

Research Article

Phytochemical Profile and Biological Activities of *Piliostigma Thonningii* Leaf Extract: Antioxidant and Anti-Inflammatory Properties

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Abstract

The study aimed to evaluate the phytochemical composition, antioxidant activity, and anti-inflammatory properties of the methanol extract of *Piliostigma thonningii* leaves to provide scientific evidence for its traditional medicinal use. Phytochemical screening was conducted using standard methods, revealing the presence of anthraquinones, alkaloids, cardiac glycosides, tannins, flavonoids, phytosterols, saponins, and steroids, while glycosides and phenols were absent. Antioxidant activities were assessed using DPPH radical-scavenging, ferric reducing antioxidant potential (FRAP), and hydrogen peroxide scavenging assays, all indicating significant antioxidant capacity that increased with extract concentration. The results indicate a dose-dependent antioxidant response across all assays, with higher concentrations of extracts exhibiting greater scavenging potential against free radicals and hydrogen peroxide-induced oxidation. While the extracts show slightly lower potency compared to ascorbic acid, they still demonstrate considerable antioxidant activity, especially at higher concentrations. The anti-inflammatory effect was evaluated using a carrageenan-induced paw edema model in rats, comparing three dosages of the extract (100, 200, and 400 mg/kg) to Diclofenac and a negative control. The 100 mg/kg dose effectively delayed peak inflammation, showing strong anti-inflammatory activity similar to, but less consistent than, Diclofenac. These findings support the traditional use of *Piliostigma thonningii* leaves in managing inflammation and pain, suggesting potential for further development as a natural therapeutic agent."

Keywords

Anti-oxidant, Anti-inflammatory, Medicine, Phytochemical, *Piliostigma Thonningii*

1. Introduction

For millennia, medicinal plants have been an essential component of traditional medical systems all across the world. They provide an abundant supply of bioactive chemicals that can be applied to the management and avoidance of a wide

range of illnesses. The various phytochemical components of medicinal plants, such as alkaloids, flavonoids, tannins, terpenoids, and saponins, are thought to have therapeutic potential. Numerous biological properties, including an-

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ti-inflammatory, antibacterial, antioxidant, and anticancer effects, are displayed by these substances [1]. Medicinal plants have attracted renewed attention as possible sources of cutting-edge therapeutic compounds in recent years. This is caused by the growing inclination of consumers toward natural products, the adverse effects of synthetic medications, and the developing resistance of infections to traditional antibiotics. Consequently, a great deal of research is being done to investigate the pharmacological characteristics and phytochemical makeup of several medicinal plants [2]

Piliostigma thonningii or Camel's foot in English, is a leguminous shrub that grows widely in tropical and subtropical regions of Africa. African traditional medicine has long employed plant parts such as leaves, bark, and roots for their alleged medicinal qualities, which include anti-inflammatory, antibacterial, and antioxidant effects [3]. To determine which bioactive substances provide medicinal plants their therapeutic effects, phytochemical study is essential. Numerous secondary metabolites, including flavonoids, tannins, saponins, and alkaloids, have been found in previous research on *P. thonningii* and are well-known for their pharmacological properties [4]. These phytochemicals are thought to play a major role in the therapeutic qualities of the plant, especially in relation to its anti-inflammatory and antioxidant capabilities. For example, it is widely known that flavonoids and tannins can scavenge free radicals and block inflammatory pathways [5].

As a complicated biological reaction to damaging stimuli, inflammation has a major role in the development of many diseases, including cancer, cardiovascular disorders, and arthritis [6]. It is possible to evaluate the anti-inflammatory properties of plant extracts using both in vitro and in vivo models. Cell lines are commonly used in in vitro research to assess the suppression of pro-inflammatory indicators like prostaglandins, cytokines, and nitric oxide (NO). To evaluate the suppression of NO generation in response to inflammatory stimuli, for example, the macrophage cell line RAW 264.7 is frequently employed [7]. In contrast, animal models are used in in vivo research to examine the extracts' general physiological effects on inflammation. A popular technique for researching acute inflammation and evaluating the effectiveness of anti-inflammatory medications is the carrageenan-induced paw edema model in rats [8]. The purpose of this study is to examine the phytochemical constituents, antioxidant potential, and anti-inflammatory properties of an extract *P. thonningii* leaves extract.

2. Material and Methods

2.1. Sampling and Sample Preparation

The plant's leaves were collected in Girei, Girei Local Government Area, of Adamawa State, Nigeria on Wednesday 7th May, 2024 during dry season period. Freshly collected plant parts were transported in a black polyethylene bags to

the Abubakar Tafawa Balewa University, Bauchi, where they were recognized and authenticated by a Mr. Danlami Adamu a taxonomist of the Department of Forestry at Abubakar Tafawa Balewa University, Bauchi. The plant samples were dried in a room with shade. Also, the samples were spread out and rotated often. Electric blender were used to grind the thoroughly dried samples into a fine powder. Before being used, the powder sample was weighed using an analytical balance and store at room temperature till further use [9].

