicate determinants.

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Table 2. Vitamins and minerals content of *Piliostigma reticulatum* dry fruit powder.

Vitamins parameters	Content (mg/100 g)	Minerals parameters	Content (mg/100 g)
Vitamin A	9.093±0.5	Ca	1640.30±50.00
Vitamin C	0.489±0.1	Mg	263.47±10.00
Vitamin D	11.632±0.3	Fe	36.25±2.00
Vitamin E	14.476±0.4	Mn	5.45±0.50
Vitamin K	1.008±0.05	Zn	3.51±0.20
Vitamin B5	1.250±0.1	Cu	2.19±0.10
		Pb	0.80±0.05

Values are means of triplicate determinants.

Table 3. Functional groups in the extracts determined by FTIR analysis of *Piliostigma reticulatum* dry fruit powder.

Wavenumber (cm^{-1})	Chemical bond	Phytoconstituents	Peaks observed (cm^{-1})
597-700	C-H bend (alkenes, alkynes)	Aromatic/Unsaturated compounds	597.95, 675.11
1085-1275	C-O stretch (ethers, alcohols)	Carbohydrates, phenols	1076.32, 1153.47, 1261.49
1330-1412	C-H bend, N-O stretch (aromatic nitro)	Phenols, nitro compounds	1330.93, 1411.94
1630-1680	C=O stretch (amides, ketones)	Proteins, aromatic ketones	1639.55
2850-2924	C-H stretch (alkanes)	Lipids, alkanes	2854.74, 2924.18
3300-3500	O-H stretch (alcohols, phenols)	Hydroxyl compounds	3429.55

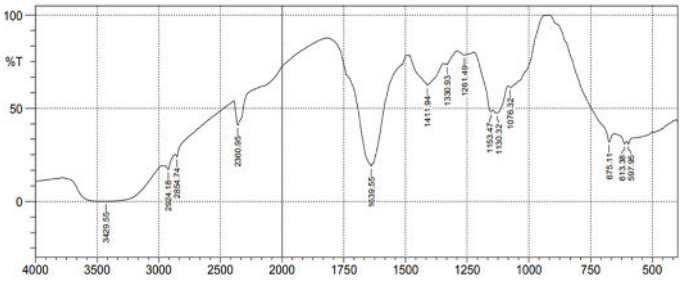

Figure 2. FTIR spectra of the *Piliostigma reticulatum* dry fruit powder.

597.95 cm^{-1} and 675.11 cm^{-1}, correspond to C-H bending vibrations associated with alkenes and alkynes. These findings suggest the presence of aromatic or unsaturated compounds, which are often associated with bioactive functionalities, such as antioxidant activities. The range 1085-1275 cm^{-1} exhibits prominent peaks at 1076.32 cm^{-1}, 1153.47 cm^{-1}, and 1261.49 cm^{-1}, attributed to C-O stretching vibrations of ethers and alcohols. These results confirmed the presence of hydroxyl compounds, such as carbohydrates and phenols. These constituents play vital roles in maintaining structural integrity and exhibiting antioxidant properties. In the 1330-1412 cm^{-1} region, the peaks at 1330.93 cm^{-1} and 1411.94 cm^{-1} are indicative of C-H bending and N-O stretching vibrations. These suggest the presence of phenols and nitro compounds, which are known for their antimicrobial and anti-inflammatory effects. A strong band at 1639.55 cm^{-1}, within the 1630-1680 cm^{-1} range, is associated with C=O stretching vibrations characteristic of amides and ketones. This observation highlights the presence of proteins or aromatic ketones, underscoring their importance in metabolic and structural processes. The absorption features in the 2850-2924 cm^{-1} range, observed at 2854.74 cm^{-1} and 2924.18 cm^{-1}, correspond to C-H stretching vibrations of alkanes. These results suggest the presence of lipids and alkanes, critical for energy storage and cellular membrane composition.

Lastly, the broadband in the 3300-3500 cm^{-1} range, with a peak at 3429.55 cm^{-1}, corresponds to O-H stretching vibrations**.** This indicates the presence of alcohols and phenols, confirming the presence of carbohydrates and phenols, which contribute to hydration and antioxidative mechanisms. Previously, studies have proven that antioxidant activity and reducing power were directly related. The reducing power and antioxidant activity of dry fruit powders are important measures of their ability to neutralize free radicals and prevent oxidative damage. Studies have shown that the antioxidant capacity of fruit powders can vary depending on the sample preparation used. Indeed, in this study, the *P. reticulatum* dry fruits were naturally dried on the tree before harvest and developed more aroma than those dried using other methods. However, the fruit pods are hard enough to retain higher levels of antioxidants, such as vitamin C and phenolic compounds. The lower the reducing power of a substance, the more it tends to be a potent oxidizing agent (i.e., readily accepts electrons). Therefore, the results of this research showed that the reducing power of *P. reticulatum* decreases as the concentration increases (Figure 3), indicating that the presence of reducers (i.e., antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. A similar observation has been reported by Badmus *et al.* (2016) on dried *Arenga pinnata* juice powder.

