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PHYTOCHEMICAL, *IN VITRO* ANTIMICROBIAL, ANTIOXIDANT PROPERTIES AND POLYPHENOLIC CONTENT OF METHANOL LEAVES EXTRACT OF *Piliostigma thonningii* SCHUM MILNE-REDHEAD (FABACEAE)

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ABSTRACT

Piliostigma thonningii leaves are used in Nigeria folk medicine for the treatment and management of diverse ailments. This study evaluated the phytochemical composition, polyphenolic content, antioxidant and antimicrobial activities of the crude extract and fractions with a view to validating its folkloric usage. While the phytochemical screening was performed using standard procedures, the phenolic, flavonoid, antioxidant and antimicrobial screening were performed using the Folin-Ciocalteu, aluminum chloride, DPPH free radical scavenging and agar well diffusion methods respectively. Qualitative phytochemical analysis showed the presence of important metabolites in the crude methanol extract and its fractions. While the ethyl acetate fraction at the highest concentration of 1 mg/mL produced the best ($p<0.05$) antibacterial activity, the n-hexane fraction had the least. The extract and its fractions showed no sensitivity against the selected fungi. Using gallic acid and quercetin equivalent plots, the crude extract contained a significantly higher concentration of total phenolic and flavonoid contents when compared with other fractions. The standard antioxidant (ascorbic acid) had superior DPPH antioxidant capacity when compared with the extract and its fractions. The ethyl acetate fraction had the least IC_{50} value when compared to other fractions. However, there was no significant correlation between the DPPH antioxidant activity and the polyphenolic contents in the extract and fractions. Our results provide evidence that the plant extract and fractions exhibited marked antibacterial and antioxidant activities thus justifying the popular use of the plant leaves to treat selected microbial infections and free radical implicated illnesses.

KEYWORDS: **Antioxidant; Antimicrobial; Phytochemical; *Piliostigma thonningii*; Polyphenol.**

INTRODUCTION

Traditional medicine has received global attention because plants produce a large number of secondary metabolites which offer defense and protection from various diseases [1]. This is why the continuous screening and scientific evaluation of medicinal plants for their claimed efficacy is not only important but could also serve as a source of novel drugs and therapeutic agents [2,3]. The increase in global chemotherapeutic failure could be attributed to the antimicrobial toxicity and undesirable side effects of the present antibiotics employed to challenge the present and emerging infections. There is therefore need for an extensive

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investigation to discover new and better drug candidates [4]. Medicinal plants have played crucial roles in shaping human health care and improving global health for thousands of years as affirmed by the World Health Organization report that claimed that over 80% of the world population rely on traditional medicine for some aspect of their primary health care needs [5-7].

Although human and aerobic organisms possess natural antioxidant defense systems which prevent oxidative damage, they are grossly inadequate on their own hence the nutritional consumption of antioxidants is highly recommended [8-10]. Natural antioxidants specifically from plant sources have been shown not only to be potent in eliminating or neutralizing free-radicals, they also act as reduction agents, pro-oxidant metal complexes and singlet oxygen quenchers [11]. They are also safer compared to synthetic antioxidants since the later has been implicated to have carcinogenic properties. There is therefore an upsurge in the scientific search for potential antioxidants with reduced side effects and economic viability [12]. Natural antioxidants protect to a greater extent the human body from free radicals hence delay the progression of many chronic illnesses like cancer, heart diseases and stroke, among others. They also boost the plasma's antioxidant ability and prevent lipid oxidative rancidity in foods [13,14].

The West African plant *Piliostigma thonningii*, Schum (Milne-Redhead) belongs to the subfamily Caesalpinioideae with about 133 genera [15]. It belongs to the legume family, Leguminosae/Fabaceae comprising of trees, shrubs or very rarely scramblers. The plant (Figure 1) is mainly found in open and moist woodlands and in savannah regions as well as wooded grasslands in low to medium altitudes [16,17]. The plant is perennial in nature, its white or pink-colored flowers are produced around November and December. The twigs are hairy while the bark is rough, longitudinally fissured as well as creamy brown in color. It has a leathery leaf of up to 15 x 17 cm, bilobed one eight to one third the way down with a small bristle notch, glossy above and heavily veined and somewhat rusty hair below. It bears hairy flat-pod fruits that turn nasty-brown and woody on maturity and usually persist on the plant until around June to September [17]. It is commonly known as monkey's bread or camel's foot tree. Locally, it is known as kalgo (Hausa), kalur (Kanuri) and inpilataki (Higgi). It has been used for the treatment of a variety of diseases in traditional medicine. The root and twig of the plant are used for the trado-medical treatment of dysentery, fever, respiratory ailments, snake bites, hookworm and skin diseases, gastrointestinal tract (GIT) problems [16-19]; the stem is used in the management of dysentery, pile and male erectile dysfunction [20] while the leaves are used for the treatment of inflammation, bilharzia, eye diseases, catarrh [21], malaria fever [22,23], wounds, chronic ulcers, diarrhea, toothache, gingivitis, cough and bronchitis [24]. The decoction of the leaves and bark has been used for the treatment of heart pain, arthritis, pyrexia, leprosy, sore throat, toothache, gingivitis, cough and bronchitis among others [25,26]. The previous investigation on the leaves of the plant resulted in the isolation of important pharmacological metabolites D-3-o-methylchiroinosital established to possesses anthelmintic activity [27], analgesic, antipyretic, antidiabetic, antioxidant, and antilipidemic activities [28,29], and C-methyl flavanols revealed to possess antibacterial and anti-inflammatory activities [25]. Based on the ethnopharmacological relevance of the plant and in the absence of any scientific report, we designed this study to evaluate and compare the phytochemical, *in vitro* antimicrobial and DPPH free radical scavenging activities as well as the polyphenolic (total phenolic and flavonoid) contents of the methanol extract and its n-hexane, ethyl acetate, n-butanol and aqueous fractions of the leaves of *P. thoninngii* found in Sokoto North-Western Nigeria.