2.2. Extraction of Plants Samples

The cold maceration extraction method was applied to the plant sample. For the extraction process, 300 grams of the material were steeped for four days in a 70% methanol and 30% water 210 ml and 70 ml solutions respectively. The mixture was filtered, then allowed to air dry. Following that, the dehydrated methanol extracts were placed in glass vials and appropriately labeled for further use [10].

2.3. Phytochemical Screening of the Extract

Qualitative phytochemical analysis

The qualitative analysis was conducted using the standard procedure as outlined by [10].

Test for anthraquinones

5ml of extract, a couple of milliliters of concentrated H_2SO_4 , and one milliliter of diluted ammonia were added. The presence of anthraquinones is confirmed by the appearance of rose pink.

Test for alkaloids

A solution of picric acid was added to about 2 ml of the extract. An orange coloration indicates the presence of alkaloids.

Test for glycosides

5 ml of the extract and 25 ml of 1% sulfuric acid were combined in a test tube, heated to a boil for 15 minutes, cooled, and neutralized with 10% sodium hydroxide. 5 ml of Fehling's solutions A and B were then added. The presence of glycosides is indicated by a brick-red precipitate of reducing sugars.

Test for cardiac glycosides

About 1.5 ml of solvent extract with 2 ml of glacial acetic, adding a drop of ferric chloride solution, and then adding 1 ml of concentrated H_2SO_4 . Cardiac glycosides are indicated by a brown ring in the interface. When acetic layers build, a green ring may also form progressively towards the layer, appearing beneath the brown ring.

Test for phenol

About 25 ml of extract was added to 2 ml of ferric chloride solution, formation of deep bluish green solution indicates the presence of phenol.

Test for tannins

On a hot plate, 2 grams of the sample were boiled for 30 minutes in 50 milliliters of purified water. After filtering the mixture, some of the filtrate was diluted 1: 4 with sterile water,

and three drops of a 10% ferric chloride solution were added. Tannins are indicated by a blue or green hue.

Test for flavonoids

About 5 g sample was weighed and fully contained in acetone. To get rid of the acetone, the mixture was heated in a water bath. Using a water bath, the residue was removed. After the mixture was filtered, the test was conducted using the filtrate. An equivalent volume of the retained water extract will be mixed with 5 milliliters of 10% sodium hydroxide. The presence of flavonoids is indicated by a yellow solution.

Test of phytosteroids

The extract is dissolved in 2 ml of acetic anhydride and to which 2 drops of concentrated H_2SO_4 is added along the sides an array of colour changes indicates the presence of phytosterols.

Test for saponins

A conical flask weighing one gram of the sample will have ten milliliters of sterile distilled water added to it before it is cooked for five minutes. After the mixture was filtered, 2.5 ml of the filtrate was put to a test tube containing 0 ml of sterile distilled water. After giving the test tube a good shake for roughly 30 seconds, it was let to stand for 30 minutes. Saponins are indicated by honeycomb froth.

Test for steroids

About 0.5g of the extracts, add around 2 ml of acetic anhydride, then 2 ml of sulfuric acid. Steroid presence is indicated by a violet hue turning blue.

2.4. Quantitative Analysis

Determination of Anthraquinones

About 10 g of dried plant material to a fine powder, and then use sonication for 30 minutes to extract it with 100 mL of methanol. Utilizing a rotary evaporator, filter the extract and then concentrate it. Prepare standard anthraquinone solutions in methanol for HPLC analysis. Then, use a methanol-water mobile phase to set up the C18 column in the HPLC system. To identify and quantify the anthraquinones, inject 20 μ L of

each standard and the sample extract, noting retention times and peak regions. Measure the absorbance of anthraquinone standards in ethanol at a certain wavelength (430 nm, for example) to create a standard calibration curve. Using the calibration curve, dilute the sample extract accordingly, measure its absorbance, and calculate the anthraquinone concentration. [11].

