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Research Article

Phytochemical Analysis and Evaluation of Ethanol and Aqueous Extracts of *Piliostigma thonningii* Leaf for *in vitro* Antioxidant Activities

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ABSTRACT

Piliostigma thonningii Linn is a leguminous plant belonging to the family *fabacea*. It is found growing abundantly as a wild uncultivated tree in many parts of Nigeria. Various studies have been done to identify antioxidants from plant sources and efforts have been taken to integrate it in conventional therapy. In our present study, *in vitro* antioxidant and phytochemical investigation was carried out using solvent extracts such as ethanol and water. The leaves were collected, washed and air-dried for twenty-one (21) days to constant weight. The leaves were pulverized and extracted using distilled water and ethanol respectively. The phytochemical (qualitative and quantitative) analysis was determined using standard procedures. *In vitro* antioxidant properties were evaluated by assessing DPPH free radical scavenging abilities, α-tocopherol, ascorbic acid and β-carotene by adopting standard methods. The results of the phytochemical depict the presence of carbohydrates, glycosides, cardiac glycosides, saponins, steroids, triterpenes, tannins, flavonoids, alkaloids and anthraquinones, while the antioxidant studies revealed that DPPH activity was higher in the ethanol and aqueous extracts when compared to the reference antioxidant. However, β-carotene, ascorbic acid and α-tocopherol levels were higher in ethanol than aqueous extract. Conclusively, *P. thonningii* leaf possesses phytochemical constituents and antioxidant effect as seen in this study.

Keywords: *Piliostigma thonningii, Phytochemical analysis, In vitro antioxidant, Free radicals*

INTRODUCTION

Traditional medicine has remained the most affordable and easily accessible source for the management of diseases. It allows one utilizes natural means as a way of improving health and finding relief from numerous diseases (Oteng *et al.*, 2019). In recent years, considerable researchers have discovered the medicinal values of many plants (Sofowara, 2008), and composed of bioactive compounds with proven efficacy as the basic raw material of drugs (Akinpelu and Obuotor, 2000).

Several reports have been put over the years by different researchers on the medicinal uses of various plants. One of which is the *Piliostigma thonningii* plant. *P. thonningii* is a leguminous plant belonging to the family *Fabaceae*. It is found growing abundantly as a wild uncultivated tree in many parts of Nigeria such as Zaria, Bauchi, Ilorin, Plateau, Lagos, and Abeokuta (Jimoh and Oladiji, 2005). Among the different tribes in Nigeria, it is called Abefe (Yoruba), Kalgo (Hausa), Okpoatu (Igbo), Bafin (Nupe), and Barkehi (Fulani). It is also called the Monkey bread or sometimes camel's foot (Jimoh and Oladiji, 2005; Offiah *et al.*, 2011; Ighodaro *et al.*, 2012). Leaves of *P. thonningii* are edible and chewed to relieve thirst. Its fruits and seeds are also edible fodder. The preparation from the bark of the tree is used in treating cough, usually taken as an infusion; it is used to stop diarrhea, dysentery, and intestinal upset (Tira-Picos *et al.*, 2010). Maceration prepared from the bark and leaves is also used in the treatment of malaria, leprosy, wounds, ulcers, gastric/heart pain, cough, gingivitis, sore throat, toothache, and, as an antipyretic (Aderogba *et al.*, 2006; Ighodaro and Omole, 2012). The leaf decoction is a laxative given to children, neonates, and is also used as an embrocating tonic to massage the abdomen of newly delivered mothers. It also

serves as a lotion for lumbago (Diallo *et al.*, 2002). The leaves are soaked in hot water and applied topically as wound dressing or to excisions in the southern part of West Africa (Okoli and Iroegbu, 2004). Different extracts of *P. thonningii* was reported to possess antipyretic, antidiabetic, antioxidant, and antilipidemic activities (Ighodaro and Omole 2012; Asuzu and Nwaehujor 2013; Nwaehujor *et al.*, 2015), antibacterial (Akinpelu and Obuotor, 2000; Ouattara *et al.*, 2020; Ighodaro *et al.*, 2012), antihelminthic (Asuzu and Onu 1994; Asuzu *et al.*, 1999), antifungal (Olela *et al.*, 2020; Ighodaro *et al.*, 2012), anti-inflammatory and analgesic activities (Ibewuike *et al.*, 1997; Aderogba *et al.*, 2006; Igbe *et al.*, 2012; Dasofunjo *et al.*, 2013). Also, leishmanial and trypanosomal studies have been reported based on the chemical constituents of *P. thonningii* (Afolayan *et al.*, 2018; Mohamed *et al.*, 2016a, b; Mostafa *et al.*, 2016; Mohamed *et al.*, 2017).

