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RESEARCH ARTICLE

Comparative Phytochemical and Antioxidant Analysis of the Leaf Extracts of Two Nigerian Medicinal Plants

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ABSTRACT

Bridelia ferruginea (Benth., Euphorbiaceae), prominently found in Savannah regions, is commonly used as an ethnomedicine for the treatment of several ailments in many parts of Africa. *Piliostigma thonningii* (Schum., Fabaceae) is used for various medicinal purposes in African countries with some pharmacological activities established from the crude extracts including antibacterial, antihelminthic, antiinflammatory and antilipidemic activities. In the present study, crude methanolic extract and fractions of *B. ferruginea* and *P. thonningii* were examined for their phytochemical constituents and antioxidant activity using standard protocols. Qualitative phytochemical screening of *B. ferruginea* crude extract revealed the presence of tannins, saponins, phlobatanins, flavonoids, steroids, alkaloids, glycosides, phenols and resins while it tested negative for triterpenoids, carbohydrates, terpenoids and anthraquinones; the quantitative phytochemical analysis showed that it contained 1.5% saponins, 5.3% flavonoids, 1.2% alkaloids and 0.9% phenols. *P. thonningii* crude extract tested positive for saponins, flavonoids, terpenoids, steroids, alkaloids, and phenols while tannins, phlobatanins, glycosides, triterpenoids, carbohydrates, resins and anthraquinones were absent. It contains 3.2% saponins, 1.6% flavonoids, 1.8% alkaloids, and 0.7% phenols. The concentration of the crude extract required for 50% inhibition of DPPH radical scavenging effect (IC_{50}) is 0.018 and 0.020 mg/ml for *B. ferruginea* and *P. thonningii*, respectively, compared to 0.015 mg/ml for vitamin C as a standard antioxidant. Both extracts showed potent inhibition of DPPH radical scavenging activity comparable to that of the Vitamin C standard and would be valuable sources of antioxidant compounds.

Keywords: antioxidant, *Bridelia ferruginea*, DPPH, *Piliostigma thonningii*, phytochemical

1. INTRODUCTION

Plants have been traditionally used as a source for secondary metabolites which play a significant role as medicines, flavoring, and recreational drugs in different parts of the world. This use contributes immensely to the enhancement of primary health care delivery (Usman et al., 2018). Plants continue to be an integral part of human survival, and this has made ethnobotany a necessary, interesting, and important research field (Thambi and Cherian, 2023). *Bridelia ferruginea* is a shrub commonly growing up to a height of 45 feet in the Savannah or in open spaces of coastal districts (Ngueyem et al., 2009). The plant is usually a

gnarled shrub which sometimes reaches the size of a tree. Bark, roots, fruits, and leaves of the plant are mainly used as decoctions (Ngueyem et al., 2009). The roots are used in Togo externally for the treatment of skin diseases and eruptions (Oliver-Bever, 1986). In Nigeria, *B. ferruginea* is used against arthritis, contusion, distortions, bites, and burns, as an antidote against arrow poison and against diabetes (Ngueyem et al., 2009) and in Southern Nigeria, it is considered as sacred and is featured in certain rituals and ceremonies (Afolayan et al., 2019).

Piliostigma thonningii Schum. is a leguminous plant that belongs to the Fabaceae family, which consists of about 133 genera (Kwaji et al., 2010). The plant is perennial in nature and its flowers, which are produced around November and December, are white or pink in colour. It bears hairy flat-pod fruits that turn nesty-brown and woody at maturity and usually persist on the plant between June and September (Jimoh, 2005). In African countries, *P. thonningii* is used for various medicinal purposes including the treatment of ulcers, wounds, heart pain, arthritis, malaria, pyrexia, leprosy, sore throat, diarrhea, toothache, gingivitis, cough, bronchitis, dysentery, fever, wound infections, cough, and skin diseases

The present investigation aims to explore the phytochemical contents and antioxidant activity of *B. ferruginea* and *P. thonningii*. These phytochemicals may be the link to the antioxidant activities of the plant and possibly the justification for the medicinal properties observed in the extracts of the plants. This could be further exploited for the isolation and characterization of biologically active chemical constituents for the treatment of infectious diseases.