Determination of Alkaloids

About 2.50 g sample of wood powder was placed in a 250 cm^3 beaker with around 200 cm^3 of 10% acetic acid in ethanol, and it was left for four hours. After diluting the extract to a quarter of its initial volume in a water bath, 15 drops of concentrated hydroxide were added to the extract dropwise till the precipitation was fully formed right away after filtering. After the mixture sediments for three hours, the supernatants are discarded, and the precipitates are cleaned with 20 cm^3 of 0.1 M ammonium hydroxide before being placed on 12.5 cm filter paper. After the residue is baked dry, the proportion of alkaloid is calculated analytically as follows [12].

$$\% \text{ Alkaloid} = \frac{\text{weight of Alkaloid}}{\text{Weight of sample}} \times 100\%$$

Determination Cardiac Glycoside

Cardiac glycoside methodology as described by [13] as reported by [14]. One gram of the powder sample was weighed into a 250 cm^3 round-bottom flask, and 200 cm^3 of distilled water was added. The mixture was then let to stand for two hours to allow autolysis to take place. After adding an antifoaming agent (tannic acid), 20 cm^3 of 2.5% NaOH (sodium hydroxide) was added to the sample. Full distillation was then performed in a 250 cm^3 conical flask. Using a microburette on a black backdrop, 100 cm^3 of cyanogenic glycoside, 8 cm^3 of 6 M NH_4OH (ammonium hydroxide), and 2 cm^3 of 5% KI (potassium iodide) were added to the distillate (s), mixed, and titrated with 0.02 M $AgNO_3$ (silver nitrate). The persistent turbidity signifies the termination point. Content of cyanogenic glycoside in the sample was calculated as

$$\text{Cyanogenic glycoside (100 g)} = \frac{\text{Titre Value (Cm3)} \times 1.08 \times \text{exact volume}}{\text{Aliquot volume (Cm3)} \times \text{sample weight(g)}} \times 100$$

Determination of Tannin

About 1.0 g of sample was weighed into a 50 ml of sample bottle, 10 ml of 70% aqueous acetone was added, and the bottle was securely closed. At 30 $^{\circ}C$, the bottle was shaken for two hours in a bath shaker. After centrifuging each solution, the supernatant was frozen and kept on ice. 0.8 ml of distilled water was added to the test tube after 0.2 ml of each solution was pipetted in. After adding 0.5 ml of Folin-Ciocateau reagent to the sample and standard, 2.5 ml of 20% Na_2CO_3 was added to create the standard tannin acid solution. At 725 nm, the solution was measured [12].

Determination of Flavonoid

The method described by [12] was used for flavonoid determination. 2.50 g of the sample were placed in a 250 cm^3

beaker, filled with precisely 50 cm^3 of 80% aqueous methanol, covered, and left to stand at room temperature for 24 hours. The residue was extracted again using the same amount of methanol after the supernatant was discarded. To filter the entire solution of every plant sample, Whatman filter paper number 42 (125mm) was utilized. The filtrate of each plant sample was put into a crucible and dried over a water bath. After being cooled in a desiccator, the contents of the crucible were weighed until a consistent weight was reached. The proportion of flavonoids were calculated as

$$\% \text{ Flavonoid} = \frac{\text{weight of Flavonoid}}{\text{Weight of sample}} \times 100\%$$

Determination Phytosteroids

Using acetic acid and sulfuric acid to hydrolyze an aliquot of the extract in ethanol, followed by a reaction with anisaldehyde reagent, is a colorimetric technique. A UV-Visible spectrophotometer is used to detect absorbance at 620 nm following incubation and cooling, and a calibration curve made from standard phytosteroid solutions is used to calculate concentrations [15].

Determination of Saponin

20 g of each sample was put into a conical flask and 100 cm³ of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4hrs with continuous stirring at about 55 °C. The mixture was filtered and the residue was re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water at about 90 °C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was then added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. 60 ml of n-butanol was added and the combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight, the saponin content was calculated as percentage [16, 17].

Determination of Steroids

1ml of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III)

chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20 °C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank [17].

2.5. Antioxidant Assay

The three methods described below were used for the antioxidant assay;

1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging activity was determined as described by [18]. 1 ml of 0.2 mM DPPH with methanol, 2.5 ml of different quantities of the different methanolic crude extracts and ascorbic acid as standard at different concentrations of 100, 200, 300, 400, and 500 µg/mL were added. The mixture was then left to react for 30 minutes at room temperature in the dark. The control consisted of 2.5 ml of methanol and 1 ml of 0.2 mM DPPH. For every concentration, three duplicates of the assay were run. Absorbance of the resultant mixture was measured using the double beam UV-visible spectrophotometer (Model T80; PG Instruments, Lutterworth, England, UK) at 517 nm. Percentage inhibitions of the methanol extract and the standard was calculated using the formula below:

$$\% \text{ inhibition} = \frac{\text{Optical Density Control} - \text{Optical Density sample}}{\text{Optical Density control}} \times 100$$

Optical Density control = The absorbance without sample,

Optical Density sample = The absorbance of methanol extract or standard.