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule (Yamagishi and Matsui, 2011). The characteristic feature of an antioxidant is the ability to scavenge the free radicals due to their redox hydrogen donators and singlet oxygen quencher (Anokwuru *et al.*, 2011; Wu *et al.*, 2011). They are essential and important for plant and animal nutrition. The sources of antioxidants include fruits, vegetables, meats, poultry, and fish. Fruits and vegetables contain a large number of flavonoids and antioxidant supplements that contribute to protection against different types of cancers and cardiovascular health problems (Hamid *et al.*, 2010). Plant-derived antioxidants have been shown to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists (Racova *et al.*, 2007). Increased awareness of the significance of medicinal plants and nutrition to the health of individuals and communities has necessitated the need for determining the antioxidant profile of medicinal plants (Halliwell, 2007). Free radicals can be scavenged by the natural (plants) and synthetic (butylated hydroxyl toluene, butylated hydroxyl anisol, and tetra butyl hydroquinone) antioxidants (Mbaebe *et al.*, 2012). But the usages of these synthetic antioxidants are now replaced because the natural antioxidants could be considered safer without any side effects (Meenakshi *et al.*, 2011). Therefore, there is a considerable need in finding antioxidants from natural sources to replace synthetic ones. So this study is aimed at analyzing the phytochemical constituents and evaluating the *in vitro* antioxidant properties of aqueous and ethanol extracts of *P. thonningii* leaf.

MATERIALS AND METHOD

Plant Materials

Fresh leaves of *P. thonningii* were harvested from a garden in Lafenwa, Abeokuta North Local Government Area, Ogun State, Nigeria. The plant material was identified and authenticated at the Forest Institute Research of Nigeria, Ibadan, Oyo State, and the voucher number; FHI 112994 was deposited for future reference.

Preparation of Plant Materials

The leaves were thoroughly washed, then air-dried at room temperature for twenty-one (21) days, macerated and pulverized into a powdery form using the electric blender, and then sieved. The resulting powder was used to prepare two (2) different extracts as highlighted below.

Extraction

Fresh leaves of *Piliostigma thonningii* were thoroughly washed, then air-dried at room temperature for twenty-one (21) days, macerated and pulverized into powdery form using the electric blender and then sieved. The resulting powder was used to prepare two (2) different extracts as highlighted below.

Aqueous Extract

Three hundred (300) grams of powdered *Piliostigma thonningii* leaves was dissolved in 150 mL of distilled water for 72 hours in a refrigerator. Therefore, it was filtered with muslin cloth and filtered using Whatman No. 1 (320 nm, 4 µm) filtered paper. The filtrate was evaporated to dryness using a water bath (40^{o}C) to obtain the slurry. The resulting slurry was persevered in a phial, labelled appropriately and stored in the refrigerator at 4^{o}C until needed for analysis.

Ethanol Extract

Three hundred (300) grams of powdered *Piliostigma thonningii* leaves was soaked in 150 mL of ethanol for 72 hours. This was followed with vacuum filtration and extract was concentrated at low pressure using a rotary evaporator water bath at 40^{o}C. The concentrate was heated over a water bath to obtain a solvent free extract, which was persevered in a phial, labelled appropriately and stored in the refrigerator at 4^{o}C until needed for analysis.

Chemicals/Reagents

All chemicals and reagents used were of analytical grade. Ethanol and methanol (HPLC grade) were obtained from E. Merck (Germany). The ascorbic acid ($C_6H_8O_6$) with 99.7% purity, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), and β-carotene were purchased from Sigma Aldrich Chemical Co. (St Louis, USA). Thiamine was obtained from Serra Heidelberg, Germany. The α-tocopherol was obtained from Fluka Chemicals (Buchs, Switzerland). All the reagents were used without any further purification.