MATERIALS AND METHODS

Chemicals and standards

Gallic acid, Folin-Ciocalteu reagent, Ferric chloride hexahydrate ($FeCl_3.6H_2O$), and DPPH were obtained from Merck (Darmstadt, Germany) and ascorbic acid was purchased from Fisher (Pittsburgh, USA). Methanol, n-hexane, ethyl acetate and n-butanol (Sigma-Aldrich, USA) were used for solvent extraction. Other chemicals and reagents used in this study were of analytical grade and were freshly prepared in the laboratory according to official specifications.

Plant material collection, authentication and preparation

The plant used in this study was identified through the ethnobotanical approach. The information on its use and preparation in the Hausa community in Sokoto metropolis Nigeria was obtained from local herbalists. Healthy and mature plant leaves were collected openly from Jabo district in Tambuwal local government area of Sokoto State during the flowering period (April/June) 2019. The taxonomic identification of the plant sample was done by

Mallam Abdulaziz Salihu of Botany Unit, Department of Biological Sciences, Faculty of Science, Usmanu Danfodiyo University Sokoto, Sokoto State Nigeria where sample herbarium specimen was deposited and voucher number UDUH/ANS/0137 issued. The plant materials were thoroughly rinsed with running tap water to remove the dirt and specks of dust before been shade dried at room temperature for 2 weeks. The dried leaves were reduced to fine powder using an electric mill machine, sieved using a fine sieve to obtain a uniform size and stored in an airtight glass container until ready for extraction.

Extraction and fractionation of plant materials

A portion of the plant material (2 kg) was cold macerated with absolute methanol (4 × 2.5 L) at room temperature for 72 hours. The mixture was filtered using filter paper and concentrated using a rotary evaporator within the controlled temperature of (45 - 50ºC) and stored in a pre-cleaned well-labeled container to obtain the crude methanol extract. A portion of the methanol crude extract (MCE) was further fractionated by liquid-liquid partitioning using 2 × 2.5 L n-hexane, ethyl acetate and n-butanol in succession to yield the n-hexane fraction (NHF), ethyl acetate fraction (EAF), n-butanol fraction (NBF) and residual aqueous fraction (RAF) respectively. All fractions were filtered, concentrated, kept in a measured well-labeled glass container, and stored in the freezer at -4ºC. The percentage yield of the extract and all the fractions was determined.

Phytochemical screening

Phytochemical screening was carried out in accordance with standard methods [30].

Determination of total phenolic content

The total phenolic content in the extract and fractions was determined according to the Folin-Ciocalteu procedure [31] with slight modifications. Each solution of the extract and fractions (0.5 ml) with a concentration of 1000 µg/ml was added to 4.5 ml of deionized distilled water and 0.5 ml of Folin Ciocalteu's reagent (previously diluted with deionized water (1:10 v/v). The mixed resultant solution was maintained at room temperature for 5 minutes followed by the addition of 5 ml of 7% sodium carbonate and 2 ml of deionized distilled water. The thoroughly mixed samples were incubated for 90 minutes at 23°C. The absorbance was measured by a spectrophotometer set at 750 nm. The total phenolic content (TPC) was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract/fraction. Gallic acid was used as the positive control. The standard curve was prepared by using gallic acid in five different concentrations (50, 100, 150, 250 and 500 mg/L).

Determination of total flavonoid content (TFC)

The total flavonoid content in the plant extract and fractions was determined by the aluminum chloride method [32]. Each of the solutions (0.5 mL) of the extract/fractions (5 g/L) was mixed with 1.5 ml of methanol, and then 0.1 ml of 10 % aluminum chloride was added, followed by 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 min. The absorbance was measured by a spectrophotometer set at 415 nm. The results were expressed as milligrams quercetin equivalents per gram of extract (mg QE/g dry extract). Quercetin was used as positive control and the standard curve was prepared by using quercetin in different concentrations (12.5, 25, 50, 80, and 100 mg/L).

