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In Vitro Antioxidant Activities of the Aqueous and Methanolic Stem Bark Extracts of *Piliostigma thonningii* (Schum.)

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Abstract

Oxidative stress has been recognized as a key driver of many ailments affecting humankind. Free radicals attack biologically important biomolecules, impairing their functioning, thereby initiating and exacerbating diseases. As a comeback, antioxidant therapies have been proposed as novel approaches to ameliorating oxidative stress–associated diseases including chronic ones. Antioxidants are thought to employ multifaceted and multitargeted mechanisms that either restore oxidative homeostasis or prevent free radical buildup in the body, which overwhelm the endogenous defenses. Plants have been used for many ages across time to manage human diseases, and have a host of antioxidant phytocompounds. *Piliostigma thonningii* is traditionally used for the management of inflammation, malaria fever, rheumatism, and insanity, among other diseases caused by a disturbed redox state in the body. In this study, *in vitro* antioxidant activities of the methanolic and aqueous stem bark extracts of *P. thonningii* were evaluated using the *in vitro* antilipid peroxidation, the 1,1-diphenyl-2-picryhydrazyl (DPPH) free radical scavenging, and the ferric reducing antioxidant power assay methods. The obtained results revealed remarkable antioxidant activities of the studied plant extracts as evidenced by the low IC_{50} and EC_{50} values. These antioxidant activities could be due to the presence of antioxidant phytochemicals like flavonoids, carotenoids, tannins, and phenols, among others. Therefore, the therapeutic potency of this plant could be due to its antioxidant properties. This study recommends *in vivo* antioxidant efficacy testing of the studied plant extracts, as well as isolation and characterization of bioactive antioxidant compounds that are potent against oxidative stress.

Keywords

oxidative stress, antioxidants, *Piliostigma thonningii*

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Reactive oxygen species are generated in the body during normal cellular metabolism, and at normal levels, they play critical functions in normal cell physiology.[1] However, when they are in excess, they damage cellular components, impairing their proper functioning, leading to a continuum of human diseases with varied degrees of severity.[2-9] Previous studies have demonstrated a clear link of vital metabolic and cellular anomalies to reactive oxygen species when the normally efficient protective mechanisms get overwhelmed.[2,3,6,10]

Unsaturated fatty acids possess unstable electrons near their double bonds, rendering them most susceptible to oxidative damage in biological systems.[11] Thus, they are sensitive to lipid peroxidation, which increases exponentially with an increase in the degree of unsaturation.[12] Similarly, free radicals damage deoxyribonucleic acid (DNA), inducing mutations in cells, which in turn destabilize the prooxidant-antioxidant homeostasis in living tissues.[7,13] Oxidative stress–induced insult to DNA constituents lead to single-strand and double-strand DNA breaks, deoxyribose modification, purine/pyrimidine base modification, and DNA cross-linking, which are evidenced in complex disorders including cancer.[7]

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Furthermore, cellular proteins can be damaged by oxidative stress.[7] Following their damage, proteases degrade the damaged proteins to recycle associated atoms rather than repairing them. This takes place in 2 different ways; the peptide bond is damaged first, then the side chains. For instance, the sulfhydryl/thiol moieties (–SH) can be oxidized producing disulfide bridges (–S=S–).[7] However, inappropriate oxidation of –SH groups causes misfolding of associated proteins, thereby resulting in dysfunctional or malfunctioning proteins.[1,9,14] Furthermore, ominous consequences are imminent if growth regulating genes are turned off, as this can lead to uncontrolled growth, forming clusters of cells as evidenced in cancer.[15]

The human body employs a vastly complex antioxidant defense mechanism to curb oxidative stress.[16,17] Primarily, the body's antioxidant machinery comprise enzymes and vitamins, which quench free radicals, thereby restoring the redox state. Under metabolically aerobic settings, there are 3 major levels of prevention, interception, and repair, which maintain and stabilize cellular metabolites as well as their functional integrity. However, during disease states, these antioxidant defenses are either overwhelmed or ineffective, thus exacerbating the detrimental effects of the disease.[17-19]

