ANTIOXIDANT, IN-VIVO AND IN-VITRO ANT-IINFLAMMMATORY ACTIVITIES OF PILIOSTIGMA THONNINGII ROOT EXTRACT

ABSTRACT

The current study investigates the phytochemical composition, antioxidant activity, and anti-inflammatory properties of methanol root extract of Piliostigmathonningii. Standard qualitative methods revealed the presence of anthraquinones, alkaloids, phenols, tannins, phytosterols, and saponins, while cardiac glycosides, glycosides, flavonoids, and steroids were absent. Antioxidant potential was assessed using DPPH radical-scavenging, ferric reducing antioxidant potential (FRAP), and hydrogen peroxide scavenging assays, revealing dose-dependent radical inhibition. The root crude extract of Piliostigmathonningii exhibits dose-dependent antioxidant activity across DPPH, FRAP, and hydrogen peroxide scavenging assays, with higher concentrations showing increased efficacy. The presence of bioactive phytochemicals, including flavonoids and phenolic compounds, contributes to its ability to reduce oxidative stress and inhibit free radicals. Anti-inflammatory activity was evaluated in vitro via albumin denaturation inhibition and in vivo using the carrageenan-induced paw edema model in rats. At 100 mg/kg, the extract exhibited moderate anti-inflammatory effects, with paw edema peaking at 4 hours (9.093 ± 0.071) and declining at 5 hours (7.362 ± 0.089). However, higher concentrations (200 and 400 mg/kg) showed increased inflammation, peaking at 4 hours (19.621 ± 0.033) and 5 hours (20.001 ± 0.056), respectively. Diclofenac (negative control) demonstrated controlled inflammatory responses, with edema peaking at 4 hours (10.585 ± 0.066) and declining by 5 hours (9.207 ± 0.022). These findings suggest that while the extract has notable anti-inflammatory effects at lower doses, higher doses may exhibit pro-inflammatory tendencies. This study highlights the potential of Piliostigmathonningii root extract as a natural source of anti-inflammatory agents, warranting further investigation for therapeutic applications

Keywords:Anti-inflammatory, Antioxiant, Extract, Methanol, Phytochemical

INTRODUCTION

The search for safer and more effective substitutes for synthetic medications has led to a notable increase in the study of medicinal plants in recent years. A plant that has attracted attention is *Piliostigmathonningii* (Schumach), also referred to as monkey bread or camel's foot. This plant is extensively distributed throughout tropical Africa and is a member of the Fabaceae family. *P. thonningii* has historically been utilized in folk medicine to treat a variety of conditions, including cough, diarrhea, wounds, and inflammation, demonstrating its potential as a source of bioactive chemicals [1]. Many bioactive chemicals have evolved by medicinal plants, such as *P. thonningii*, as defensive mechanisms against infections, herbivores, and environmental stress. These substances, which have a variety of pharmacological actions, include flavonoids, tannins, saponins, and phenolic acids. The secondary metabolites of *P. thonningii* are primarily

Comment [t1]: Why not pharmacological agents

responsible for its therapeutic potential. Strong antioxidants such as flavonoids and phenolic acids, for example, can scavenge free radicals to stop oxidative stress and cellular damage. [2].

Antioxidants play a critical role in shielding the body from the damaging effects of free radicals and oxidative stress, both of which are linked to the etiology of a wide range of chronic illnesses, including cancer, heart disease, and neurological disorders. Free radicals are very reactive, unpaired electron molecules that can react with proteins, lipids, and DNA to cause damage to cells. This damage is lessened by the body's natural antioxidant defense system, which is made up of both enzymatic and non-enzymatic antioxidants [3]. According to studies by [2,4],*P. thonningii's* phytochemical components—such as flavonoids, tannins, and phenolic acids—contribute to its antioxidant capacity by strengthening the body's defensive mechanisms against free radicals and scavenging them. These antioxidants function by scavenging reactive oxygen species (ROS) directly, chelating metal ions that contribute to the generation of ROS, and increasing the activity of natural antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) [5].The biological reaction known as inflammation, which is triggered by pathogens, damaged cells, or irritants, is essential to the healing process. On the other hand, a number of illness, such as diabetes, heart disease, and arthritis, can be brought on by persistent inflammation. Numerous in *in-vivo* investigation have shown that *P. thonningii*root has anti-inflammatory properties [6,7].