Determination of DPPH free radical scavenging activity

The ability of plant extract and fractions to scavenge 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals was estimated as previously described [33] with slight modifications. Each of the solutions of the extract and fractions (3 ml) with five different concentrations (12.5, 25.0, 50.0, 100.0, and 200 µg/ml) was mixed with 1 ml of a 0.1 mM methanol solution of DPPH. The absorbance was measured with a spectrophotometer set at 517 nm at 30 minutes intervals against a blank (pure methanol). The percentage of radical scavenging activity was calculated using the formula:

$$\text{DPPH radical inhibition (\%)} = [1 - (A_{test} / A_{control})] \times 100$$

Where $A_{control}$ is the absorbance of the control and A_{test} is the absorbance of the tested extract/fraction at the end of the reaction.

Ascorbic acid was used as positive control with the same concentrations. The antioxidant activity of the sample was also expressed in terms of IC_{50} value, which represents the concentration of the sample required to inhibit 50% formation of DPPH radical.

Antimicrobial assay

Antibacterial and antifungal screening of the extracts was carried out using the agar well diffusion method previously described [34,35]. The extract and fractions were challenged with confirmed laboratory strains of *Staphylococcus aureus; Bacillus subtilis; Pseudomonas aeruginosa; Salmonella species;*

Klebsiella pneumoniae, Candida albicans, and *Aspergillus niger* procured from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University Awka Anambra State. A concentration of 1 mg/mL was prepared for the crude extract and fractions by dissolving them in 100% DMSO. A volume of 20 mL of molten Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) (for the antibacterial and antifungal tests respectively) were poured into sterile Petri plates (90 mm) and were allowed to set. Standardized concentrations (McFarland 0.5) of overnight cultures of test isolates were swabbed aseptically on the agar plates and holes (6 mm diameter) were made in the agar plates using a sterile metal cork-borer. A volume of 20 µL of the extract, fractions and controls were put in each hole under aseptic condition, kept at room temperature for one hour to allow the agents to diffuse into the agar medium, and then incubated accordingly. For positive controls, ciprofloxacin (5 µg/mL) and miconazole (50 µg/mL) were used in the antibacterial and antifungal tests respectively; while 100% DMSO was used as the negative control in both tests. The MHA plates were then incubated at 37^{o}C for 24 h while the SDA plates were incubated at room temperature (28^{o}C) for 3 days. The inhibition zone diameters (IZDs) were measured and recorded. The size of the cork borer (6 mm) was deducted from the values recorded for the IZDs to get the actual diameters. The procedure was conducted in triplicate, and the mean IZDs calculated.

Determination of minimum inhibitory concentration (MIC)

The MICs of the active extract/fractions were determined by the agar dilution method described above with some modifications. The MIC was determined for the microorganisms that showed reasonable sensitivity to the test extract and fractions. Stock solution (4,000 mg/mL) of the active extract and fractions were made followed by 2-fold serial dilutions to get graded concentrations (2,000, 1,000, 500, 250 mg/mL) of the extract and fractions. Agar plates were prepared by pouring 9 mL of molten double strength MHA into sterile Petri plates containing 1 mL of the various dilutions of the extract making the final plate concentrations to become 400, 200, 100, 50, and 25 mg/mL respectively. The test isolates which were grown overnight in broth were adjusted to McFarland 0.5 standard and streaked onto the surface of the agar plates containing dilutions of the extract. The MHA plates were then incubated at 37°C for 24 hours after which all plates were observed for growth. The minimum dilution (concentration) of the extracts completely inhibiting the growth of each organism was taken as the MIC. Each experiment was performed in triplicate [36].

Statistical analysis

Results obtained in triplicates were expressed as means ± standard error of the mean (SEM). Statistical comparisons were made using the student t-test, one-way analysis of variance (ANOVA) using SPSS statistics 17.0 software package. The confidence limit was set at $p < 0.05$.

RESULTS

The results of the extractive yield of the MCE as well as fractions after liquid-liquid fractionation of a portion of the crude extract are presented in Table 1 using 2.00 kg powdered leaf sample of *P. thonningii* after 72 h of cold maceration in methanol.

Preliminary phytochemical analyses showed the presence of major classes of secondary metabolites as presented in Table 2.

The total phenolic and flavonoid contents of the methanol crude extract and its fractions were determined from the gallic acid (R^2 = 0.9919) and quercetin (R^2 = 0.9866) calibration plots respectively. The results showed that while the MCE had the highest flavonoid and phenolic contents of 251.406 mgQE/g extract and 317.413 mgGAE/g extract respectively, the ethyl acetate had the highest polyphenolic contents of 151.404 mgQE/g fraction and 239.19 mgGAE/g fraction compared to the other fractions as presented in Figure 2.