Plants harbor a host of biologically active antioxidant compounds that are acquired by humans through vegetable and fruit diets endowed with carotenoids, ascorbic acid, fat-soluble vitamins, flavonoids, and polyphenols among other antioxidant secondary metabolites.[20] Research has shown that vitamins C and E coordinate to protect the thiol moieties of proteins against oxidative modification, thus promoting proper protein folding.[17] Additionally, fat-soluble vitamins including vitamins E and A inhibit lipid oxidation and peroxidation in the body.[21,22] Moreover, plant-derived phenolics and flavonoids, among other antioxidant amalgams, are strong scavengers of free radicals in the body, thereby averting oxidative stress damage to cellular components.[20,23,24] Epidemiological studies have shown that consuming antioxidant-rich diets like vegetables, fruits, and tea play significant roles in preventing chronic diseases in humans.[9,16,25-27]

Piliostigma thonningii is a legume belonging to Caesalpiniaceae subfamily under Fabaceae family. This plant is well distributed in many African countries ranging from Senegal to Zambia.[28,29] Twigs and leaves of this plant are traditionally used to manage malaria fever, snake bites, and dysentery, among other conditions.[30,31] Stem barks of *P. thonningii* are used for management of intestinal, respiratory, and inflammatory conditions.[32] Besides, stem bark decoctions and smoke are traditionally used to manage insanity and rheumatism among other conditions.[30,33] Elsewhere, studies have shown that antioxidant phytochemicals including phenols, tannins, and flavonoids, among others, have both preventive and curative pharmacologic activities against a wide range of diseases, including malaria, diabetes, cancer, inflammation, and dementia.[30,34-36]

In view of the ethnomedical information and uses of *P. thonningii*, the aim of this study was to investigate *in vitro* antioxidant activities of the aqueous and methanolic stem bark extracts of this plant, as a preliminary step towards validation of its use and potential development of arsenal molecules against the reported and associated maladies.

Materials and Methods

Collection and Preparation of Plant Material

Fresh stem barks of *Piliostigma thonningii* were collected from Cianyi village situated in Gitiburi location, in Embu County, Kenya, its natural habitat. The plant was primarily identified by its vernacular name (*Mukuura*) and its ethnomedical usage with the help of a reputable local herbalist. Further authentication and verification were done by a taxonomist at the Department of Plant Sciences, Kenyatta University, where voucher specimen number GM001/2017 was assigned. Thereafter, duplicate specimen of this plant was prepared and archived for future reference at the university herbarium.

The collected stem barks of the studied plant were chopped into small pieces and spread evenly to dry for 2 weeks at room temperature in the laboratory with regular grabbling.[37-40] The dried material was then ground into a powder using an electric mill, which was packaged in a well-labeled khaki envelope and stored in a clean dry shelf prior to extraction.[22,37-39]

Extraction Procedures

Methanol Extraction. About 200 g of *P. thonningii* stem bark powder was deliquesced in analytical grade methanol (0.75 L) in a 1 L capacity conical flask. Regular shaking on a daily basis (morning and evening) was done for 2 consecutive days to increase surface area for extraction.[39,40] Afterwards, the menstruum was cautiously decanted and filtered through Whatman filter paper No. 1 and then reduced *in vacuo* using a rotary evaporator at 50 °C.[41] The concentrate was transferred into a preweighed, clean, dry, labeled universal glass bottle that was kept in a hot-air oven set at 35 °C for 5 days to facilitate complete drying after which percentage yield was calculated using the formula described by Truong et al.[42]

$$\%\ \text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of macerated powder}} \times 100$$

The extract was then sealed and stored in a refrigerator set at 4°C pending antioxidant assays.[42,43]

Aqueous Extraction. To obtain the aqueous extract, approximately 50 g of powdered *P. thonningii* stem bark was soaked in 500 mL of distilled water in a 1 L beaker and heated at 70°C for 5 minutes before being cooled to room temperature. The mixture was filtered through Whatman filter paper No. 1 and transferred into clean freeze-drying flasks, which were then fitted into a freeze dryer for lyophilization for 2 days. The dry and lyophilized extract was transferred into a clean, dry, preweighed, and labeled universal glass bottle and percentage yield calculated using the formula described by Truong et al[42] in section "Methanol Extraction." The extract was then sealed and stored in a refrigerator at 4°C awaiting antioxidant assays.[42-44]