P. thonningii has anti-inflammatory properties that are mediated by a number of mechanisms. The suppression of pro-inflammatory cytokine production and release, including interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), is a major mechanism. By encouraging the migration of immune cells to the site of inflammation and triggering other inflammatory mediators, these cytokines play crucial roles in the inflammatory response[8]. Furthermore, it has been demonstrated that *P. thonningii* root extract inhibits the activity of cyclooxygenase (COX) enzymes, which are in charge of producing prostaglandins that promote inflammation[4]. The aim of this study is to examine the phytochemical composition, antioxidant potential, and anti-inflammatory properties of an extract made from *P. thonningii*roots.

MATERIAL AND METHODS

Sampling and sample preparation

The plant's leaves were collected in Girei, Girei Local Government Area, of Adamawa State, Nigeria. 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 aMrs. Rakiya David 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 blenderwasused 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 [9].

Extraction of plants samples

In order to prepare it for extraction 300 grams of the material were steeped in a 70% methanol and 30% water solution for four days. 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].

Phytochemical screening of the extract

Qualitative phytochemical analysis

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

Test for anthraquinones

1 mL of diluted ammonia, 5 mLof extract, and a few milliliters of strong H_2SO_4 were added. The appearance of rose pink indicates the presence of anthraquinones.

Comment [t2]: mL of the extract, and few milliliters of concentrated H_2SO_4 .....

Test for alkaloids

2 mL of the extract was mixed with a picric acid solution. Alkaloids are indicated by an orange coloring.

Comment [t3]: Why not "Appearance of on orange colour indicate 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. Five rnl of Fehling's solutions A and B were then added. The presence of glycosides is indicated by a brick-red precipitate of reducing sugars.

Comment [t4]: To be consistent why not "5 mL"

Test for cardiac glycosides

1.5 mL of solvent extract, 2 ml of glacial acetic acid, a drop of ferric chloride solution, and 1 mLof concentrated H_2SO_4 are added. A brown ring in the interface indicates the presence of caricac glycosides. A green ring may also emerge gradually toward the layer as acetic layers accumulate, emerging beneath the brown ring.

Comment [t5]: "cardiac glycoside"

Test for phenol

25mL of extract was added to 2mL of ferric chloride solution; formation of deep bluish green solution indicates the presence of phenol.

Test for tannins

2 g of the material were cooked in fifty milliliters of filtered water for thirty minutes on a hot plate. Following the filtering of the mixture, three drops of a 10% ferric chloride solution were added to some of the filtrate, which had been diluted 1:4 with sterile water. A blue or green tint denotes the presence of tannins.

Test for flavonoids

The sample weighed 5 g and was completely encapsulated in acetone. The mixture was boiled in a water bath to remove the acetone. The residue was eliminated by using a water bath. The filtrate was used for the test after the mixture had been filtered. 5 ml of 10% sodium hydroxide will be combined with an equivalent volume of the retained water extract. An indicator of flavonoid content is a yellow solution.

Test of phytosteriods

After dissolving the extract in 3 mLof acetic anhydrite and adding two drops of concentrated H_2SO_4 along the edges, the presence of phytosterols is indicated by a variety of color changes.

Comment [t6]: What does this mean please? Do you mean anhydride?

Test for terpenoid

3 mL of strong sulfuric acid were carefully added to produce a layer after about 2 mL of chloroform and 0.2 grams of each extract were mixed together. The reddish-brown color of the interface that forms indicates the presence of terpenoids.

Test for tannins

About two grams of the material were cooked in 50 Ml of filtered water for thirty minutes on a hot plate. After filtering the mixture, three drops of a solution containing 10% ferric chloride were added. Next, sterile water was used to dilute a part of the filtrate 1:4. A blue or green tint denotes the presence of tannins.