In vitro Antioxidant Activities

DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging activity of ethanol and aqueous extracts of *Piliostigma thonningii* leaf was determined by the method described by Brand-Williams *et al.,* (1995) with some modifications. In this method, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was used as a free radical. A stock solution was prepared by dissolving 2.4 mg of DPPH free radical in 100 mL methanol. The solution was kept at 20ºC until required. The working solution was prepared by diluting the DPPH stock solution with methanol till the absorbance was noted to be 0.980 ± 0.02 at 517 nm. Then, 3 mL of the working solution was mixed with 100 µL of the ethanol and aqueous extract of the medicinal plant (1 mg/mL). After incubating the mixture in the dark for 30 minutes, absorbance was read at 517 nm. Ascorbic acid was used as a standard antioxidant in this method. The ability of DPPH radical scavenging activity was calculated by using the following formula:

$$DPPH\ scavenging\ effect\ (\%\ inhibition) = \frac{A\ control - A\ test}{A\ control} \times 100$$

The half-maximal inhibitory concentration (IC_{50}) of the extracts was computed from a plot of percentage DPPH free radical inhibition versus the extract concentration.

β-carotene Assay

The plant materials were analyzed for β-carotene according to the method described by Karau *et al.,* (2012). HPLC system fitted with a UV/VIS detector was used. The mobile phase consisted of 90:10, methanol: acetonitrile with 0.05 % (v/v) of triethanolamine (TEA). The flow rate was set at 2.5 mL/min, the oven temperature at 25°C, and the detector wavelength at 451 nm. β-carotene (2 mg) ≥ 93 % pure standard was dissolved in 10 mL absolute ethanol and actual concentration was determined spectrophotometrically. The wavelength of maximum absorbance was recorded and the mean absorbance and the molar extinction coefficient of β-carotene in absolute ethanol were used in the determination of the actual concentration of the working standard, and the 2560 m^2/mol as the molar extinction coefficient of β-carotene in absolute ethanol. Samples and the standards were analyzed in triplicates. The mobile phase for HPLC was prepared by mixing methanol; acetonitrile and tetrahydrofuran in the ratio of 70:25:5 (v/v), respectively. The mixture was sonicated to remove air bubbles. The extraction solution was prepared by mixing methanol and tetrahydrofuran in 50:50 (v/v), and it was also used as a blank. The Waters Spherisorb HPLC column was conditioned at an oven temperature of 25ºC, a flow rate of 1.0 mL/min, and wavelength 451 nm (β-carotene). The standard and the samples were analyzed in triplicates. Peak areas were used in the determination of the amounts. Single point calibration was used in quantization and the amounts were recorded as µg/100 g of dry matter.

α–tocopherol (Vitamin E) Assay

Two (2) grams of the plant slurry were suspended in 50 mL of methanol and into this suspension, 0.25 g of ascorbic acid and 5 mL of 50 % sodium hydroxide were added. The mixture was blanketed with nitrogen and saponified in a water bath at 60ºC for an hour with intermittent shaking after every 20 minutes. After saponification, the flasks were cooled in a running stream of cold water. Then, 50 mL of distilled water was added to the sample. α–tocopherol was extracted from the sample using 70 mL of n-hexane containing 30 mg/kg of BHT. The phases were allowed to separate and the aqueous phase drained to the round-bottomed flask and then n-hexane layer into a conical flask covered with aluminum foil. The procedure was repeated twice with 50 mL of n-hexane. The organic phase containing the α–tocopherol was evaporated in a rotary evaporator under reduced pressure and at a temperature below 50ºC. The resulting extract was dissolved in 10 mL of methanol, filtered and 10 µL was used in the determination of α–tocopherol by HPLC-UV/VIS method. The standard was prepared by dissolving 100 mg of α–tocopherol in 100 mL of absolute ethanol. The absolute concentration of the standard stock was determined by the use of a UV-VIS spectrophotometer at 291 nm. The molar coefficient of extinction of α–tocopherol in absolute ethanol is 75.6 m^2/mol. The concentration of the standard stock solution was determined from three (3) absorbance as detailed below;

The HPLC system was set as follows; flow rate of 1.2 mL/min, column oven temperature of 25ºC, injection volume of 10 µL, and run time of 12 minutes. The mobile phase consists of HPLC-grade water and methanol mixed in the ratio of 70:30 (v/v). For the fluorescence detector, the excitation and emission wavelengths were set at 290 and 330 nm respectively. Between the standards and samples, a blank comprising of filtered mobile phase was injected to prevent carryover. The standard working solution and the samples were injected three times and the mean peak area was used in the determination of the concentrations.