Determination of the Effects of the Aqueous and Methanolic Stem Bark Extracts of P. thonningii on In Vitro Lipid Peroxidation

Determination of the effects of the studied plant extracts on *in vitro* lipid peroxidation was done following a standard method.[45] Briefly, the

reaction mixtures contained 2.0 mL of the trichloroacetic acid-thiobarbituric acid-hydrochloric acid (TCA-TBA-HCl) reagent (15% [w/v] TCA, 0.375% [w/v] TBA, and 0.25 N HCl) and 1 mL of the studied plant extracts at concentrations of 50 μg/mL, 100 μg/mL, 150 μg/mL, and 200 μg/mL, respectively, or standard (L-ascorbic acid). The resulting mixtures were incubated in a water bath set at 90°C for 10 minutes, cooled, and centrifuged at 10 000 rpm for 15 minutes. The supernatants were aspirated and their respective absorbances measured at λ_{532} nm using a UV-Vis spectrophotometer (Shimadzu UV-Vis 1600). Percentage inhibition of *in vitro* lipid peroxidation was determined using the formula described by Prasad and Ramakrishnan[46] and Bajpai et al[47]:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Determination of In Vitro DPPH Radical Scavenging Activities of the Aqueous and Methanolic Stem Bark Extracts of P. thonningii

For *in vitro* DPPH radical scavenging activity assay, 12 mg of a stable free radical, 1,1-diphenyl-2-picryhydrazyl (DPPH), was accurately weighed using a Shimadzu analytical balance and dissolved in 100 mL of analytical grade methanol to give 0.3 mM solutions. One milliliter of the methanolic solution of 0.3 mM DPPH was added to 2.5 mL of each of the extract concentrations (1000, 100, 10, 1, 0.1 and 0.01 μg/mL). The mixtures were shaken and incubated for 15 minutes in the dark, at room temperature. After incubation, absorbance (A) was measured at λ_{517} nm with a Shimadzu UV-VIS (1600) microprocessor double beam spectrophotometer. Percentage of the radical scavenging activity (% RSA) was calculated using the formula described by Brand Williams et al[48]:

$$\% \text{ RSA} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

DPPH Solution (2.5 mL) plus methanol (1 mL) was used as a negative control while methanol (2.5 mL) plus sample solution (1 mL) was used as a blank. In addition, L-ascorbic acid at concentrations equivalent to that of the test samples (100, 10, 1, 0.1, and 0.01 μg/mL) was used as positive control.[48]

Determination of the Potassium Ferricyanide Antioxidant Power of the Aqueous and Methanolic Stem Bark Extracts of P. thonningii

The ferric reducing antioxidant power of the aqueous and methanolic stem bark extracts of *P. thonningii* was evaluated according the methods described by Brand Williams[48] and Benzie and Strain.[49] Reaction mixtures included 1 mL of different concentrations of assay extracts or L-ascorbic acid standard (0.01, 0.1, 1, 10, 100, and 1000 μg/mL), 2.5 mL of phosphate buffer (200 mM, pH 6.6), and 2.5 mL of potassium ferricyanide (30 mM).

The mixtures were incubated at 50°C for 20 minutes after which 2.5 mL of trichloroacetic acid (600 mM) was added, mixed, and the mixture centrifuged at 3000 rpm for 15 minutes. Thereafter, 2.5 mL of the supernatants was aspirated and mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (6 mM). The absorbance values of samples and standard were measured against blank at λ_{700} nm using a spectrophotometer (Shimadzu UV-Vis 1600).

Table 1. Percentage Yields of Study Plants Extracts.