The radical scavenging property of the MCE and its NHF, EAF, NBF and RAF fractions at the highest investigated concentration of 200 µg/mL are 82.82, 63.19, 92.06, 72.17 and 89.11% respectively as represented in Figure 3. Their 50% inhibition concentration values as shown in Table 3 are in the order EAF < RAF < MCE < NBF < NHF. Statistically significant differences were observed in the DPPH radical scavenging activities of the extract and fractions compared to the reference standard (ascorbic acid). Figures 4 and 5 show lines of best fit for both total phenolic and flavonoid contents versus the IC_{50} obtained from the DPPH scavenging assay. There was thus no significant correlation between the DPPH antioxidant activity and the phenolic and flavonoid contents in the plant extract and fractions.

The results presented in Tables 4 and 5 show the *in vitro* susceptibility of the microbial isolates and the MIC of the crude extract and fractions of *P. thonningii* in comparison to ciprofloxacin (5 µg/mL) and miconazole (50 µg/mL) standards respectively. The EAF exhibited a better microbial inhibition compared

Figure 1: *Piliostigma thonningii* in its natural habitat.

Table 1: Percentage yield of crude extract and fractions of *P. thonningii*

Extract/Fractions	Percentage Yield
MCE*	13.63
NHF**	1.80
EAF**	31.40
NBF**	16.90
RAF**	47.30

Key: MCE = methanol crude extract; NHF = n-hexane fraction; EAF = ethyl acetate fraction; NBF = n-butanol fraction; RAF = residual aqueous fraction; * = crude extract; ** = fractions obtained from MCE.

Table 2: Phytochemical screening of *P. thonningii* leaves crude extract and fractions

s/n	Phytoconstituents	Extract/Fractions				
		MCE	NHF	EAF	NBF	RAF
1	Alkaloid	+	-	+	+	+
2	Saponin	+	-	+	-	+
3	Tannin	+	-	+	-	+
4	Flavonoid	+	+	+	+	+
5	Steroid/Phytosterols	-	-	-	-	-
6	Terpenoid	+	+	+	+	+
7	Phenols	+	+	+	+	+
8	Protein	+	-	+	-	+
9	Cardiac glycoside	+	-	+	+	+
10	Reducing sugar	+	-	+	-	+
11	Carbohydrate	+	+	+	+	+

Key: MCE = methanol crude extract; NHF = n-hexane fraction; EAF = ethyl acetate fraction; NBF = n-butanol fraction; RAF = residual aqueous fraction; + = present; - = absent .

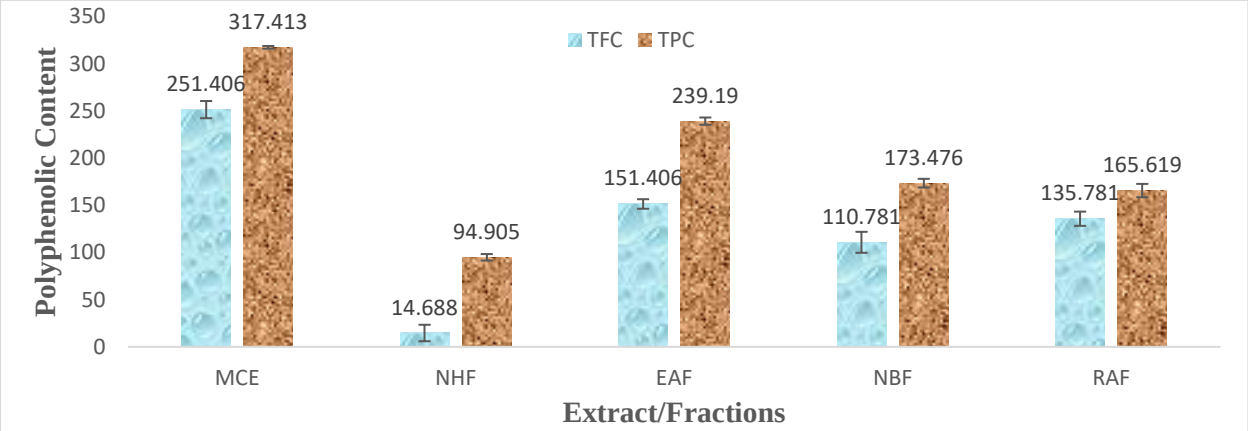

Figure 2: Polyphenolic content of crude extract and fractions of *P. thonningii*.
Key: MCE = methanol crude extract; NHF = n-hexane fraction; EAF = ethyl acetate fraction; NBF = n-butanol fraction; RAF = residual aqueous fraction.