Ascorbic Acid (Vitamin C) Assay

Ascorbic acid was determined in the extracts as total L+ and D+ ascorbic acids in 2 % metaphosphoric acid using HPLC fitted with a UV/VIS detector. The plant material was extracted in 10 mL of 2 % metaphosphoric acid for an hour. The extraction was done in amber flasks covered with aluminum foil and sonicated at room temperature. The

extract was filtered with Whatman filter paper No. 540 and further filtered with 0.54 µm membrane, then filtered is ready for analysis with HPLC. The mobile phase comprised of 50 mM potassium dihydrogen phosphate at pH 7.4. It was filtered and then sonicated to remove air bubbles before use. The wavelength was set at 265 nm, flow rate at 2.0 mL/min, and oven temperature at 15ºC. A Phenomenex column (C18) 175 × 3.20 mm × 5 µm internal diameter was used. Ascorbic acid (99.7 % pure) was used as a standard. Serial dilutions of the ascorbic acid standard ranging from 0.4 to 5.3 mg/100g were used in the construction of the calibration curve. Then, 10 µL of each sample and the standard level was injected into the HPLC system and peak areas were recorded.

Preliminary Qualitative Analysis of Phytochemical Constituents

Preliminary qualitative analysis of phytochemical constituents was carried out to identify the secondary metabolites present in the ethanol and aqueous extracts of *Piliostigma thonningii* leaf. The qualitative analysis of the various phytochemicals was carried out by using Dragendroff's and Meyer's reagents (alkaloids). Other tests carried out include the modified Keller-Killiani test for cardiac glycosides, Lieberman Burchard's Test (steroids and triterpenes), Frothing test (saponins), ferric chloride test (tannins and Phenols), Shinoda test (flavonoids), Molisch test (carbohydrates), Fehling's test (glycosides) and Borntrager's test (anthraquinones) (Evans and Trease, 1996; Harborne, 1984).

Quantitative Analysis of Phytochemical Constituents

Determination of Alkaloids

Alkaloid was quantitatively determined according to the method of Obadoni and Ochuko (2001), and the percentage of total alkaloid content was calculated as:

$$\text{Total alkaloids (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Determination of Flavonoid

The total flavonoid content was quantitatively determined using the procedure described by Boham and Kocipai-Abyazan (1994), with slight modification by Ejikeme *et al.*, (2014). The total flavonoid content was calculated as:

$$\text{Total flavonoid (\%)} = \frac{\text{Weight of flavonoid}}{\text{Weight of sample taken}} \times 100$$

Determination of Saponin

Saponin quantitatively determination was carried out using the method described by Obadoni and Ochuko (2001) and the saponin content is calculated as a percentage:

$$\text{Total saponin (\%)} = \frac{\text{Weight of saponin}}{\text{Weight of sample taken}} \times 100$$

Determination of Tannin

Tannin content was determined using the method of Van-Burden and Robinson (1981) with slight alteration as illustrated by Kaur and Arora (2009), using tannic acid as standard. About 500 mg of the sample was weighed into a 50 mL plastic bottle, 50 mL of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. 5 mL of the diluted sample was pipetted out into a test tube and mixed with 2 mL of 0.10M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes. The quantification was carried out based on the 7-point standard calibration curve of tannic acid (20, 40, 60, 80, 100, 140, 200 mg/L) in distilled water. The tannin content was articulated as tannic acid equivalents (TAEs) in milligram per 100 g of the dry material.

Determination of Phenols

Phenols content was determined using the method of Keay *et al.*, (1964) with slight modification by Kaur and Kapoor (2002). Defatting of 2 g leaves powder sample was carried out for 2 hours in 100 mL of ether using a Soxhlet apparatus. The defatted sample (0.50 g) was boiled for 15 minutes with 50 mL of ether for the extraction of the phenolic components. Exactly 10 mL of distilled water, 2 mL of 0.1N ammonium hydroxide solution, and 5 mL of concentrated amyl alcohol were also added to 5 mL of the extract and left to react for 30 minutes for colour development. The optical density was measured at 505 nm. 0.20 g of tannic acid was dissolving in distilled water and diluted to the 200 mL mark (1 mg/cm^3) in preparation the for phenol standard curve. Varying concentrations (0.2–1.0 mg/mL) of the standard tannic acid solution were pipetted into five different test tubes to which 2 mL of NH_3OH, 5 mL of amyl alcohol, and 10 mL of water were added. The solution was made up to 100 mL volume and left to react for 30 minutes for color development. The optical density was determined at 505 nm.