2. MATERIALS AND METHODS

2.1. Plant sample collection and identification

B. ferruginea leaves were collected at the end of the raining season from Eruwa, Oyo state, Nigeria, in November 2016, while *P. thoningii* leaves were collected in the rainy season (June 2016) from the medicinal plants garden of Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria. Both samples were identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo state, Nigeria, with voucher reference number FHI-110711 for *B. ferruginea* and FHI-110688 for *P. thoningii*. A voucher specimen of each sample was deposited at FRIN for further reference in the herbarium section.

2.2. Preparation of plant samples

Leaves of the plants (2 kg each) were air-dried at room temperature under shade for 7 days, after which they were milled into powder with the aid of an electric hammer mill model TRAPP TRF 80 Hammer mill foliage and finally stored in a moisture-free environment until required for further use.

2.3. Extraction of plant samples

Powdered leaves were separately extracted with aqueous methanol. Briefly, 7 L of 10% aqueous methanol was added to 1.5 kg of the powdered leaves in separate stoppered glass containers and tightly covered. The mixture was left for 3 days at room temperature with periodic daily stirring and the extract finally filtered through cotton wool and Whatman 125 mm filter paper No. 1. The methanol extract was concentrated using a rotary evaporator model Stuart RE 300B W13 at reduced pressure. The extracts were each evaporated to dryness with methanol distilled at 40ºC to obtain the crude methanolic extract for each

sample. The dry extracts were later kept in tightly stoppered bottles in a refrigerator until required for further analysis.

2.4. Fractionation of Crude Extracts

The crude methanolic extract of *B. ferruginea* (140 g) was loaded on a reverse phase column under reduced pressure and eluted in order with 1 L of H_2O (100% - BFA, 31.8 g), H_2O-MeOH (1:1-BFB, 27.2 g), H_2O-MeOH (1:3-BFC, 23.3 g) and MeOH (100% - BFD, 35.7 g) to yield 4 fractions. All fractions were concentrated over a water bath at 40 ^{O}C (using a vacuum pump) to distill off methanol and then in a freeze dryer to remove water. The 1:3 H_2O-MeOH fraction (BFC), 10 g was suspended in water and successively extracted with dichloromethane and ethyl acetate in a separating funnel to afford the corresponding dichloromethane (BFE), ethyl acetate (BFF) and aqueous (BFG) soluble sub-fractions respectively. All sub-fractions were concentrated on a water bath at 40ºC. The crude methanolic extract of *P. thoningii*, 80 g was triturated with 100 cm^3 of H_2O-MeOH (1:1) mixture and extracted with equal volume of DCM and separated using a separating funnel. The DCM layer (lower layer) was collected in a beaker and the process repeated two more times. The DCM layers were pooled and evaporated to dryness over a water bath at 40℃ to give the DCM soluble fraction (PTA). EtOAc 100 cm^3 was added to the remaining H_2O layer and more H_2O added to aid separation. The EtOAc layer (upper layer) was collected after shaking vigorously and left to settle. This process was repeated twice and the EtOAc layers were pooled and evaporated to dryness over a water bath at 40 ^{O}C to give the EtOAc soluble fraction (PTB). BuOH was also used as a fractionating solvent to give the BuOH soluble fraction (PTC). The residue after fractionation was dried in a freeze drier and kept as the aqueous soluble fraction (PTD). The EtOAc fraction (PTB), 8 g was loaded on the silica gel column (200 g) and eluted with 1 L of the solvent mixture DCM, EtOAc, MeOH and H_2O in varying proportions to yield 3 sub fractions (PTE-PTG). The solvent ratios were determined based on TLC. PTE was eluted with 8:15: 4:1, PTF was eluted with 6: 10: 4:1, and PTG was eluted with 4:6:4:1 of DCM: EtOAc: MeOH: H_2O respectively.