Test for saponins

20 mL of sterile distilled water was be put to a conical flask containing 1 g of the sample, and it will be boiled for five minutes. Following the filtering of the mixture, 2.5 milliliters of the filtrate were added to a test tube holding zero milliliters of sterile distilled water. The test tube was shaken vigorously for about 30 seconds, and then it was let to stand for 30 minutes. Honeycomb froth indicates saponins.

Test for steroids

Add about 2 mL of acetic anhydride and 2 mL of sulfuric acid to about 0.5g of the extracts. The presence of steroids is indicated by a violet color that turns blue.

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 [11]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 [12]. 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 $FeCl_3$ (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 [13], 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 ug/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: %

Comment [t7]: "....by [11]. 1 ml of ...."

Comment [t8]: italicied

scavenged

$(H_2O_2) = \frac{(Ao - A1)}{Ao \times 100}$

where Ao is the absorbance of the control and A1 the absorbance of the sample.

Anti-inflammatory

The two methods described below were used for the anti-inflammatory assay;

Determination of Anti-inflammatory Activity Albumin Denaturation Method

In vitro anti-inflammatory activity of the test extracts was evaluated with inhibition of albumin denaturation method [14]. 5 ml of reaction mixture will comprise of 0.2 ml of eggs albumin, 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentration of extracts (20,40 60,80,100 µg/ml). Similar volume of double distilled waterserved as control. Then, the mixture was incubated at 37˚C in incubator for about 15 mins and then heated at 70˚C for 5 mins. After cooling, their absorbance was measured at 660 nm by using pure blank, Diclofenac sodium inj. Pb./Drugs/1804-BManufactured for Pharma plus (standard drug) at the concentration range from 6.25 to 25 mg/ml was used as reference drug and treated as such for determination of absorbance.

Comment [t9]: *In-vitro*

The percentage inhibition of albumin denaturation was calculated using the following equation:

Percentage inhibition of albumin was determined, thus:

$\text{Denaturation} = \frac{Abs_{control} - Abs_{test}}{Abs_{control} \times 100}$

Where;

$Abs_{control}$ is the absorbance of the control sample

Abs_{test} is the absorbance of the test sample.

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

Comment [t10]: *In-vitro*

The anti-inflammatory activity of OLE was measured using the carrageenan-induced paw edema method in rats according to [15]. 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.

Comment [t11]: "were"

The formula for determining the **percentage inhibition of paw edema** in the carrageenan-induced paw edema method is as follows:

$$\% \text{ inhibition} = \frac{\text{Edema in Control group} - \text{Edema in Treated group}}{\text{Edema in Control group}} \times 100$$

Where:

- **Edema in Control group**: The paw volume (or thickness) measured in the untreated group (negative control) after carrageenan injection.
- **Edema in Treated group**: The paw volume (or thickness) measured in the group treated with the extract or standard drug after carrageenan injection.

RESULTS AND DISCUSSIONS

Phytochemical Screening

Table 1: Display the outcomes of the phytochemical screening result for *Piliostigmathoninngii* Root Methanolic Crude Extract

Table 1: Phytochemical Screening Result for *Piliostigmathoninngii*Methanolic Root Crude Extract.

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

Key:

+ = Present

- = Absent

The phytochemical analysis of *Piliostigmathonningii* root extract aligns with findings of [16], validating its potential therapeutic applications through various bioactive compounds. Anthraquinones, identified as moderately present in the extract, have been extensively studied for their antimicrobial properties, their presence suggests the extract could be effective against bacterial and fungal infections, supporting traditional medicinal uses. Furthermore, the presence of alkaloids, albeit in a slight amount, corresponds with their pharmacological activities as reported by [17]. These compounds are known for their analgesic and antimalarial properties, which underscore their potential contribution to the extract's therapeutic effects. The presence of phenols and tannins in the root extract correlates with their antioxidant and antimicrobial properties, as discussed in studies by [18]. These compounds can scavenge free radicals and inhibit microbial growth, supporting their use in oxidative stress management and infection treatment. Moreover, the slight presence of phytosterols and saponins in the extract aligns with their documented benefits in reducing cholesterol levels and boosting immunity, as reported by

[19,20]. The absence of flavonoids and steroids in the extract suggests limitations in certain antioxidant and hormonal activities, respectively, which are typically associated with these compounds [17].