Statistical Analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) test using a statistical package program (SPSS 10.0). The resulting data were represented as mean ± standard deviation of triplicate determinations. GraphPad Prism 9® (Version 9.0.1, GraphPad Software Inc., San Diego, United States of America) software was used for the graphical analysis.

RESULTS AND DISCUSSION

Result

The results below depict the phytochemical and *in vitro* antioxidant activity of aqueous and ethanol extracts of *P. thonningii* leaf. The qualitative phytochemical screening of the *P. thonningii* indicates the presence of carbohydrates, glycosides, cardiac glycosides, saponins, tannins, steroids, triterpenes, flavonoids, alkaloids, and anthraquinones (Table 1).

Table 1. Qualitative Phytochemical Screening of Aqueous and Ethanol Extracts of *P. thonningii* leaf.

Constituents	Aqueous	Ethanol
Carbohydrate	+	+
Glycoside	+	+
Anthraquinone	+	+
Cardiac glycoside	+	+
Saponin	+	+
Steroid	+	+
Triterpene	+	+
Tannin	+	+
Flavonoid	+	+
Alkaloid	+	+

Key: + Present, - Absent.

More so, the quantitative phytochemical screening of aqueous and ethanol extract of *P. thonningii* leaf revealed that alkaloids, flavonoids, saponins, tannins, and phenols were significantly ($P<0.05$) higher in ethanol extract compared to aqueous extract (Table 2).

Table 2. Quantitative Phytochemical Screening of Aqueous and Ethanol Extracts of *P. thonningii* Leaf.

Constituents	Aqueous (mg/100ml)	Ethanol (mg/100ml)
Alkaloid	2.1±0.01	2.4±0.04
Flavonoid	2.3±0.02	2.2±0.02
Saponin	3.2±0.02	3.4±0.02
Tannin	1.2±0.10	1.5±0.54
Phenol	2.3±0.34	2.4±0.60

Values were performed in triplicates and represented as means ± standard derivations ($P<0.05$).

Likewise, the *in vitro* antioxidant activity indicates that % DPPH inhibition was higher in ethanol and aqueous extracts compared to the reference antioxidant (ascorbic acid) (Figure 1 and 2). However, β-carotene, ascorbic acid, and α-tocopherol were significantly ($P<0.05$) higher in ethanol extract compared to aqueous extract (Figure 3, 4, and 5).

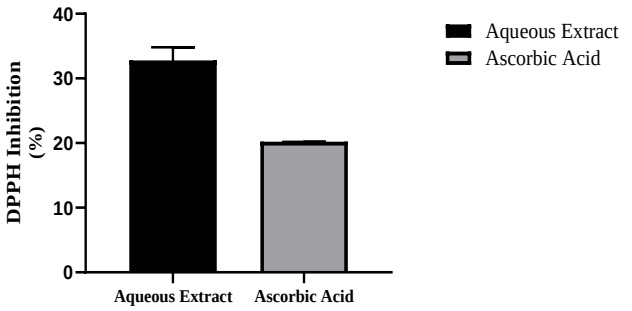

Figure. 1. Determination of DPPH Level of Aqueous Extracts of *P. thonningii* Leaf.

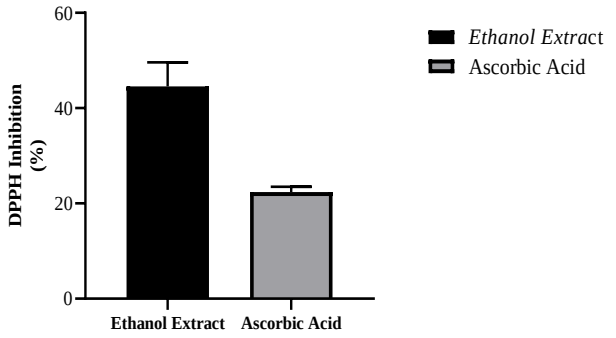

Figure 2. Determination of DPPH level of Ethanol Extracts of *P. thonningii* leaf**.**