2.5. Qualitative phytochemical screening

Phytochemical tests were carried out on the crude methanolic extracts to identify the constituents using standard procedures as described by Asekun et al. (2013). The screening tests carried out are discussed below:

Test for tannins - About 2 mL of the crude extract was stirred with 2 ml of distilled water and a few drops of 5% ferric chloride ($FeCl_3$) solution were added. The formation of a dirty green precipitate was an indication for the presence of tannins.

Test for saponins - The crude extract, 5 mL was shaken vigorously for a few minutes with 5 mL of distilled water in a test tube and warmed. The formation of stable foam (frothing) which persists on warming was taken as an indication for the presence of saponins.

Test for phlobatannins - About 2 mL of crude extract was added to 2 mL of 1% hydrochloric acid (HCl) and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

Test for flavonoids - To 1 mL crude extract was added 1 mL of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.

Test for anthraquinones - The crude extract, 3 mL of crude extract was shaken with 3 mL of benzene, filtered, and 5 mL of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet colour in the ammonical (lower) phase indicates the presence of free anthraquinones.

Test for terpenoids - To 0.5 mL acetic anhydride was added 1 mL of sample extract and a few drops of concentrated sulphuric acid (H_2SO_4). A bluish green precipitate indicates the presence of terpenoids.

Test for steroids - A red colour produced in the lower chloroform layer when 2 mL of crude extract was dissolved in 2 mL of chloroform and 2 ml concentrated sulphuric acid added indicates the presence of steroids.

Test for alkaloids - Test extract was acidified with 1% hydrochloric acid (HCl) and was then treated with a few drops of Mayer, Wagner, and Dragendroff's reagents separately in different test tubes. A creamy white (Mayer), reddish brown (Wagner), or orange brown (Dragendroff's) precipitate indicates the presence of alkaloids.

Test for resins - Copper acetate solution, 5 mL was added to 5 mL of the crude extract. The resulting solution was shaken vigorously and allowed to separate. A green coloured solution is an evidence of the presence of resin.

Test for phenols - The crude extract, 0.2 g was dissolved in $FeCl_3$ solution. A green or dirty green precipitate indicates the presence of phenolic compounds.

2.6. Quantitative phytochemical analysis

Saponin determination - Plant sample (2 g) was dispersed in 20 mL of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55℃. The mixture was filtered and the residue re-extracted with another 20 mL of 20% ethanol. The combined extracts were reduced to 40 mL over a water bath at about 90ºC. The concentrate was purified by transferring into a 250 mL separating funnel and 20 mL of diethyl ether added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This process was done twice. Next, 60 mL of the ethanol extract was washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated as a percentage of the initial sample (Ajuru et al., 2017).

Total phenols determination - The fat-free sample was boiled with 50 mL of ether for the extraction of the phenolic component for 15 min. Next, 5 mL of the extract was pipetted into a 50 mL flask, then 10 mL of distilled water was added, and then 2 mL of ammonium hydroxide solution and 5 mL of concentrated *n*-pentanol were also added. The samples were made up to the mark and left to react for 30 min for colour development. The absorbance of the sample was measured with a spectrophotometer at 505 nm. The absorbance read was used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid (Ajuru et al., 2017).

Alkaloid determination - The crude extract sample, 1 g was weighed into a 250 mL beaker and 200 mL of 10 % acetic acid in ethanol was added, covered, and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried, weighed, and expressed as a percentage of the sample (Ajuru et al., 2017).

Flavonoid determination - The crude extract sample, 1 g was extracted repeatedly with 100 mL of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath to a constant weight, which is the amount of flavonoids present in the sample. The total flavonoid content was then expressed as a percentage of the sample (Ajuru et al., 2017).