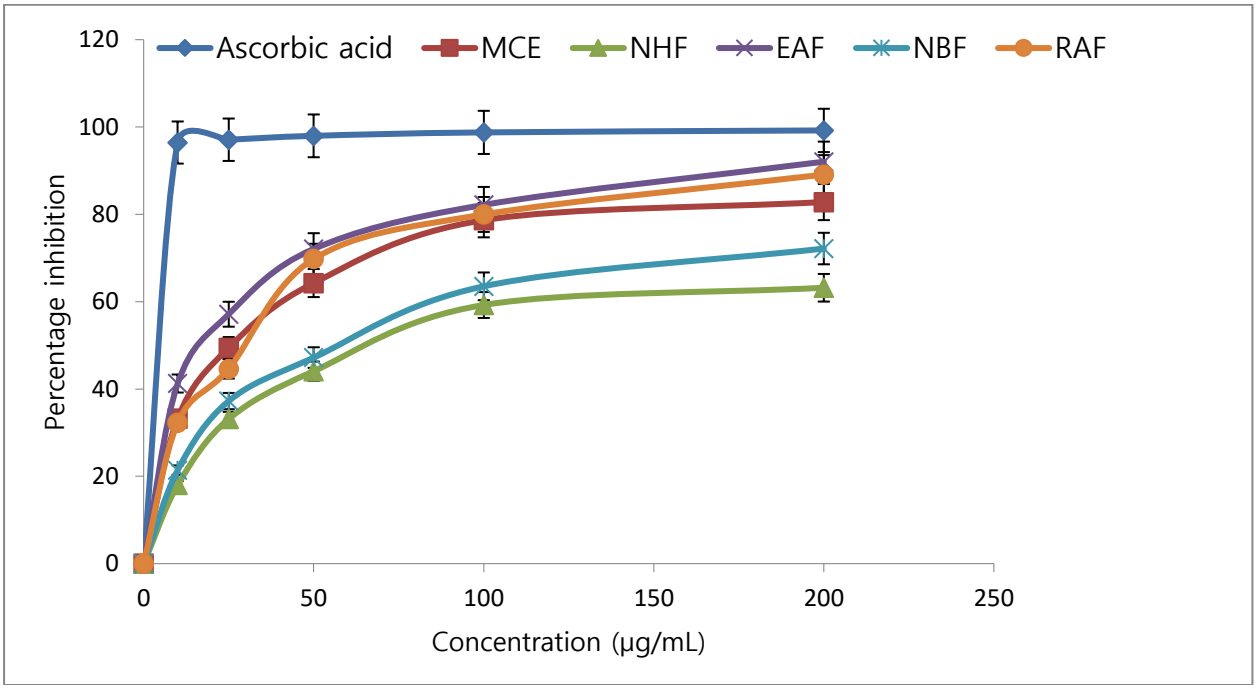

Figure 3: DPPH radical scavenging activity of crude *P. thonningii* extract, its fractions and the synthetic antioxidant (ascorbic acid) at different concentrations. Each point is a mean from triplicate measurements.

Table 3: 50% inhibitory concentration of crude *P. thonningii* extract, its fractions and the synthetic antioxidant (ascorbic acid)

Sample	IC_{50} value (µg/mL)
AA	5.42± 0.04
MCE	24.49± 0.10**
NHF	71.97± 0.12***
EAF	15.90± 0.14*
NBF	52.72± 0.37***
RAF	24.89± 0.07**

Data represent mean ± standard error of mean of triplicate analysis. * = P < 0.05, ** = P < 0.01, *** = P < 0.001 compared to ascorbic acid (standard).