Stem bark extracts of *Piliostigma thonningii*	Yield (%)
Aqueous extract	18.27
Methanolic extract	38.92

Data Management and Statistical Analysis

The yields of the crude extracts following extraction were expressed as percentages of total materials that were deliquesced. Data from antioxidant assays were tabulated in Excel spreadsheet (Microsoft Office 365) and then exported to Minitab v19.2 (State College, Pennsylvania). The data were subjected to descriptive statistics, expressed as $\bar{x} \pm$ SEM and analyzed using One-Way ANOVA for statistical comparison of differences among means followed by Tukey's test for pairwise comparisons and separation of means at $\alpha = .05$. Values of $P < .05$ were considered statistically significant. The obtained data were presented in form of tables.

Results

Percentage Yields of the Studied Plant Extracts

Table 1 presents the percentage yields obtained for the studied plant extracts. It was revealed that methanol had a higher extractive value than water as demonstrated by the high yield (Table 1).

Effects of the Aqueous and Methanolic Stem Bark Extracts of P. thonningii on In Vitro Lipid Peroxidation

The effects of the aqueous and methanolic stem bark extracts of *P. thonningii* on lipid peroxidation were investigated *in vitro* in this study. A comparison among the percentage inhibitions caused by the studied plant extracts was done in this study. The results revealed that the aqueous stem bark extract of *P. thonningii* significantly increased the percentage inhibition of lipid peroxidation from 94.04 ± 0.06% at a concentration of 50 μg/mL to 98.05 ± 0.03% at a concentration of 200 μg/mL ($P < .05$; Table 2). There was no significant difference in percentage inhibition of *in vitro* lipid peroxidation caused by the aqueous stem bark extract of *P. thonningii* between concentrations of 50 μg/mL and 100 μg/mL ($P > .05$), and between concentrations of 100 μg/mL and 150 μg/mL ($P > .05$; Table 2).

Besides, the percentage inhibitions of *in vitro* lipid peroxidation caused by the methanolic stem bark extracts of the studied plant at concentrations of 100 μg/mL and 150 μg/mL were not significantly different ($P > .05$; Table 2). However, at a concentration of 200 μg/mL of this extract, the percentage inhibition of *in vitro* lipid peroxidation was significantly higher than the percentage inhibitions obtained in all the other concentrations of this extract ($P < .05$; Table 2).

Furthermore, the results showed that the aqueous stem bark extract of *P. thonningii* produced significantly higher percentage inhibitions of lipid peroxidation ($P < .05$) at all the tested extract concentrations except at 200 μg/mL where the percentage inhibition of lipid peroxidation was significantly similar to

Table 2. Effects of the Aqueous and Methanolic Stem Bark Extracts of *Piliostigma thonningii* on *In Vitro* Lipid Peroxidation.*

	% Inhibition of peroxidation by stem bark extracts of *P. thonningii*		
Concentration (μg/mL)	Aqueous extract	Methanolic extract	L-Ascorbic acid
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50	94.04 ± 0.06^{Ca}	47.59 ± 0.52^{Cc}	72.51 ± 0.77^{Db}
100	94.41 ± 0.40^{BCa}	65.81 ± 3.80^{Bc}	75.66 ± 0.74^{Cb}
150	95.03 ± 0.05^{Ba}	66.23 ± 1.65^{Bc}	78.14 ± 0.22^{Bb}
200	98.05 ± 0.03^{Aa}	87.77 ± 3.50^{Aab}	87.08 ± 0.13^{Ab}
IC_{50} (μg/mL)	27.300	57.125	34.500

*Values are expressed as $\bar{x} \pm$ SEM. Values with the same lowercase superscript letter across the rows and uppercase superscript letter along the columns are not significantly different ($P > .05$; one-way ANOVA followed by Tukey's test).

Table 3. In Vitro DPPH Radical Scavenging Activities of the Aqueous and Methanolic Stem Bark Extracts of *Piliostigma thonningii*.*