Ferric Reducing Antioxidant Potential Assay

Ferric reducing assay was carried out using the method described by [19]. 1 ml of a methanol extract of *P. thonningii* plant (leaves) at varying concentrations was placed into test tubes together with 2.5 ml of a solution of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1% w/v). After 30 minutes of incubation at 50 °C, 2.5 ml of 10% w/v trichloroacetic acid was added to the reaction solutions. After centrifuging the reaction mixtures for ten minutes at 3000 rpm, the top layer of the solution was separated and saved. 2.5 ml of the supernatant solution, 2.5 ml of distilled water, and 0.5 mL of FeCl₃ (0.1% w/v) were combined. The absorbance was then measured at 700 nm against blank sample using the double beam UV-visible spectrophotometer (Model T80; PG Instruments, Lutterworth, England, UK). Ascorbic acid was used as the standard.

Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide scavenging assay was determined according to [20], 10 mg of allantoin, betaine and nicotinamide were weighed on a balance and diluted in 1000 of distilled water. Allantoin, betaine, and nicotinamide were prepared at different concentrations (100, 200, 300, 400 and 500 µg/ml).

In new Eppendorf tubes we added 100 µL of each sample, and added 400 µL of phosphate buffer. Same was performed with the standard solution containing ascorbic acid. Then we added 600 µL of 40mM hydrogen peroxide solution to the tubes, vortexed them and incubated for 10 minutes. We added 100 µL of each sample in triplicates in a 96-well plate and measured absorbance at 230 nm on the Microplate reader (ThermoFisher Scientific, USA) against a blank containing only phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard/positive control. Samples without hydrogen peroxide were used as a negative control. The abilities to scavenge the hydrogen peroxide were calculated using the equation: % scavenged

$$(H_2O_2) = \frac{(A_0 - A_1)}{A_0 \times 100}$$

Where A₀ is the absorbance of the control and A₁ the absorbance of the sample.

Anti-inflammatory Assay

The method described below was used for the anti-inflammatory assay;

Determination of in vivo anti-inflammatory activity Carageenan-induced paw edema method

The anti-inflammatory activity of OLE was measured using

the carrageenan-induced paw edema method in rats according to [21]. For all groups of rats, a sub-planter injection of 0.1 ml of a freshly made 1% carrageenan suspension was used to cause paw edema and inflammation in the right footpad of the hind paw. The left hind paw was used as a control to determine how much the thickness of the paw changed despite not receiving any treatment. Visible redness and noticeable swelling were brought on by carrageenan; these effects established fully within three hours and continued until the experiment's conclusion. Using a Vernier digital caliper, the progression of paw edema was assessed at 0, 1, 2, 3, 4, and 5 hour intervals. Each of the formerly adapted animals was split up into five groups (n = 6). The rats were pretreated with OLE at dosages of 100, 200, and 400 mg/kg to the first three groups, respectively, one hour before the carrageenan injection. The reference medications for the remaining two groups were diclofenac (10 mg/kg) and distilled water (10 ml/kg), respectively. Compared to the control groups, received the vehicle, the percent of the edema's inhibition was calculated as per the formula shown below: Where the change of paw thickness values was calculated from the difference between the left and the right paw volumes.

3. Results and Discussions

Table 1 show the phytochemical screening result for *Piliostigma thonningii* leaves Methanolic Crude Extract

Table 1. Quantitative analysis of *P. thonningii* leaves of methanol extracts.

Phytochemical	Leaves
Anthraquinones	+
Alkaloids	+
Glycoside	-
Cardiac Glycoside	+
Phenol	-
Tannins	++
Flavonoids	++
Phytosteriod	+
Saponins	+
Steriods	+

Keys + =Presents, - = Absent

Table 1 showed that anthraquinones, alkaloids, cardiac glycoside, tannins, flavonoids, phytosteroids, saponins, and steroids were present in the *P. thonningii* leaves extract, but glycoside and phenols were absent.