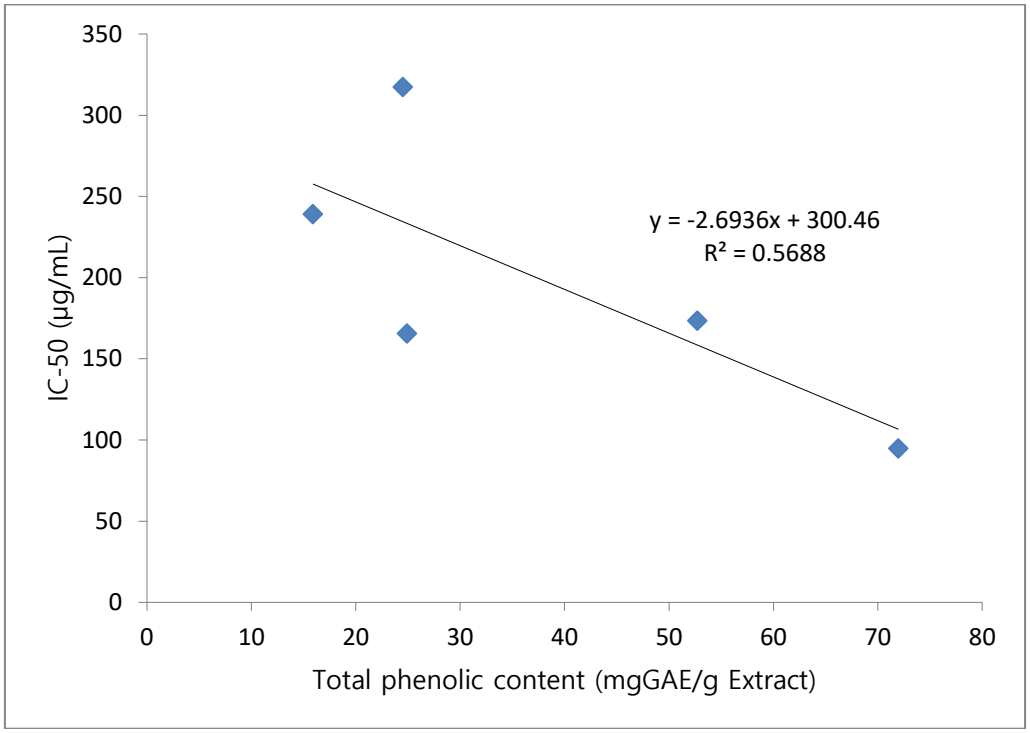

Figure 4 : Corrélation of phenolic content with IC_{50} values.

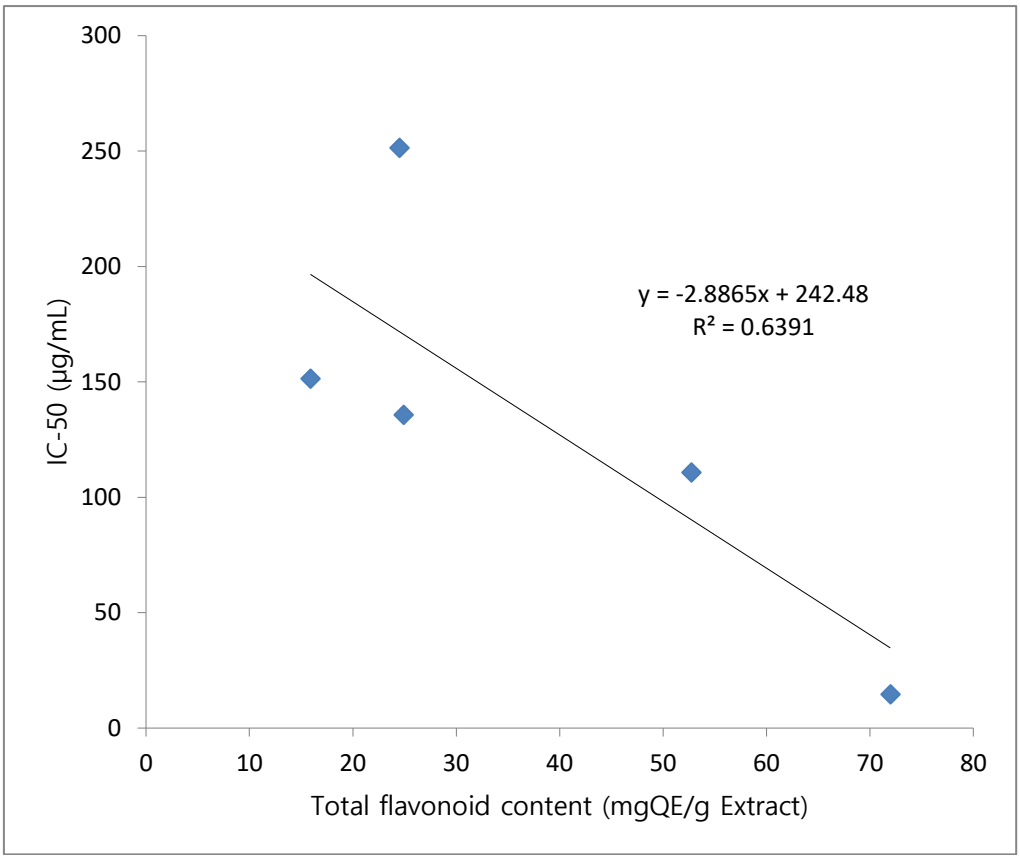

Figure 5: Corrélation of flavonoid content with IC_{50} values.

Table 4: Result of antimicrobial activities (inhibition zone diameter) of the crude extract and fractions (1.00 mg/mL) of ***P. thonningii***

Microorganisms	IZDs (mm) at 1.00 mg/mL						
	Extract/Fractions					Positive Control	Negative Control
	MCE	NHF	EAF	NBF	RAF	Ciprofloxacin (5 µg/mL)	DMSO
S. aureus	6±0.7[a]	0	7±0.7[a]	0	0	15±0.7[b]	0
B. subtilis	5±0[a]	3±0[a]	5±1.4[a]	0	4±0.7[a]	17±0[b]	0
E. coli	7±0[b]	4±1.4[a]	9±0.7[b]	5±0[a]	5±0.7[a]	18±0.7[c]	0
P. aeruginosa	6±0.7[b]	2±0[a]	8±0.7[b]	3±0[a]	3±0[a]	11±0[c]	0
Sal. spp	8±0.7[a]	5±0.7[a]	7±0[a]	5±0[a]	6±0[a]	15±0.7[b]	0
K. pneumoniae	6±0.7[a]	4±0.7[a]	7±0.7[a]	4±0.7[a]	5±0.7[a]	15±0.7[b]	0
						Miconazole (50 µg/mL)	
C. albicans	0	0	0	0	0	14±0	0
A. niger	0	0	0	0	0	11±0.4	0