2.7. Antioxidant assay

The radical scavenging activity of each extract was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay according to Afolayan et al. (2014). The decrease in the absorption of DPPH solution after the addition of an antioxidant was measured at 517 nm. Ascorbic acid (10 mg/mL DMSO) was used as reference. The DPPH solution (1 mM) was prepared by dissolving 40 mg of DPPH in 100 mL of ethanol. Concentrations of 0.0625, 0.125, 0.25, 0.5, and 1.0 mg/mL of the extracts in methanol (Analar grade) were prepared. Vitamin C (Ascorbic acid) was used as the antioxidant standard at the same concentrations as (0.0625, 0.125, 0.25, 0.5, and 1.0) mg/mL. For the crude extract, 1 mL was placed in a test tube and 3 mL of methanol was added, followed by 0.5 mL of 1 mM DPPH in methanol. After 10 min, the decrease in absorption was measured on a UV-Visible Spectrophotometer at 517 nm. A blank/control solution was prepared containing the same amount of methanol and DPPH. The actual decrease in absorption was measured against the control and the percentage inhibition was calculated. All experiments were run in duplicates and the results obtained were averaged. The radical scavenging activity (RSA) was calculated as the percentage inhibition of DPPH discolouration using the equation: % inhibition = {[Ab-Aa]/Ab} × 100; where Ab is the absorption of the blank sample and Aa is the absorption of the extract. Results were expressed as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

Powdered *B. ferruginea* leaves (1.5 kg) extracted with 7 L of 10% aqueous methanol gave 158.1 g solvent-free, dark green crude extract representing 10.54% yield. Powdered *P. thonningii* leaves (1.5 kg) extracted with 7 L of 10% aqueous methanol gave 235.6 g solvent-free, dark brown crude extract representing 15.71% yield. The results obtained from the qualitative phytochemical analysis are presented in Table 1. Both crude extracts contained saponins, flavonoids, steroids, alkaloids, and phenols, while anthraquinones, carbohydrates, and triterpenoids were absent in both. Meanwhile, *B. ferruginea* crude extract contains tannins, phlobatannins, resins, and glycosides which are absent in *P. thonningii* crude extract. *P. thonningii* crude extract tested positive for terpenoids and was absent in *B. ferruginea* crude extract.

Table 1. Qualitative phytochemical screening of *B. ferruginea* and *P. thonningii* extracts and fractions

Test Phytochemical	BF	BFA	BFB	BFC	BFD	PT	PTA	PTB	PTC
Tannins	+	+	+	-	-	-	-	-	-
Saponins	+	-	+	+	+	+	+	+	+
Phlobatannins	+	+	-	-	-	-	-	-	-
Flavonoids	+	-	+	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	+	+	+	-
Steroids	+	-	-	-	+	+	+	-	-
Alkaloids	+	-	+	+	-	+	+	+	+
Carbohydrates	-	-	-	-	-	-	-	-	-
Glycosides	+	+	-	+	+	-	-	-	-
Triterpenoids	-	-	-	-	-	-	-	-	-
Phenols	+	+	+	+	-	+	-	+	+
Resins	+	+	-	-	-	-	-	-	-

Key: + = Present; - = Absent

The results revealed that *B. ferruginea* leaf contains high percentage of total flavonoids while *P. thonningii* leaf has high total saponin contents as shown in Table 2.

Table 2. Quantitative phytochemical analysis results of *B. ferruginea* and *P. thonningii* extracts

Test Phytochemical	*B. ferruginea*	*P. thonningii*
Total Saponins (%)	1.5 ± 0.01	3.2 ± 0.01
Total Phenols (mg gallic acid g^{-1})	133.2 ± 0.02	128.3 ± 0.03
Total Alkaloids (%)	1.2 ± 0.01	1.8 ± 0.01
Total Flavonoids (%)	5.3 ± 0.01	1.6 ± 0.02

The results of the antioxidant assay (percentage DPPH inhibition) on the crude methanolic extracts of *B. ferruginea* and *P. thonningii* presented in Table 3 showed that both extracts had good DPPH radical scavenging activity quite comparable to the standard Vitamin C. The antioxidant activities recorded for both extracts were also observed to be dose dependent with activity increasing as the concentration increases.