The effectiveness of anthraquinones as antibacterial agents was demonstrated in studies by [22], which demonstrated notable action against bacterial strains. The traditional use of plants containing anthraquinone for laxative effects was also confirmed by [23]. These results imply that *P. thonningii* might be a useful natural source for creating remedies for microbiological and gastrointestinal ailments. The pharmacological effects of alkaloids are well-known, and they include antihypertensive, antimalarial, and analgesic actions. The fact that *P. thonningii* contains alkaloids is consistent with its possible medicinal uses. Plant extracts rich in alkaloids have been shown by [24] to have strong analgesic properties, which makes them useful for pain management. Alkaloids have been used in traditional medicine to cure malaria. Studies by [25] demonstrated these capabilities. The aforementioned studies bolster *P. thonningii*'s potential for the development of antimalarial and pain management treatments. *P. thonningii* leaves lack glycosides, which means that a portion of the plant might not be appropriate for uses where glycosides are known to be advantageous, like some anti-inflammatory or anti-diabetic medications. Because of their many medicinal qualities, such as their capacity to control blood sugar levels and lessen inflammation, glycosides frequently have a significant impact on the pharmacological activities of plants. Studies by [26], found that the anti-diabetic effects of glycosides in medicinal plants are noteworthy and can help in the management of diabetes mellitus. In a studies by [27] emphasized the therapeutic utility of cardiac glycosides in the treatment of heart failure and their significance in contemporary medicine. [28] provided more evidence of their efficacy in the management of arrhythmias. *P. thonningii* has the potential to be used in cardiac therapy since it contains cardiac glycosides, which provide a natural source for the development of heart medicines. Strong antioxidants like phenolic compounds are well known for their ability to lower inflammation and oxidative damage. The *P. thonningii* leaves extract's absences of phenols is in line with research showing how different plant species' phenolic contents can be depending on the extraction technique used [29]. The therapeutic potential of medicinal plants is enhanced by phenols, which are important antioxidants that scavenge free radicals and guard against oxidative stress [30]. *P. thonningii* leaves possesses a high content of tannins. Tannins have been thoroughly researched for their antibacterial, anti-inflammatory, and wound-healing characteristics, they also have astringent qualities [31, 32]. These characteristics highlight their historical use in folk medicine as well as their possible uses in contemporary pharmacology. The presence of flavonoids in the extract is in line with research that shows how common they are in plants and how they have a variety of pharmacological effects. Due to their well-known cardioprotective, anti-inflammatory, and antioxidant properties, flavonoids are valuable ingredients in herbal remedies [33, 32]. Their existence in *P. thonningii* lends credence to its application in conventional medical

procedures. The pharmacological significance of phytosterols is highlighted by their acknowledged ability to decrease cholesterol and have possible cardiovascular benefits [23]. Saponins possess diverse biological activities, including antimicrobial, anti-inflammatory, and immune-modulating effects, which contribute to their therapeutic potential [34]. Their presence in *P. thonningii* supports its traditional use in treating various ailments. Steroids were found in the extract, in addition to their potential uses in anti-inflammatory treatments, steroids are essential for several physiological processes [35].

Table 2. Quantitative Phytochemical Screening of *Piliostigma thonningii* Plant (Leaves, Stem bark and Root) in mg/g.

Parameters	(mg/g)
Alkaloids	4.000
Anthraquinones	0.015
Cardiac Glycoside	0.464
Flavonoid	11.450
Phytosteroids	0.020
Saponins	1.270
Steroids	0.140
Tannins	0.100

Numerous bioactive chemicals with varied amounts are found in *Piliostigma thonningii* leaves, according to a quantitative phytochemical investigation, underscoring the potential medical uses for these substances.

At 4.000 mg/g, the leaves have a considerable concentration of alkaloids. The analgesic, anti-inflammatory, and antibacterial qualities of alkaloids are widely recognized. Given

their high concentration, *Piliostigma thonningii* leaves may be a valuable source for medicines incorporating these bioactivities [36]. Anthraquinones have known laxative, antimicrobial, and antiviral properties. Despite the low amount, their presence indicates potential, albeit limited, therapeutic applications [37]. The leaves also have cardiac glycosides (0.464 mg/g), which are heart condition helpful. This concentration implies that the leaves could be employed in herbal therapies for heart-related disorders [38]. At 11.450 mg/g, flavonoids are the most prevalent phytochemicals found in leaves. The qualities of flavonoids—antioxidant, anti-inflammatory, and anticancer—are well known. The high concentration highlights the leaves' potential as a potent antioxidant source and their application in the management of inflammation- and oxidative stress-related ailments [33, 39]. Phytosterols, which are good for heart health and cholesterol reduction, are present in the leaves in amounts of 0.020 mg/g. The leaves have some promise for treating cholesterol-related problems even though they are present in modest concentrations [40]. The leaves contain saponins, which have a concentration of 1.270 mg/g and are useful for a variety of medical applications due to their immune-stimulating, anti-inflammatory, and cholesterol-lowering properties [41]. The amount of steroids in the leaves is 0.140 mg/g. These substances are necessary for the structural function they play in cell membranes as well as for the treatment of some malignancies and inflammations [38]. The leaves contain 0.100 mg/g of tannins, which are recognized for their astringent qualities, ability to heal wounds, and ability to prevent diarrhea. Given their concentration, the leaves may prove beneficial in treatments that call for these characteristics [42, 43].

4. Antioxidant Activities

The result below shows the antioxidant activities of the methanol leaves extract of *Piliostigma thonningii* plant.