Key: *Staphylococcus aureus; Bacillus subtilis; Escherichia coli; Pseudomonas aeruginosa; Salmonella species; Klebsiella pneumoniae; Candida albicans; Aspergillus niger*; MCE = methanol crude extract; NHF = n-hexane fraction; EAF = ethyl acetate fraction; NBF = n-butanol fraction; RAF = residual aqueous fraction. Values are presented as mean ± standard error of mean of three determinations; values with different superscript in the same row are significantly different at $p < 0.05$.

Table 5: Result of the minimum inhibitory concentration of the methanol crude extract and fractions of *P. thonningii*

Test organisms	Minimum Inhibitory Concentration (mg/mL)				
	MCE	NBF	RAF	NHF	EAF
S. aureus	>1.00	>1.00	>1.00	0.5	0.5
B. subtilis	1.00	>1.00	0.5	1	0.5
E. coli	0.13	0.13	0.13	0.13	0.13
P. aeroginosa	1	1	0.5	1	0.25
Sal. spp	0.13	0.5	0.25	0.25	0.13
K. pneumoniae	0.5	1	1	1	0.5

Key: *Staphylococcus aureus; Bacillus subtilis; Escherichia coli; Pseudomonas aeruginosa; Salmonella species; Klebsiella pneumoniae; Candida albicans; Aspergillus niger*; > = greater than; MCE = methanol crude extract; NHF = n-hexane fraction; EAF = ethyl acetate fraction; NBF = n-butanol fraction; RAF = residual aqueous fraction.

to the extract and fractions at the highest investigated concentration of 1.00 mg/mL for all the microorganisms except for *Salmonella spp* where the MCE had a better inhibitory activity. There were significant differences between the IZDs of the standard antibiotics and the extract and fractions. The extract and fractions had no antifungal activity when tested on *C. albicans* and *A. niger*.

DISCUSSION

The 13.63, 1.80, and 31.40% extractive yields obtained in this study of methanol, n-hexane, and ethyl acetate are lower than the 16.82 and 2.09% for methanol and n-hexane but greater than 0.71% of ethyl acetate reported by Halilu *et al*. [20]. The extractive yields of solvents are due to their different polarities which lead to differences in the extracted bioactive compounds [37,38]. Crude extracts contain complex mixtures of biologically active compounds some of which might exhibit genotoxic or antigenotoxic effects [39]. It therefore becomes imperative to carry out phytochemical analysis to know the potential health hazards that may occur as a result of using the plant extracts for medicinal purposes [40]. Preliminary screening of medicinal plants for their phytoconstituents is also a valuable step in the detection of the bioactive principles which are often explored in drug discovery and development [41]. The extract and fractions of the leaf of *P. thonningii* reveal the presence of various phytochemicals such as carbohydrates, reducing sugar, glycosides, proteins, alkaloids, flavonoids, steroids, terpenoid, phytosterols, tannins, saponins and phenols in the investigated extract and fractions (Table 2). The results agree with the findings by Dluya et al. [24] and Ighodaro *et al*. [26] for the presence of phenols, alkaloids, flavonoids, saponins, steroids and tannins in the leaf extracts of *P. thonningii*. The findings are also in total agreement with the findings of Ewansiha *et al*. [42] for the n-hexane and aqueous fractions except for steroids and glycosides observed for the n-hexane extract as well as flavonoid and glycosides for the aqueous extract. The result also agreed with Halilu *et al*. [20] for the presence of alkaloid, saponin, tannin and glycosides in the methanol, n-hexane and ethyl acetate extract and fractions, while disparity existed in the report of steroids and flavonoids among the extract and fractions. This disparity could be attributed to factors like ecotype, chemotype, phenophases, variations in environment conditions such as location of the plant, temperature, relative humidity, irradiance and photoperiod as well as genetic background all of which affect the secondary metabolites of plants [43-45].