Table 3. Percentage inhibition of DPPH by *B. ferruginea* (BF) and *P. thonningii* (PT) extracts

Extracts	Concentration (mg/mL)				
	0.0625	**0.125**	**0.25**	**0.5**	**1.0**
BF (%)	63.47 ± 0.02	81.80 ± 0.03	84.47 ± 0.01	86.41 ± 0.03	89.56 ± 0.03
PT (%)	76.82 ± 0.05	79.40 ± 0.02	79.03 ± 0.01	79.77 ± 0.02	81.24 ± 0.02
Vitamin C (%)	81.80 ± 0.01	87.74 ± 0.03	89.96 ± 0.01	97.35 ± 0.02	93.81 ± 0.02

The above result is further illustrated in Figure 1 to show how the extracts compare with the standard (Vitamin C). Both extracts exhibited good antioxidant activity which was dose dependent comparable with the Vitamin C standard. It is also observed that *B. ferruginea* extract had better antioxidant activity than the *P. thonningii* extract except at a concentration of 0.0625 mg/ml with Vitamin C having higher antioxidant activity at all concentrations. IC_{50} values were calculated for all three samples as 0.018, 0.020, and 0.015 mg/mL for the *B. ferruginea* extract, *P. thonningii* extract and Vitamin C respectively.

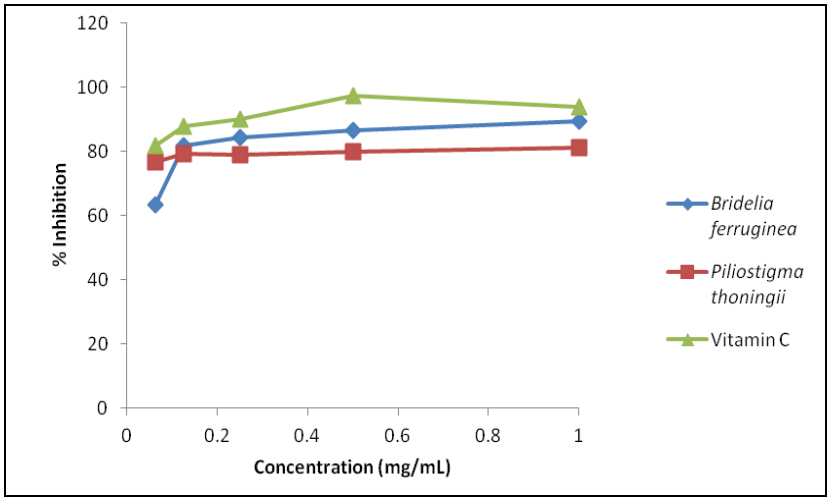

Figure 1. Antioxidant activities of *B. ferruginea*, *P. thonningii* extracts and vitamin C standard

Phytochemicals, also called secondary metabolites, are chemical compounds formed by plants and they are accountable for their biological, therapeutic, or toxicological activities. These phytochemicals help plants blossom against competitors, predators, or pathogens (Mendoza and Silva, 2018). Phytochemical analysis of the crude methanolic extract of *B. ferruginea* showed the presence of tannins, saponins, phlobatannins, flavonoids, steroids, alkaloids, glycosides, phenols and resins. This is in tandem with an earlier report of the presence of flavonoids, tannins, cardiac glycosides, and phlobatannins in the crude extract

(Adebayo and Ishola, 2009). Quantitative phytochemical analysis of the crude extract of *B. ferruginea* revealed a very high flavonoid content of 5.3%. Meanwhile, *P. thonningii* crude extract had saponins, flavonoids, terpenoids, steroids, and alkaloids. This result is comparable to that reported by Bello et al. (2013) where the methanol leaf extract of *P. thonningii* was found to contain alkaloids, flavonoids, tannins, cardiac glycosides and saponins. The quantitative analysis revealed that *P. thonningii* crude methanolic extract has a higher saponin content of 3.2%.