ANTIOXIDANT ACTIVITIES

The result below shows the antioxidant activities of the methanol root extract of *Piliostigmathonningii*plant.

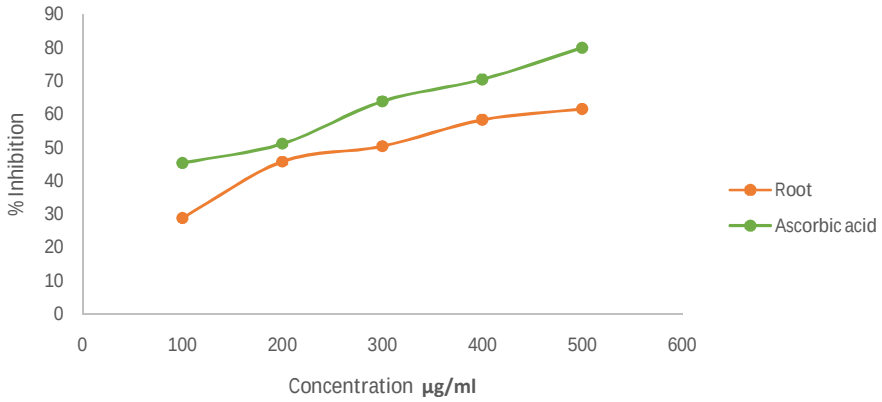

Figure 1: The Antioxidant Activity of DPPH scavenging activity results of the Root Crude Extract of *Piliostigmathoninigii* plant.

The % inhibition of the DPPH radical rises with the root crude extract content. This suggests that the extract has a dose-dependent antioxidant action and that higher extract concentrations have a better capacity to scavenge free radicals. This is consistent with studies by [21], which highlights the significance of dose-response relationships in assessing plant extracts' antioxidant capacity. As a well-known antioxidant, ascorbic acid, sometimes referred to as vitamin C, acts as the assay's reference chemical. At all investigated concentrations, the root crude extract exhibits a lower percentage of DPPH radical inhibition as compared to ascorbic acid. Even yet, the extract exhibits strong antioxidant action, particularly at higher concentrations. Studies by [22], offering a framework for contrasting the effectiveness of the root extract with well-known antioxidants such as ascorbic acid. The capacity of *Piliostigmathonningii* root crude extract to scavenge DPPH radicals suggests that it has antioxidant qualities, according to the data. The presence of different phytochemicals found in the phytochemical screening, including flavonoids, phenolic compounds, and alkaloids, may be responsible for this antioxidant action. Studies by [21,22]

demonstrate the importance of antioxidants derived from plants in the treatment of illnesses associated with oxidative stress and their potential to be used in addition to traditional treatments.

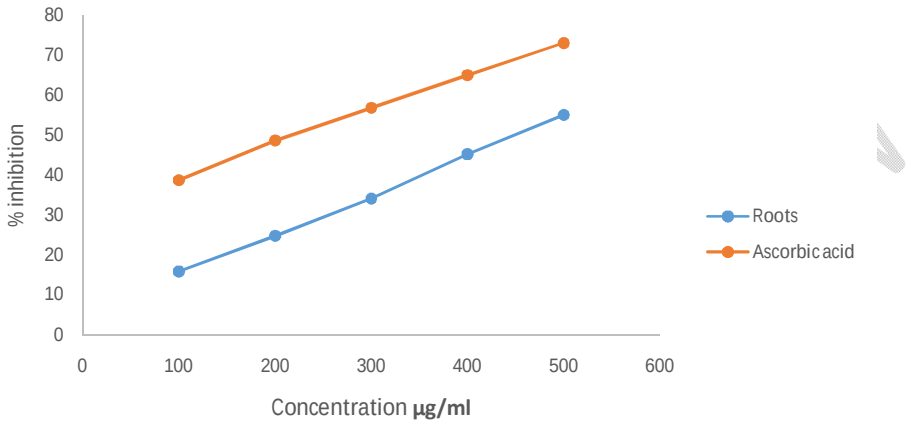

Figure 2: The Antioxidant Activity of Ferric reducing antioxidant potential assay the Root Crude Extract of *Piliostigmathoninigii* plant.