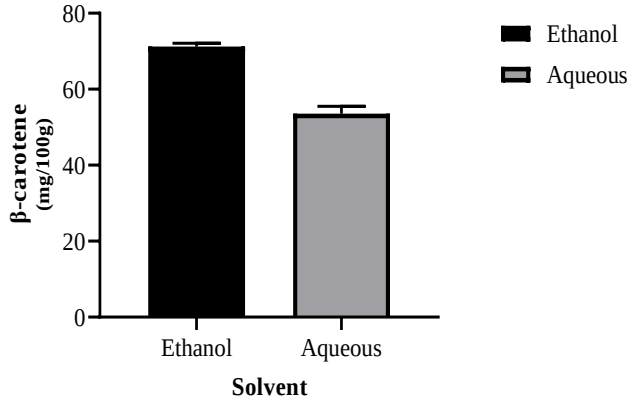

Figure 3. Determination of β-carotene Level of Aqueous and Ethanol Extract of *P. thonningii* Leaf.

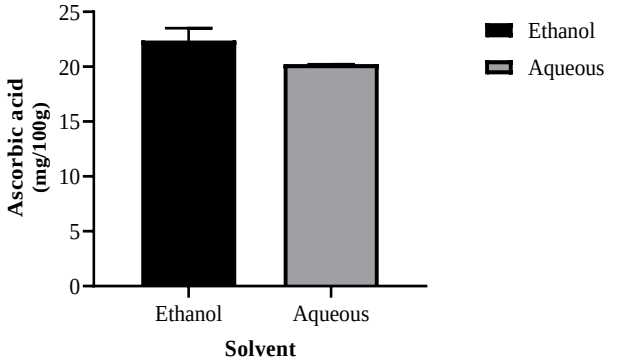

Figure 4. Determination of Ascorbic acid (Vitamin C) Level of Aqueous and Ethanol Extracts of *P. thonningii* Leaf.

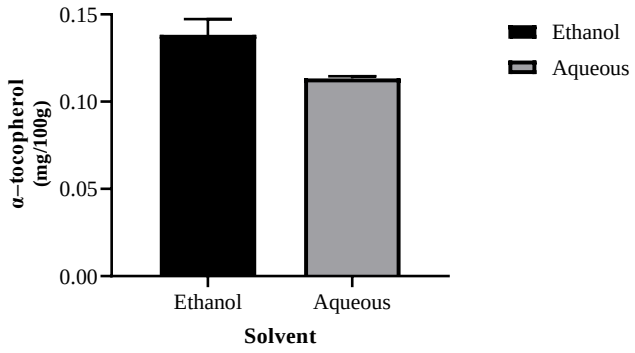

Figure 5. Determination of α-tocopherol (Vitamin E) Level of Aqueous and Ethanol Extracts of *P. thonningii* Leaf.

DISCUSSION

Phytochemicals are bioactive compounds and are responsible for the definite physiological effects exerted on the human body by various parts of the plant (Ighodaro *et al*., 2009). The result of the phytochemical screening of the *P. thonningii* extracts indicates the presence of carbohydrates, glycosides, cardiac glycosides, saponins, steroids, triterpenes, tannins, flavonoids, alkaloids, and anthraquinones. The presence of these secondary metabolites in *P. thonningii* leaf is, therefore, a strong indication that the leaves possess valuable medicinal properties which are yet to be explored. Saponins exhibit cytotoxic effect on cancer cells through induction of apoptosis, protects against microbial attack in plants; it is also useful in treating yeast and fungal infections (Moses *et al*., 2014; Elekofehinti *et al*., 2021). Saponins have hypolipidemic properties as they lower cholesterol and low-density lipoprotein levels and may be helpful in the treatment of dyslipidemia (Ejelonu *et al*., 2017). Flavonoids are found in plants, and thus commonly consumed in the diets of humans (Delage, 2015). This study revealed that the ethanol and aqueous extracts of *P. thonningii* leaf contain appreciable quantities of saponins and flavonoids. It has been reported to exert multiple biological effects including decreased risk of cardiovascular diseases, antiviral, antibacterial, antitoxic, anticancer (breast and prostate cancers), anti-inflammatory activities, and improved endothelial function thereby reducing blood pressure (Wang *et al*., 2014; Delage, 2015; Rodriguez-García *et al*., 2019).