These phytochemical constituents are considered accountable for a variety of biological activities. The presence of saponins, flavonoids, alkaloids, phenols, tannins, terpenoids, and steroids in both extracts indicates that the extracts would be of biological or pharmacological importance (Ekhaise et al., 2010). Phenolic compounds, which are abundant in both extracts, are known to show a wide range of biological activities such as antioxidant, free radical scavenging activity and antimicrobial activity (Volluri et al., 2011). Steroids are of great consequence in pharmaceutical studies because of their connection with compounds used as hormone supplements. They are also reported to have antimicrobial properties (Okwu, 2001). Plant terpenoids are used for their aromatic qualities. They are well utilized in local herbs and are continuously employed for antibacterial, antineoplastic, and other pharmaceutical functions (Michael, 2009). Alkaloids are reputed to be one of the main bioactive phytochemicals in plants. They are identified to possess analgesic, antimicrobial, and antihypertensive activities, among others (Zongo et al., 2009). Tannins are plant metabolites well reputed for their antimicrobial properties (Adeyanju et al., 2011). Flavonoid compounds have been recognized to add to the biological activity of plant materials and they have been reported to possess broad spectrum antimicrobial activity. They also have anti-inflammatory activity (Ogundaini, 2005). Therefore, the abundance of flavonoids, phenols, alkaloids, and other phytochemicals of biological value are probable to contribute to the pharmacological significance of the crude extracts and fractions of *B. ferruginea* and *P. thonningii*.

Antioxidants are essential compounds that restrain or prevent oxidation. Oxidation in the human body is a chemical process that releases free radicals leading to chain reactions which may destroy cells in the human body. Antioxidants are consequently active compounds that can defend plant and animal tissues from damage by these free radicals (Scheibmeir et al., 2005). Phenolic compounds are reputed to consist of compounds with high antioxidant activity due to their redox properties, which can perform a considerable role in adsorbing and deactivating free radicals, reducing singlet and triplet oxygen, or destroying peroxides (Velioglu et al., 1998). Phytochemicals with antioxidant activity can destroy reactive chemical species and decrease the oxidative pressure resulting from unnecessary light energy acquaintance. Numerous plants' metabolites are major constituents of both human and animal foods and they are not dangerous for consumption (Argolo et al., 2004). Medicinal plants have been considered to hold numerous antioxidants competent of stabilizing or destroying free radicals before they attack cells. Polyphenols, including flavonoids, are secondary metabolites of plants that are generally involved in defense against ultraviolet radiation, aggression by pathogens, and in the last decades as an antioxidant due to their proton donation activity. Several current research reports substantiate that flavonoids are even more effective antioxidants than vitamins C and E (Oloyede et al., 2012).

The antioxidant assay of the methanol crude extracts of *B. ferruginea* and *P. thonningii* revealed that both extracts exhibited good dose-dependent radical scavenging activity against DPPH. However, the crude extract of *B. ferruginea* had better percent inhibition at almost all concentrations tested with up to 89.56% inhibition at 1.0 mg/mL. These results obtained can be linked to their phytochemical constituents. The qualitative phytochemical screening carried out on both extracts showed that both extracts contained flavonoids and phenols. These classes of compounds are known to be natural antioxidants and are able to decolorize DPPH

solutions with their high hydrogen donating capacity and could act as free radicals scavenging serving as a primary antioxidant (Alabri et al., 2014). Moreover, the quantitative phytochemical analysis revealed that the crude extract of *B. ferruginea* had a higher flavonoid and phenol content than *P. thonningii* hence, the observed higher antioxidant activity for *B. ferruginea*. The impressive antioxidant activity of these extracts is also likely connected to the specific constituents that have been isolated from them. The results obtained are consistent with that reported by Lagnika et al. (2012) where the methanol extract of *B. ferruginea* leaves showed good antioxidant activity against DPPH in a dose-dependent manner with a percentage inhibition of 86.09% at 1.0 mg/mL and aligns with that reported by Oloyede et al. (2012) for *B. ferruginea* stem bark.

4. CONCLUSION

This study indicates *B. ferruginea* and *P. thonningii* contain essential phytochemicals and possess remarkable antioxidant activity, which would make them valuable sources of antioxidant compounds. It supports the pharmacological basis for these plants as traditional medicine for the treatment of many alignments. Further investigations are needed to exploit both plants for valuable therapeutic use.

Declaration of Interest
The authors declare that there is no conflict of interest.

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