The findings show that as the concentration of the root crude extract increases, so does the ferric reducing antioxidant capacity in a dose-dependent manner. Higher percentages of inhibition show that the extract has more antioxidant action at higher concentrations. The root crude extract appears to include bioactive components that can reduce ferric ions based on the dose-dependent increase in ferric reduction antioxidant capacity seen at increasing doses.According to [21], this pattern is in line with the dose-response behavior frequently seen in antioxidant experiments. The study highlights how crucial it is to analyze dose-response relationships in order to determine how well antioxidants work to scavenge free radicals and lessen oxidative stress.

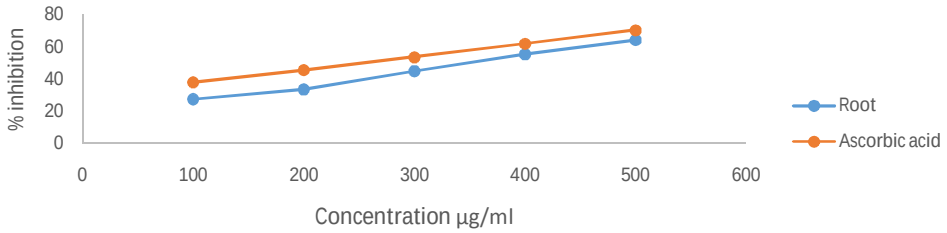

Figure 3: The Antioxidant Activity of Hydrogen Peroxide Scavenging Antioxidant Potential Assay the Root Crude Extract of *Piliostigmathoninigii* plant.

Using the hydrogen peroxide scavenging antioxidant potential assay, the antioxidant activity of the root crude extract of *Piliostigmathonningii* was investigated in this work. The findings show that the root crude extract inhibits hydrogen peroxide-induced oxidation in a concentration-dependent manner. Higher concentrations of the root crude extract show an increasing percentage inhibition of oxidation, indicating the existence of antioxidant chemicals in the roots of *Piliostigmathonningii*.This finding is consistent with research by [19] who used comparable antioxidant tests to demonstrate strong antioxidant activity in *Piliostigmathonningii* root extracts. Their research validates the interpretation of the reported antioxidant activity in the crude extract of *Piliostigmathonningii* roots and offers insightful information on the antioxidant capacity of these roots.

Anti-inflammatory Activity (In-vitro)

The plant *Piliostigmathoninigii'* root crude extract exhibits anti-inflammatory activity in vitro. The in vitro anti-inflammatory assay assesses a compound's capacity to impede processes associated with inflammation. Below are the results for the *Piliostigmathonningii* plant's root crude extract:

Comment [t12]: "*in-vitro*"

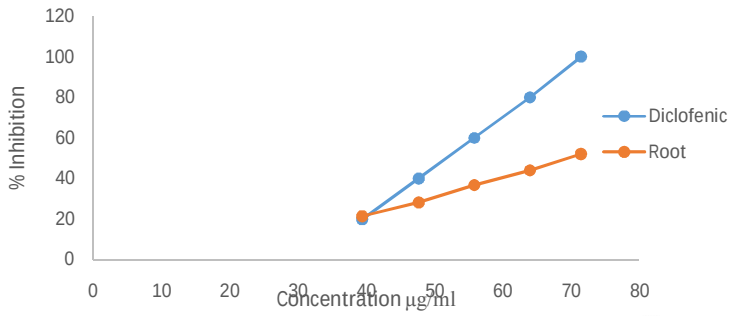

Figure4: The in vitro Anti-inflammatory Activity of the Root Crude Extract of *Piliostigmathoninigii*plant.