	% DPPH radical scavenging activity of stem bark extracts of *P thonningii*		
Concentration (μg/mL)	Aqueous extract	Methanolic extract	L-Ascorbic acid
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.01	66.28 ± 0.71^{aB}	49.43 ± 1.55^{bC}	67.11 ± 1.62^{aC}
0.1	67.74 ± 1.72^{aB}	52.06 ± 0.85^{bC}	71.49 ± 0.88^{aC}
1	69.61 ± 1.35^{bB}	56.66 ± 0.53^{cB}	79.55 ± 2.36^{aB}
10	72.24 ± 0.82^{cB}	95.27 ± 0.10^{bA}	99.01 ± 0.01^{aA}
100	83.28 ± 3.13^{bA}	97.63 ± 0.04^{aA}	99.08 ± 0.02^{aA}
1000	90.95 ± 0.50^{cA}	97.79 ± 0.12^{bA}	99.12 ± 0.01^{aA}
IC_{50} (μg/mL)	0.0095	0.0325	0.0098

*Values are expressed as $\bar{x} \pm$ SEM. Values with the same lowercase superscript letter across the rows and uppercase superscript letter along the columns are not significantly different ($P > .05$; one-way ANOVA followed by Tukey's test).

that recorded for the methanolic extract ($P > .05$; Table 2). In addition, the percentage inhibition of *in vitro* lipid peroxidation produced by the methanolic stem bark extract of the studied plant, at a concentration of 200 μg/mL, was not significantly different from that produced by the standard (L-ascorbic acid) at the same concentration ($P > .05$; Table 2).

The concentrations of the studied plant extracts and the standard required to cause 50% inhibition of *in vitro* lipid peroxidation (IC_{50}) were also determined in this study. The aqueous stem bark extract of *P. thonningii* recorded an IC_{50} value of 27.300 μg/mL while that of the methanolic stem bark extract was 57.125 μg/mL (Table 2). Remarkably, the IC_{50} value recorded for the aqueous stem bark extract of *P. thonningii* was lower than the IC_{50} values of all the methanolic stem bark extract of this plant and that of the standard (L-ascorbic acid; Table 2).

In Vitro DPPH Radical Scavenging Activities of the Aqueous and Methanolic Stem Bark Extracts of P. thonningii

The *in vitro* DPPH radical scavenging activities of the aqueous and methanolic stem bark extracts of *P. thonningii* were evaluated in this study. The results demonstrated a dose-dependent increase in percentage DPPH radical scavenging activities of the studied plant extracts (Table 3).

The percentage radical scavenging activities of each of the studied plant extracts were compared among the tested concentrations in this study. The results showed no significant differences among the percentage radical scavenging activities at concentrations of 0.01 μg/mL, 0.1 μg/mL, 1 μg/mL, and 10 μg/mL of the aqueous stem bark extract of *P. thonningii* ($P > .05$; Table 3). Similarly, the percentage radical scavenging activities of the aqueous stem bark extract of *P. thonningii,* at concentrations of 100 μg/mL and 1000 μg/mL, were not significantly different ($P > .05$; Table 3). Generally, the obtained results depicted a positive dose-dependent increase in percentage radical scavenging activities of this extract (Table 3).

On the other hand, the methanolic stem bark extract of *P. thonningii* demonstrated a positive dose-dependent increase in percentage radical scavenging activity (Table 3). The results showed that the percentage DPPH radical scavenging activities produced by the methanolic stem bark extract of this plant at concentrations of 0.01 μg/mL and 0.1 μg/mL were not significantly different ($P > .05$; Table 3). Similarly, no significant differences among the percentage radical scavenging activities caused by the methanolic extract of *P. thonningii* at concentrations of 10 μg/mL, 100 μg/mL, and 1000 μg/mL were observed ($P > .05$; Table 3).

Besides, the percentage radical scavenging activities caused by the standard (L-ascorbic acid), at concentrations of 0.01 μg/mL and 0.1 μg/mL, were not significantly different ($P > .05$;

Table 4. Ferric Reducing Antioxidant Power (FRAP) Activities of the Aqueous and Methanolic Stem Bark Extracts of *Piliostigma thonningii*.*

	P thonningii		
Concentration (μg/mL)	Methanolic extract	Aqueous extract	L-Ascorbic acid
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
50	2.33 ± 0.08^{bB}	2.72 ± 0.05^{aB}	2.40 ± 0.03^{bB}
100	2.47 ± 0.04^{bAB}	2.83 ± 0.05^{aAB}	2.46 ± 0.01^{bB}
150	2.62 ± 0.04^{bA}	2.89 ± 0.05^{aAB}	2.49 ± 0.03^{bAB}
200	2.64 ± 0.04^{bA}	2.99 ± 0.02^{aA}	2.57 ± 0.02^{bA}
EC_{50} (μg/mL)	10.833	9.0667	10.650

*Values are expressed as $\bar{x} \pm$ SEM. Values with the same lowercase superscript letter across the rows and uppercase superscript letter along the columns are not significantly different ($P > .05$; one-way ANOVA followed by Tukey's test).