The aqueous extract of *P. reticularum* pod powder showed significant antioxidant activity compared to both methods used and at all concentrations tested, in a dose-

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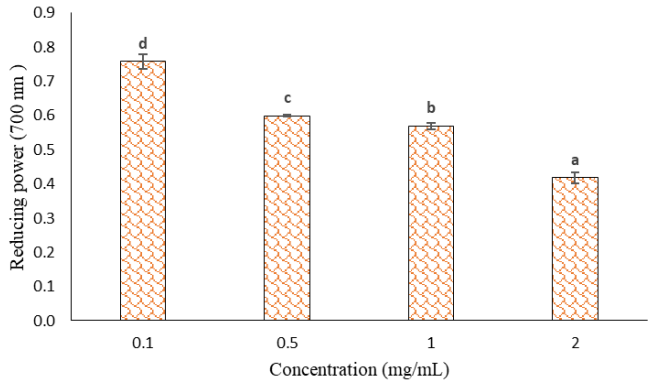

Figure 3. Reducing power of *Piliostigma reticulatum* dry fruit powder used at different concentrations. Values are means ± standard deviation of three determinations. Histograms with different letters indicate statistical differences ($P < 0.05$).

dependent manner ($p < 0.05$). Thus, we have the inhibition percentages (IP) ranging from 20.73% to 73.71% and from 28.03% to 63.37% for diphenyl 1-picrylhydrazyl (DPPH) and Hydroxyl radical scavenging activity (HO), respectively (Table 4). Several studies have reported the antioxidant activity of other organs of *P. reticulatum*, such as bark and leaves (Dieng *et al.,* 2017; Boualam *et al.,* 2021; Daniel and Temikotan, 2021). On the other hand, for concentrations of 0.1, 0.5, and 1 mg/mL tested, the inhibition percentages obtained by the DPPH test are slightly lower (in the order of 8 to 10%) than those of the hydroxyl test. Moreover, when the concentration reaches 2 mg/mL, the opposite is observed with DPPH, which exceeds hydroxyl by approximately 10% (Table 4). This leads to say that the higher the concentration, the more the powder of *P. reticulatum* pods shows better antioxidant activity against the DPPH radical. However, scavenging hydroxyl radicals is an important antioxidant activity due to the very high reactivity of the OH radical, allowing it to react with a wide range of molecules present in living cells, such as sugars, amino acids, lipids, and nucleotides (Sowndhararajan and Kang, 2013).

Table 4. Antioxidant activity of *Piliostigma reticulatum* dry fruit powder.

Concentration (mg/mL)	DPPH (%)	HO (%)
0.1	20.73 ± 3.96^{a}	28.03 ± 0.75^{a}
0.5	29.09 ± 4.68^{b}	40.32 ± 1.02^{b}
1	42.81 ± 2.90^{c}	52.92 ± 0.97^{c}
2	73.71 ± 0.75^{d}	63.37 ± 0.28^{d}

Values are means±standard deviation of triplicates. Values with different superscripts in the same row are statistically significantly different ($p<0.05$). DPPH: diphenyl 1-picrylhydrazyl, HO: Hydroxyl radical.

The calculated IC_{50} (median inhibitory concentration) values confirmed that the *P. reticulatum* pod powder exhibits moderate antioxidant activity, with IC_{50} values of 127 mg/mL and 129 mg/mL against DPPH and HO, respectively (Figure 4). The IC_{50} is inversely related to a compound's antioxidant capacity, as it expresses the amount of antioxidant required to reduce the concentration of the DPPH free radical by 50%. Lower IC_{50} values indicate a sample's effectiveness and more potent antioxidant activity (Maguirgue *et al.,* 2023). For ascorbic acid, considered the reference antioxidant, the IC_{50} is around 32.60 mg/mL (Addab *et al.,* 2020). Furthermore, oxidative stress is associated with chronic diseases such as diabetes, cardiovascular disease, and cancer. Plant antioxidants, such as phenolic acids and flavonoid compounds, protect against this oxidative stress and associated diseases by scavenging free radicals, inhibiting lipid peroxidation, and other mechanisms. Thus, scavenging the OH radical is crucial for protecting living systems.

Nowadays, the use of synthetic antioxidant molecules is being questioned due to potential toxicological risks. However, plants contain a large number of bioactive compounds with vigorous natural antioxidant activity and few or no side effects. This has sparked interest in researching natural sources of antioxidants, which are known for their beneficial effects on health and are also used as additives in the food, pharmaceutical, and cosmetic industries (Gheffour *et al.,* 2015; Halliwell, 2024).

4. Conclusion

This study provides a detailed examination of phytochemicals and nutritional contents in the powder of *P. reticulatum*. The findings reveal that the powder is rich in essential nutrients, particularly vitamins A, C, and E, which are known for their antioxidant properties. Additionally, the presence of bioactive compounds such as saponins, tannins, and flavonoids underscores the plant's potential medicinal benefits, including antibacterial, antihypertensive, and anti-inflammatory effects. The proximate analysis reveals a significant carbohydrate and protein content, making the powder a valuable source of energy and protein. The mineral analysis further emphasizes the high levels of calcium and magnesium, suggesting that the powder may play a role in addressing conditions related to mineral deficiencies. Additionally, the FTIR findings confirmed the plant's potential applications in the pharmacological and nutraceutical fields, highlighting its diverse biological activities. Overall, the study highlights the significance of P. reticulatum as a valuable medicinal and nutritional resource, particularly in underdeveloped regions where traditional medicine plays a vital role in healthcare. Further research is needed to elucidate its

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bioactive components, explore its therapeutic potential, and develop new food products.

Conflict of interest

The authors declare no conflict of interest.

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