Tannin is one of the major active ingredients found in plant-based medicines, and polyphenolic biomolecules that bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids. Tannins are known to possess antiviral, antibacterial, and antitumor activity (Khanbabaee and van Ree, 2001). Therefore, the ethanol and aqueous extracts of *P. thonningii* leaf have potential in the provision of tannin. Cardiac glycoside has been used in the treatment of congestive heart failure due to its direct action which increases the force of myocardial contraction (Braunwald *et al*., 1961). The aqueous and ethanol extracts of *P. thonningii* leaf in this study were shown to contain glycosides which could be exploited for their medicinal properties. Phenols are antioxidants in humans and plants (Dillard and German, 2000). The aqueous and ethanol extracts of *P. thonningii* leaf may be exploited for their glycosides contents.

Alkaloids consist of chemical compounds that contain mostly basic nitrogen atoms which occur naturally, mainly, in plants but may be produced by bacteria, fungi, and animals (Sodipo *et al*., 2000). In this study, it is revealed that alkaloids are present, thus suggesting that the plant extracts could serve as a firm means of affording alkaloids. Alkaloids have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine), antibacterial (e.g., chelerythrine), and antihyperglycemic activities (e.g., piperine) (Cushnie *et al*., 2014; Kittakoop *et al*., 2014; Qiu *et al*., 2014). The phytochemicals result obtained from this study is in agreement with previous studies by Egharevba and Kunle (2010), Haliliu *et al*., (2017), Ibrahim *et al*., (2019), Marquardt *et al*., (2020), and Boualam *et al*., (2021) on different extracts of the leaves of the plant.

From this study, the leaf of *P. thonningii* has been found to be rich in ascorbic acid, α-tocopherol, and β-carotene as revealed in the results. There is an increasing body of evidence that natural antioxidants such as ascorbic acid, α-tocopherol, and β-carotene protect the body against a number of degenerative diseases such as atherosclerosis, aging, and certain types of cancer (Pratt, 1990). The substantial level of these molecules in *P. thonningii* leaf is an indication of the plant's nutritional and medicinal significance. More so, free radical scavenging activity was assessed and the experimental method used to evaluate this activity was by determining the efficiency of *P. thonningii* to

scavenge DPPH radicals. This method is based on the reduction of DPPH, a stable free radical that accepts electrons of hydrogen radical to become stable diamagnetic molecules (Bijaya and Bikash, 2013). Freshly prepared DPPH solution exhibits purple colouration. When an antioxidant is present in the medium, it donates an electron or hydrogen atom to the radical resulting in scavenging of the radical by hydrogen atom rendering the formation of a colourless complex (Thambiraj and Paulsamy, 2012; Sumathy *et al*., 2013). The degree of discolouration is measured to evaluate the antioxidant activity (Shah *et al*., 2010). From the results, it is shown that both the ethanol and aqueous extracts of *P. thonningii* leaf exhibited strong antioxidant activity when compared to the reference antioxidant (ascorbic acid) as previously demonstrated by Vivek *et al*. (2013), Abdel-Farid *et al*. (2014), Patil *et al*. (2010) and Guchu *et al*. (2020). It was observed that the ethanol extract has the highest activity than the aqueous extract. This is because the ethanol extract has the least IC_{50} value, the lower the IC_{50} value of a compound, the higher its radical scavenging activity (Maisuthisakul *et al*., 2007). However, the half-maximal (IC_{50}) value for both extracts were lower than 50 mg/ml, which according to Fidrianny *et al*. (2015), rendered them very strong. This is an indication that the plant possesses *in vitro* antioxidant effect.

CONCLUSION

This study suggests that the *P. thonningii* leaf possesses phytochemical constituents and antioxidant effects especially the ethanol extract of the plant. The outcome of this study is supportive of the medicinal uses of *P. thonningii* leaf and its ability to prevent oxidative damage.

AUTHORS' CONTRIBUTIONS

This research idea was conceived by SGU, and the experiments were performed under the close supervision of OOD, who also performed the interpretation and analysis of data. All authors reviewed and approved the final manuscript for publication.

FUNDING STATEMENT

None

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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