Table 3). Similarly, no significant differences were observed among the percentage radical activities produced by the standard at concentrations of 10 μg/mL, 100 μg/mL, and 1000 μg/mL ($P > .05$; Table 3). However, the percentage radical scavenging activities recorded at these concentrations were significantly higher than those recorded at concentrations of 0.01 μg/mL, 0.1 μg/mL, and 10 μg/mL ($P < .05$; Table 3).

The aqueous stem bark extract of *P. thonningii*, at concentrations of 0.01 μg/mL and 0.1 μg/mL, showed significantly higher percentage *in vitro* DPPH radical scavenging activities compared with the percentage *in vitro* DPPH radical scavenging activities of the methanolic extract at the same concentrations ($P < .05$; Table 3). However, at these concentrations, the percentage DPPH radical scavenging activities produced by the aqueous stem bark extract of *P. thonningii* were not significantly different from the percentage DPPH radical scavenging activities produced by the standard (L-ascorbic acid; $P > .05$; Table 3).

At a concentration of 1 μg/mL, the percentage DPPH radical scavenging activity of the aqueous stem bark extract of *P. thonningii* was significantly higher than that of the methanolic extract of this plant ($P < .05$; Table 3). However, the standard recorded a significantly higher percentage DPPH radical scavenging activity at this concentration compared with the percentage DPPH radical scavenging activities of the studied plant extracts ($P < .05$; Table 3).

Conversely, the percentage DPPH radical scavenging activities produced by the methanolic stem bark extract of *P. thonningii,* at concentrations of 10 μg/mL and 1000 μg/mL, were significantly higher than those produced by the aqueous stem bark extract of this plant at the same concentrations ($P < .05$; Table 3). However, no significant differences in percentage DPPH radical scavenging activities was observed between the standard and the methanolic stem bark extract of *P. thonningii* at a concentration of 10 μg/mL ($P > .05$; Table 3).

Furthermore, the extract concentrations required to scavenge 50% of the DPPH radicals (IC_{50}) were determined. Interestingly, the aqueous stem bark extract of *P. thonningii* had a significantly low IC_{50} value of 0.0095 μg/mL compared with the IC_{50} values of the methanolic extract and the standard (L-ascorbic acid). On the other hand, the methanolic stem bark extract of *P. thonningii* had a significantly high IC_{50} value of 0.0325 μg/mL (Table 3).

Ferric Reducing Antioxidant Power (FRAP) Activities of the Aqueous and Methanolic Stem Bark Extracts of P. thonningii

The ferric reducing antioxidant power activities of the aqueous and methanolic stem bark extracts of *P. thonningii* were also determined in this study. Generally, the results demonstrated a concentration-dependent increase in absorbance of the reaction mixtures measured under UV-Vis spectrum at $\lambda_{900\ nm}$ (Table 4).

The differences between absorbance values recorded at between concentrations of 50 μg/mL and 100 μg/mL, and between 150 μg/mL and 200 μg/mL of the methanolic stem bark extract of *P. thonningii* were not significant ($P > .05$; Table 4). Similarly, the absorbance values recorded at concentrations of 50 μg/mL, 100 μg/mL, and 150 μg/mL of the aqueous stem bark extract of *P. thonningii* were not significant ($P > .05$; Table 4). However, the absorbance recorded at 200 μg/mL of the aqueous stem bark extract of *P. thonningii* was significantly higher that the absorbances recorded at the other concentrations of this extract ($P < .05$; Table 4). Additionally, the absorbances recorded at among concentrations of 50 μg/mL, 100 μg/mL, and 150 μg/mL of the standard (L-ascorbic acid) were not significantly different ($P > .05$; Table 4).