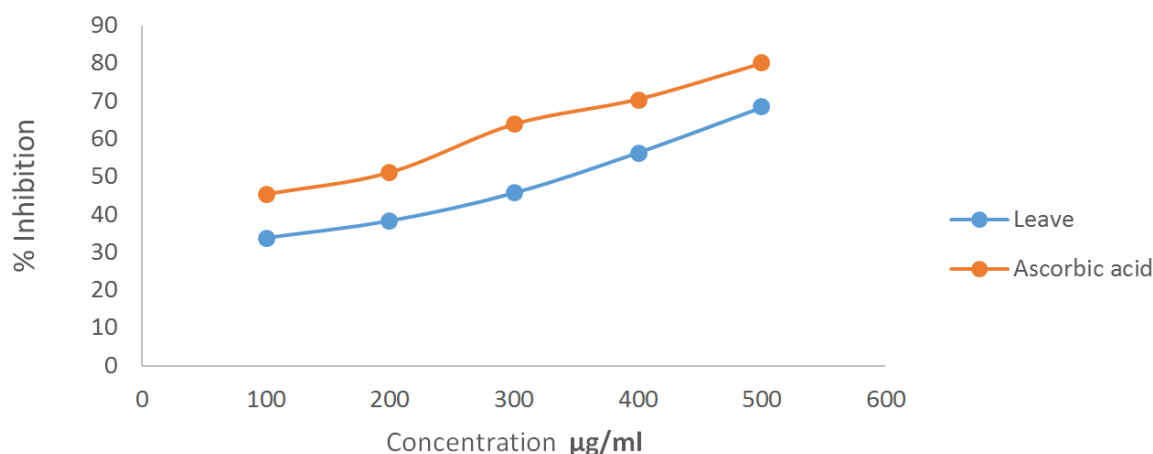


Figure 1. The Antioxidant Activity of DPPH scavenging activity results of the leaves Crude Extract of *Piliostigma thonningii* plant.

The percentage inhibition of the DPPH radical increases with increasing concentrations of the leaves methanolic extract, according to the data, in a dose-dependent manner. Studies by [44], which examines the dose-dependent behavior of antioxidants in scavenging free radicals. The assay uses ascorbic acid, a well-known antioxidant, as a positive control. The methanolic extract of leaves and the % inhibition of DPPH radical at different concentrations are compared. The extract is not as potent

as ascorbic acid, even though it has strong antioxidant action, particularly at higher concentrations. This comparison establishes a baseline for the extract's activity and emphasizes its relative effectiveness as an antioxidant. Studies by [23], which examine the antioxidant activity of medicinal plants and compare them with known antioxidants, support the standard control used in antioxidant tests, which is ascorbic acid.

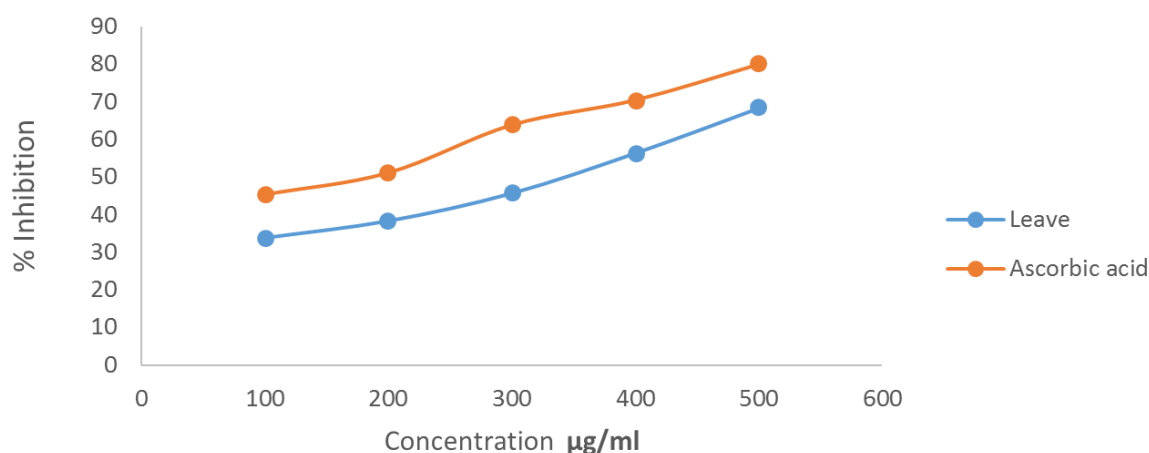


Figure 2. The Antioxidant Activity Ferric reducing antioxidant potential assay results of the leaves Crude Extract of *Piliostigma thoninigi* plant.