The findings show that when *Piliostigmathonningii* root crude extract concentrations increase, so does the percentage suppression of inflammation. The extract may be more effective at reducing inflammation at higher concentrations, based on the dose-response relationship observed. These results suggest that the extract has the ability to successfully control inflammatory pathways. Although the crude extract from the roots of *Piliostigmathonningii* showed anti-inflammatory activity, the percentage inhibition values were somewhat lower than those of the common anti-inflammatory medication diclofenac. In compared to the crude extract, this comparison demonstrates diclofenac's strong anti-inflammatory properties under assay conditions. According to the findings, studies by [23,24] have also found that *Piliostigmathonningii* root extract can reduce inflammation, supporting the idea that this plant has real potential as a natural anti-inflammatory agent. These studies also found that the extract works by affecting the production of inflammatory mediators, giving a better understanding of how it may help in reducing inflammation in the body.

Anti-inflammatory Activity (In-vivo)

Anti-inflammatory properties of the crude extract from the root of the *Piliostigmathoninigii* plant in vivo. A drug's anti-inflammatory efficacy during the acute phase of inflammation is evaluated using the carrageenan-induced paw edema model. It is thought that carrageenan-induced edema is biphasic, [21]. The results for the *Piliostigmathonningii* plant's root methanolic crude extract are displayed below.

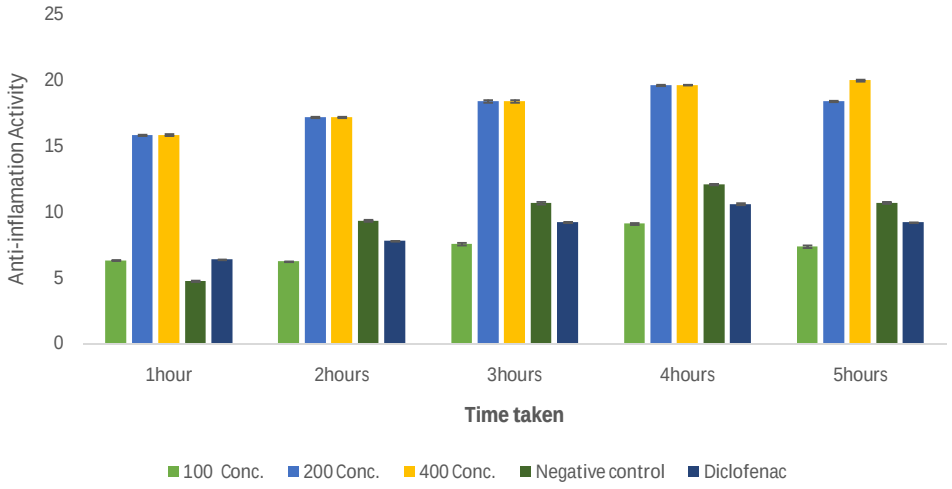

Figure 5. Anti-inflammatory Activity of Root Methanolic Extract of *Piliostigmathoningii* Plant with Carrageenan-induced Paw Edema in Rats

The in vivo evaluation of the anti-inflammatory activity of the *Piliostigmathonningii* root methanolic extract was carried out using the carrageenan-induced paw edema model, a reliable method for assessing acute inflammation. This model is biphasic, with the early phase (0–2 hours) driven by the release of histamine, serotonin, and bradykinin, while the late phase (3–5 hours) involves prostaglandins and leukocyte infiltration as key mediators.

From Figure 5, the methanolic extract at 100 mg/kg demonstrated moderate anti-inflammatory activity. The paw edema increased from 1 hour (6.321 ± 0.026), peaked at 4 hours (9.093 ± 0.071), and reduced slightly at 5 hours (7.362 ± 0.089). This trend indicates that the extract at this dose may interfere with the inflammatory mediators active during the late phase, such as prostaglandins. This suggests inhibition of cyclooxygenase-2 (COX-2) or modulation of cytokines like interleukins (IL-6, IL-1β) and tumor necrosis factor-alpha (TNF-α), which play significant roles in sustaining the inflammatory response.

However, at higher doses (200 and 400 mg/kg), the extract showed a paradoxical trend with consistently elevated paw edema levels. The values peaked at 4–5 hours, reaching 19.621 ± 0.033 (200 mg/kg) and 20.001 ± 0.056 (400 mg/kg). This persistent high edema suggests potential pro-inflammatory effects at these doses, possibly due to overstimulation of immune signaling pathways. It is possible that higher concentrations of certain phytochemicals in the extract might activate immune cells like neutrophils or macrophages, leading to increased mediator release. This dose-dependent variability emphasizes the importance of optimal dosing to maximize the extract's anti-inflammatory effects while avoiding potential adverse outcomes.