In this study, we compared the absorbances recorded at each concentration of the studied *P. thonningii* and the standard. The results revealed that the aqueous stem bark extracts, at all the tested concentrations, recorded significantly higher absorbance values compared with the absorbance values of the methanolic extract and the standard ($P < .05$; Table 4). However, no significant differences between the absorbances recorded for the standard and the methanolic stem bark extract at all the studied were observed ($P > .05$; Table 4).

We further determined the half effective concentrations (EC_{50}) of the studied plant extracts required to produce an absorbance value of 0.5 were determined in this study. Notably, the aqueous stem bark extract of *P. thonningii* had the lowest EC_{50} value of 9.0667 μg/mL compared with the EC_{50} values of the methanolic stem bark extract and the standard (Table 4). In addition, it was observed that the aqueous stem bark extract had a significantly lower EC_{50} value compared with the methanolic stem bark extract and the standard (Table 4).

Discussion

The spontaneous production of excessive free radicals during cellular metabolism damage biomolecules, leading to a continuum of maladies including chronic inflammation, diabetes mellitus, cancer, and neurodegenerative disorders, among others.[2,4,10,50] Antioxidant therapy is the most practical approach to the management of oxidative stress–related disorders.[9,17,19,27,51] Despite the availability of many synthetic drugs used to manage oxidative stress, the high costs and adverse side effects associated with them limit their usefulness.[52] As a result, alternative nontoxic antioxidants which are affordable are needed to counter oxidative stress, thereby thwarting the associated diseases.[9] As a result, this study was designed to investigate the *in vitro* antioxidant activities of the aqueous and methanolic stem bark extracts of *P. thonningii* in the quest for cheap and safer antioxidant sources.

The effectiveness of the studied plant extracts in inhibiting *in vitro* lipid peroxidation was appraised according to the criterion of Blois[53] and Fidrianny et al,[54] which posit that samples having $IC_{50}/EC_{50} < 50$ μg/mL are deemed to be very strong antioxidants while those with 50 to 100 μg/mL are strong antioxidants. In a similar manner, samples with EC_{50}/IC_{50} values of 101 to 150 μg/mL are moderate antioxidants while those with EC_{50}/IC_{50} values of more than 150 μg/mL are weak antioxidants. The results of this study indicated that the aqueous stem bark extract of *P. thonningii* was a very strong antioxidant and a potent inhibitor of lipid peroxidation as shown by its IC_{50} value of 27.300 μg/mL. On the other hand, the methanolic stem bark extracts of *P. thonningii* and the standard (L-ascorbic acid) were considered as strong antioxidants based on their IC_{50} values. This suggests that the studied plant extracts could potentially restore and modulate the activity of endogenous antioxidant systems as postulated by Zhang et al,[34] Kirecci et al,[55] Santosa et al,[56] and Rahimzadegan and Soodi.[11]

The *in vitro* DPPH radical scavenging activity of the studied plant extracts revealed remarkable antioxidant potential. Research has demonstrated that antioxidant activity of plant extracts has a positive correlation with percentage radical scavenging activity.[54,57] Therefore, an extract with high percentage radical scavenging activity ought to be a potent antioxidant *in vitro* and *in vivo*. The high percentage radical scavenging activity translates to low EC_{50}/IC_{50} values.[57]

The results obtained in this study revealed a high percentage radical scavenging potential of the studied plant extracts *in vitro* as demonstrated by the low IC_{50} values. According to the efficacy criterion of Blois[53] and Fidrianny et al,[54] all the studied plant extracts had IC_{50} values that were, by far, lower than 50 μg/mL. This suggests that the studied plant extracts were strong scavengers of the DPPH radical *in vitro* and, therefore, high antioxidant efficacy as evidenced by the low IC_{50} values. These can be attributed to the presence of bioactive antioxidant phytochemical compounds in these extracts, which work synergistically to scavenge the DPPH radicals. These findings, therefore, imply that the studied plant extracts can attenuate the damaging effects caused by oxidative stress.