The use of natural antioxidants in the management of disorders associated with oxidative stress is becoming an attractive alternative in recent times [46,47]. Phenolic compounds are known to scavenge radicals such as singlet oxygen, superoxide, and hydroxyl because of their phenolic hydroxyl groups [48-51]. They have redox properties that allow them to act as antioxidants [20]. Flavonoids, on the other hand, are a group of plant polyphenolic compounds ubiquitous in photosynthesizing cells possessing broad biological properties notably involved in uncontrolled lipid peroxidation. The capability to interact with protein phosphorylation, iron chelating and free radical scavenging activity may account for the wide pharmacological profile of flavonoids [52]. Flavonoids and other plant phenolics such as phenolic acids, stillbenes, tannins, lignins among others are especially common in leaves, flowering tissue, and woody parts such as stems and barks. The antioxidant activities of plant extracts which contain various kinds of compounds are due to their abilities to be donors of hydrogen atom or electrons and thus able to capture free radicals [53] which are implicated in various pathological conditions such as cancer, cardiovascular disorders, arthritis, inflammation and liver diseases [54]. DPPH is a stable free radical in aqueous, methanol and ethanol solutions and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH is a stable free radical with purple color, which changes into a stable yellow compound on reacting with an antioxidant. The extent of the reaction depends on the hydrogen-donating ability of the antioxidant. The concentration of the antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure antioxidant activity. The role of medicinal plants in decreasing free radical–caused tissue injury reveals their antioxidant activity [13].

The results of the DPPH, TFC, and TPC content showed that the methanol crude extract exhibited greater radical scavenging activity as well as a higher polyphenolic content, compared to the fractions. This is in agreement with the findings of Pinelo *et al*. [55] who reported methanol to be specifically effective in extracting polyphenols because of its polarity. The IC_{50} values obtained were lower for MCE and EAF but higher for NBF than those reported by Aderogba *et al*. [56] (Table 3). There was no appreciable correlation of the polyphenolic contents of the extracts with their antioxidant activities (Figures 4 and 5) in agreement with the findings of several researchers [57,58]. This suggested that the antioxidant activity of the plant

extracts may also be partly due to some other polyphenolics such as anthocyanidins, carotenoids, steroids among others. This agreed with the report of several researchers who stated that plants with high contents of phenolics and flavonoids had high antioxidant activities [59-64]. The order of the phenolic and flavonoid contents among the extract and fractions was in agreement with the findings of Arya and Yadav [65] and Priyadharshini and Sujatha [66] respectively who reported the order to be methanol (polar) > ethyl acetate > aqueous > chloroform > hexane (non-polar). The superior antioxidant activity of the ascorbic acid standard compared to the methanol extract and fractions may probably be due to its higher purity [10].

The antibacterial activity test as depicted by the inhibition zone diameter was seen to be concentration-dependent. The reported antimicrobial activity for the extract and fractions may be due to the presence of the detected phytochemicals (Table 2) especially terpenoids, which have been shown to possess antibacterial, antifungal, and antitubercular activities [67]. Their reported mechanism of action is proposed to involve the disruption of the membrane by the lipophilic compounds [68]. Other phytochemicals like phenols, flavonoids, tannins, saponins, cardiac glycosides among others that were all detected in the extract and fractions have been shown to have antibacterial, anthelmintic, and antineoplastic activities [69]. While saponins are surface-active agents which alter the permeability of the cell wall of organisms thus facilitating the entry of toxic materials or leakage of vital constituents from the cell [70], tannins are reported to exhibit antibacterial, antiviral and antitumor activities [71, 72] and alkaloids reported to possess antibacterial, antiarrhythmics effect, anti-inflammatory and anti-asthmatic activity [73].

MIC results give a clue as to the required minimum dose that could visibly inhibit the growth of test organisms if implicated in any infection. MIC values less than or equal to 200 mg/ml is considered effective in microbial growth inhibition [74]. The far less MIC reported in this study (Table 5) is an indication that the leaf extracts of *P. thonningii* exhibited significant antibacterial activity against the tested organisms except when challenged with *C. albicans* and *A. niger* where no activity was observed for all concentrations. The recorded activity for all the challenged Gram-negative bacteria in this study is of pharmaceutical relevance since they are known to evolve a sophisticated permeability barrier with an additional outer membrane comprising a highly hydrophilic lipopolysaccharide layer which restricts penetration of hydrophobic and amphipathic compounds that encompass many drug compounds [75]. The difference in activity that was evident among tested strains in both crude and fractions could be a result of genetic differences between different strains that provide proof for the necessity of antibiogram prior to prescription as a precautionary measure in mitigating drug resistance development [76]. Ciprofloxacin and miconazole, the positive controls in this study had higher activity against the test organisms compared to the extract and fractions. This could also be attributed to the fact that the antibiotics are in their pure form, unlike the extract and fractions which are still in the unpurified form and thus needed to be purified to remove all inhibitory substances to its activity.

CONCLUSION

The promising *in vitro* antioxidant and antibacterial activities of these plant extracts might be due to the presence of supportive phytochemicals reported. This study lends scientific credence in support of the folkloric use of the leaves of *Piliostigma thonningii* plant for the investigated activities. Studies are still ongoing by the authors to isolate and characterise the particular compound(s) that is/are involved in the antioxidant and antimicrobial activities, and ascertain their potency *in vivo*.

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