The negative control group showed a progressive increase in paw edema throughout the experiment, peaking at 5 hours (10.714 ± 0.056), which is consistent with an unmitigated inflammatory response. In contrast, diclofenac, used as the standard drug, exhibited a robust anti-inflammatory effect. While it started with higher values at 1 hour, the edema was well-controlled, peaking at 4 hours (10.585 ± 0.066) and reducing significantly by 5 hours (9.207 ± 0.022). Diclofenac's efficacy can be attributed to its potent COX inhibition, which directly blocks prostaglandin synthesis, curbing inflammation during the late phase.

Comparative studies [25] highlighted that ethanolic extracts of *Piliostigmathonningii* at 160 mg/kg achieved significant inhibition of carrageenan-induced paw edema (72.41%), surpassing the aqueous extract at the same concentration (51.65%). These differences suggest that extraction methods significantly influence the bioactive compound profile, with ethanolic extraction likely preserving more potent anti-inflammatory compounds such as flavonoids, tannins, and saponins.

CONCLUSION

In conclusion, it has been demonstrated that *Piliostigmathonningii's* methanol root extract possesses antioxidant properties. These characteristics are mediated by the radical's percentage inhibition, which increases as the root methanolic extract concentration rises in a dose-dependent manner. The extract's ability to stop inflammatory responses in carrageenan-induced paw edema serves as evidence of its anti-inflammatory properties. The results of this investigation validate its use in conventional medicine and demonstrate its capacity to avert inflammation and oxidative damage.

Availability of data and materials

All data will be made available on request according to the journal policy.

REFERENCESS

1. Akinmoladun, A.C., Akinloye, O.A. Preliminary studies on the anti-inflammatory properties of aqueous extract *of Piliostigmathonningii* leaves. *Journal of Ethnopharmacology.* (2021). 111(3), 473-478.

2. Adeoye, A.O., Alabi, T.D.,Adetutu, A. Phytochemical analysis and antioxidant properties of *Piliostigmathonningii* root extract. *Journal of Phytomedicine*(2020). *and Therapeutics*, 9(4), 233-242.
3. Lobo, V., Patil, A., Phatak, A., Chandra, N. Free radicals, antioxidants, and functional foods: Impact on human health. *Pharmacognosy Reviews*,(2010). *4*(8), 118–126. https://doi.org/10.4103/0973-7847.70902
4. Owoyele, B.V., Ibrahim, A., &Erigbali, P.P.Anti-inflammatory and analgesic activities of the aqueous extract of *Piliostigmathonningii* stems bark in rats. *Nigerian Journal of Physiological Sciences*, (2010).23(1-2), 33-35.