The results show a dose-dependent increase in the percentage inhibition of Ferric reducing antioxidant potential assay with increasing concentrations of the leaves methanolic extract. This suggests that higher concentrations of the extract possess greater antioxidant activity, as they are more effective at scavenging free radicals. This observation aligns with the general principle of dose-response relationships in pharmacology and is supported by numerous studies, including [44], which discusses the dose-dependent behavior of antioxidants in scavenging free radicals. Ascorbic acid, a well-known antioxidant, serves as a positive control in the assay. The

percentage inhibition of DPPH radical by the leaves methanolic extract is compared to that of ascorbic acid at various concentrations. While the extract demonstrates significant antioxidant activity, especially at higher concentrations, it exhibits lower potency compared to ascorbic acid. This comparison highlights the relative efficacy of the extract as an antioxidant and provides a benchmark for its activity. The use of ascorbic acid as a standard control is common in antioxidant assays and is supported by studies carried out by [23], which discusses the antioxidant activity of medicinal plants and their comparison with established antioxidants.

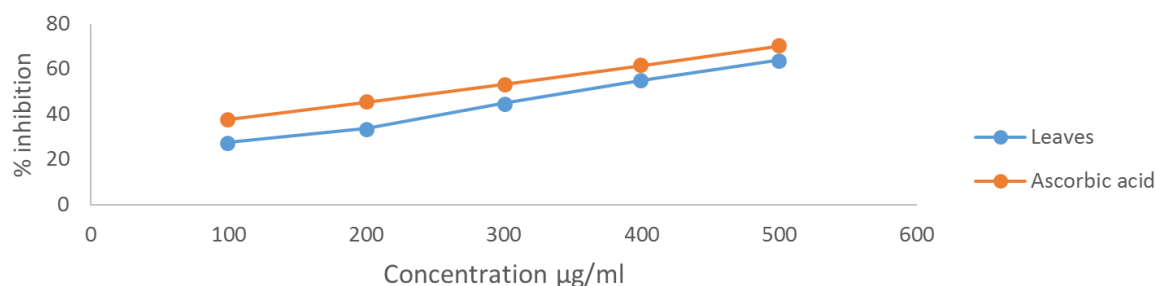


Figure 3. The Antioxidant Activity hydrogen peroxide scavenging antioxidant potential assay results of the leaves Crude Extract of *Piliostigma thoninigi* plant.

The hydrogen peroxide scavenging antioxidant potential assay was used in this study to determine the antioxidant

activity of the crude extract of *Piliostigma thonningii* leaves. The results show that the crude extract from the leaves inhibits

hydrogen peroxide-induced oxidation in a concentration-dependent manner. Higher doses of the crude leaf extract exhibit an increasing percentage inhibition of oxidation, indicating the existence of antioxidant chemicals in the leaves of *Piliostigma thonningii*. This observation is in line with studies by [45], who used hydrogen peroxide scavenging assays to show strong antioxidant activity in *Piliostigma thonningii* leaf extracts. The interpretation of the observed antioxidant activity in the leaves' crude extract is supported by their findings, which offers insightful information about the

antioxidant capacity of *Piliostigma thonningii* leaves.

Anti-inflammatory Activity (In-vivo)

The leaves' in vivo anti-inflammatory activity *Piliostigma thonningii* plant crude extract. The model of paw edema caused by carrageenan is used to test a drug's anti-inflammatory properties during the acute phase of inflammation. Carrageenan-induced edema is thought to be biphasic [44]. The provided results for the leaves methanolic crude extract of *Piliostigma thonningii* plant are shown below:

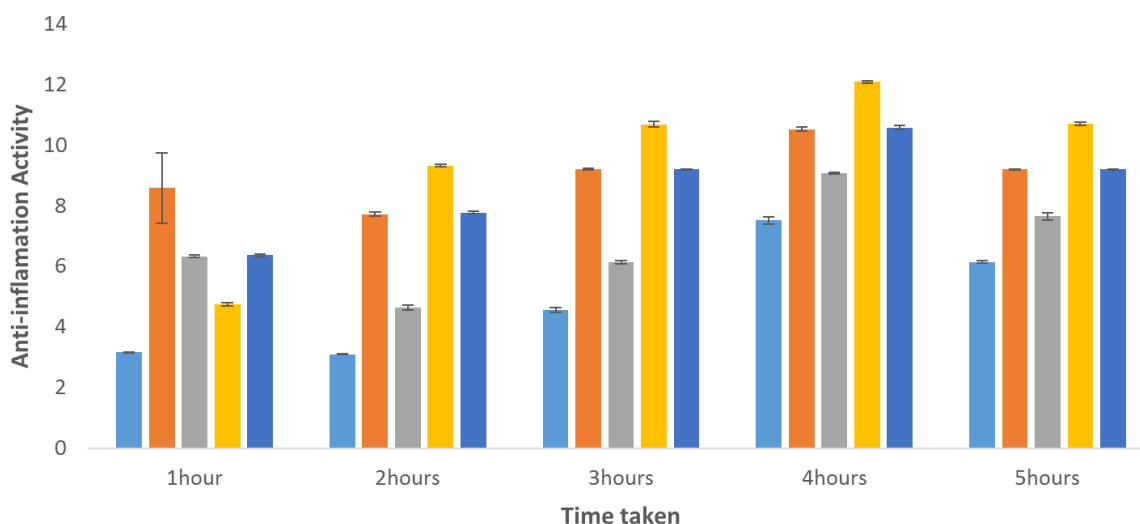


Figure 4. Leaves methanolic extract of *Piliostigma thonningii* plant with carrageenan-induced paw edema in rats.

Figure 4 presented the anti-inflammatory effects of the leaves methanolic extract of *Piliostigma thonningii*, using the carrageenan-induced paw edema model in rats. It includes data for different extract concentrations (100, 200, and 400 mg/kg) compared to a negative control and Diclofenac.

It is observed that the leaves at 100 mg/kg shows a gradual increase in paw edema, starting at 3.176 ± 0.014 at 1 hour, peaking at 4 hours (7.526 ± 0.121), and slightly decreasing at 5 hours (6.154 ± 0.033) whereas at 200 mg/kg it shows a high initial values at 1 hour (8.598 ± 1.167) and consistently high values, peaking at 4 hours (10.539 ± 0.065) before a slight decrease at 5 hours (9.210 ± 0.022). However, at 400 mg/kg, the initial moderate values at 1 hour (6.336 ± 0.042), a decrease at 2 hours (4.650 ± 0.068), then increasing again, peaking at 4 hours (9.080 ± 0.029) and slightly decreasing at 5 hours (7.658 ± 0.132). For Negative Control, similar to Figure 4, shows a steady increase in paw edema from 1 hour (4.754 ± 0.055) to 5 hours (10.714 ± 0.056). The Standard Drug (Diclofenac) shows higher initial values but more controlled increases, peaking at 4 hours (10.585 ± 0.066) and decreasing at 5 hours (9.207 ± 0.022).

Figure 4; revealed that the 100 mg/kg dose of the leaves extract shows a slower progression of inflammation, with a peak at 4 hours followed by a decrease. The 200 mg/kg dose shows consistently high inflammation levels, suggesting less

effectiveness at this concentration. The 400 mg/kg dose shows an initial reduction in inflammation at 2 hours but increases similarly to the other doses, peaking at 4 hours and then decreasing.

The leaves methanolic extract of *Piliostigma thonningii* also demonstrates anti-inflammatory activity, with the 100 mg/kg dose showing the most promise by delaying the peak inflammation. The higher doses do not show a consistent reduction, indicating that the optimal concentration may be lower. Comparatively, Diclofenac remains more effective at controlling inflammation across all time points.

Studies by [46], reported a significant anti-inflammatory effects of both ethanolic and aqueous extracts. The ethanolic extract at 160 mg/kg exhibited a substantial 72.41% inhibition of carrageenan-induced paw edema, demonstrating strong anti-inflammatory activity. The aqueous extract at the same concentration showed a 51.65% inhibition, indicating moderate anti-inflammatory effects compared to the ethanolic extract. Similarly, another study by [45], showed that 200 mg/kg of OLE achieves 42.31% inhibition, higher than the 30% at 50 mg/kg in this study. At 100 mg/kg, this study shows 45% inhibition, close to the 46.99% at 400 mg/kg of OLE [46], suggesting comparable anti-inflammatory activity at a lower dose. At 200 mg/kg, this study reports 50% inhibition, similar to the 51.65% of the aqueous extract and lower than the 72.41%

of the ethanolic extract at 160 mg/kg in studies by [44].

These results indicate that while the methanolic extract shows significant activity, the ethanolic extract by [46], is more effective at similar concentrations. Increasing the concentration or using ethanolic extraction may yield stronger anti-inflammatory effects.

5. Conclusion

In conclusion, it has been shown that the methanol leaf extract of *Piliostigma thonningii* has antioxidant properties. These properties are mediated by the percentage inhibition of the radical, which rises in a dose-dependent way with increasing concentrations of the leaf methanolic extract. The extract's anti-inflammatory properties are demonstrated by its capacity to prevent inflammatory reactions in paw edema induced by carrageenan. These findings support its application in traditional medicine for the management of pain and inflammatory diseases including toothaches and backaches.

Abbreviations

DPPH	1, 1-diphenyl-2-picryl hydrazyl
FRAP	Ferric Reducing Antioxidant Potential Assay
OLE	Olive Leaf Extract

Conflict of interest

The authors declare no conflicts of interest.

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