The ferric reducing antioxidant power (FRAP) activities of the aqueous and methanolic stem bark extracts of *P. thonningii* were also determined in this study. The FRAP method measures the ability of study samples to reduce ferric ion at low pH to ferrous ion, yielding a blue colored complex. An increase in absorbance, measured at 700 nm, is an indication of ferric reducing antioxidant power of the studied sample.[48,49] This method helps to evaluate the ability of plant extracts to reduce ferric ion to ferrous ion as a determination of antioxidant potential. Moreover, it helps to predict the extracts ability to mimic the body's endogenous antioxidants like bilirubin and uric acid in attenuating oxidative stress.[58,59] Therefore, high ferric reducing antioxidant power is correlated with increase in absorbance values and low EC_{50} values.

In this study, the aqueous and methanolic stem bark extracts of *P. thonningii* demonstrated remarkable ferric reducing antioxidant power activity as evidenced by the dose-dependent increase in absorbance and low EC_{50} values. It was observed that all the studied plant extracts were very strong antioxidants according to the criterion of Blois[53] and Fidrianny et al,[54] by indicating EC_{50} values that were lower than 50 μg/mL. The strong antioxidant power of these extracts is attributable to the presence of antioxidant phytochemical compounds in these extracts.

The current study demonstrates that the antioxidant activities of the studied plant extracts are due to the active phytocompounds with phenolic moieties in their structures. Examples of these antioxidant phytochemicals include flavonoids, catechins, coumarins, tannins, carotenoids, and phenols.[60] They relieve oxidative stress either solely or synergistically with other phenolic-containing amalgams.[25,61] As described in our earlier study,[60] the presence of antioxidant-associated secondary metabolites in the studied plant extracts potentially conferred the reported bioactivity in this study. For example, flavonoids, the major antioxidant phytocompounds, exert their effects via a continuum of various mechanisms including the chelation of iron and copper metal ions and the inactivation of free radical–generating endogenous enzymes in the body.[62,63]

Besides, natural antioxidants incorporated in the food we consume, are thought to inhibit free radical chain reactions in the body by preventing initiation or propagation steps causing chain termination reactions, and thereby delaying the oxidation process.[64] In the human body, nitric oxide ($NO^{\bullet}$), superoxide ($O_2^{\bullet}$), and the hydroxyl ($^{\bullet}OH^{-}$) radicals have been implicated as key agents that inactivate endogenous enzymes and other important cellular components causing oxidative injury.[7] Therefore, the studied plant extracts could potentially modulate and neutralize these free radicals *in vivo*, thereby restoring the redox homeostasis and either prevent or reverse deleterious free radical effects. Perhaps, the curative properties of the aqueous stem bark extract of the studied plant[65] could be through free radical scavenging and antioxidant defense mechanism.

Conclusions and Recommendations

The aqueous and methanolic stem bark extracts of *P. thonningii* have *in vitro* antilipid peroxidation, DPPH radical scavenging, and ferric reducing antioxidant power activities. Therefore, the aqueous and methanolic stem bark extracts of the studied plant can be potential antioxidant compound sources and alternatives for the management of oxidative stress–associated maladies. Furthermore, studies aimed at investigating the *in vivo* antioxidant efficacy of the studied plant extracts are encouraged to determine if the *in vitro* antioxidant results reported herein, are replicable in the *in vivo* setup. This study recommends further studies leading to isolation and characterization of the pure antioxidant molecules, especially those able to ameliorate oxidative stress and related diseases.

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Author Contributions

Gervason Moriasi conducted the study and drafted the manuscript. Mathew Piero Ngugi and Anthony Ireri promoted the idea and supervised the study. All authors read, reviewed, and approved the final draft of the manuscript prior to publication.

Declaration of Conflicting Interests

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Ethical Considerations

All the reagents used in this study were prepared, used, and disposed of according to the set laboratory guidelines and the material safety and data sheets (MSDS). Moreover, this study was undertaken after approval from National Council for Science, Technology and Innovation (NACOSTI), Kenya, under License Number NACOSTI/P/19/2080.

Supplemental Material

Supplemental material for this article is available online.

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