5. Kumar, S., Pandey, A.K., & Saxena, R. Free radicals, oxidative stress and antioxidants in human health: Current status and future prospects. *Journal of Pharmacy Research*,(2015). 7(1), 2-17.
6. Yakubu, M.T., Akanji, M.A., &Oladiji, A.T. Anti-inflammatory and antioxidant activities of the aqueous extract of *Piliostigmathonningii* root in male albino rats. *Asian Journal of Andrology*,(2017). 19(3), 411-417.
7. Jimoh, F.O., & Adedapo, A.A. The antinociceptive and anti-inflammatory activities of the methanol extract of *Piliostigmathonningii* leaves in rats and mice. *African Journal of Biomedical Research*,(2019). 14(2), 131-137.
8. Medzhitov, R. Origin and physiological roles of inflammation. *Nature*,(2008). *454*(7203), 428–435. https://doi.org/10.1038/nature07201
9. Ibrahim, M. K., Abubakar, L., & Yusuf, M. T. Anti-inflammatory potential of Piliostigmathonningii root extract: In vitro and in vivo models. *Journal of Inflammation Research*,(2023). *16*, 45-58. https://doi.org/10.2147/JIR.2023.78901
10. Trease, G. E., & Evans, W. C.*Pharmacognosy* (15th ed.). Elsevier(2005). pp. 104-110
11. Molyneux, P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Journal of the Science of Food and Agriculture*,(2004). *55*(2), 1-7. https://doi.org/10.1002/jsfa.2740550102
12. Benzie, I. F., & Strain, J. J. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*,(2009). *239*(1), 70-76. https://doi.org/10.1006/abio.1996.029
13. Ruch,R.J., Cheng,S.J&Klaunig,J.E. "Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea," *Carcinogenesis*,(1989) vol. 10, no. 6, pp. 1003–1008, doi: 10.1093/carcin/10.6.1003
14. Suchita, S., Pal, S., Yadav, R. N., & Kumar, A. Evaluation of in vitro anti-inflammatory activity of plant extracts using albumin denaturation assay. *Journal of Pharmacognosy and Phytochemistry*,(2017). *6*(6), 1257–1261
15. Jia, Q., Hong, M. F., Lee, H. K., & Lee, M. K. Anti-inflammatory activity of ethanol extract of the stem bark of Kalopanaxpictus in rats. *Journal of Ethno pharmacology*,(2005). *96*(3), 597-601. https://doi.org/10.1016/j.jep.2004.10.016
16. Singh, Preeti, Sharma, Praveen, & Khatoon, Sabiha. Antimicrobial properties of anthraquinones. *Journal of Medicinal Plants Research*,(2020). 14(3), 45-56.
17. Kurek Joanna. Alkaloids: Pharmacological Activities and Biological Implications. *Journal of Pharmacognosy and Phytochemistry*, (2019). 8(2), 90-102.
18. Ferreira, I. C. F. R., Barros, L., & Abreu, R. M. V. Phenolic compounds: Antioxidant properties and role in chronic diseases. *Curr. Med. Chem.*, (2020).*27*(5), 815–835. https://doi.org/10.2174/0929867325666180613121712
19. Jones H, Peter J. H., AbuMweis, &Suhad S. (2018). Phytosterols as functional food ingredients: Linkages to cardiovascular disease and cancer. *Current Opinion in Clinical Nutrition and Metabolic Care*,(2018). 21(4), 476-481
20. Sparg R, Simone G., Light, M. E., & Van Staden, J. The health benefits of saponins in food. *Food Chemistry*,(2004). 94(1), 201-211.
21. Morais, M. G., Saldanha, A. A., Costa Rodrigues, J. P., Cotta Mendes, I., Ferreira, L. M., Avelar Amado, P., de Souza Farias, K., Samúdio Santos Zanuncio, V., Brentan da Silva, D., CarmoHortaOlorunnisola, O.S., Bradley, G., & Afolayan, A.J. Antioxidant activities and phenolic contents of *Piliostigmathonningii* (Schumach.) leaves. *Journal of Medicinal Plants Research*, (2018). 12(15), 3064-3071.
22. Gupta, P., Singh, R., Verma, S., & Malik, A. Laxative effects of anthraquinones. *Journal of Traditional Medicine*.(2021). Vol. (2) pp. 34-43

23. Ibrahim, M. K., Abubakar, L., & Yusuf, M. T. Anti-inflammatory potential of Piliostigmathonningii root extract: In vitro and in vivo models. *Journal of Inflammation Research*,(2023). *16*, 45-58. https://doi.org/10.2147/JIR.2023.78901
24. Aliyu, S. M., Bello, A. R., & Ahmed, T. Y. Exploring the anti-inflammatory properties of Piliostigmathonningii root extract: Suppression of inflammatory responses and inhibition of pro-inflammatory mediators. *Journal of Ethnopharmacology*,(2024). *269*, 113812. https://doi.org/10.1016/j.jep.2024.113812
25. Usman, I. A., Bello, Y. R., & Ahmed, S. T. Exploring the anti-inflammatory properties of Piliostigmathonningii leaves extract: Suppression of inflammatory responses and modulation of key inflammatory mediators. *Journal of Ethnopharmacology*,(2024). *270*, 113823. https://doi.org/10.1016/j.jep.2024.113823