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Antioxidant And Anti-Inflammatory Effects Of Ethanolic And Aqueous Root Extracts Of *Piliostigma Thonningii* (Schumach.) Milne-Redhead

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Abstract

Piliostigma thonningii is a tree with many ethnomedical and medico-religious uses. In Ivory Coast the roots associated with the leaves are used as an antidote for snakebites and for the treatment of gonorrhea and rheumatism. 2,2-Diphenyl 1-picrylhydrazyl is a free radical. This compound is used to measure the antioxidant activity of a plant by its ability to scavenge free radicals from DPPH. Antioxidants help protect our body against free radicals and thus prevent many diseases. The pharmacological importance of *Piliostigma thonningii* seems to be ignored by a large part of the population. The study of anti-inflammatory activity *in vivo* which consists of provoking an acute inflammation induced in rats by injection of carrageenan. AEPT and EEPT show significant anti-inflammatory activity. They very significantly inhibit the development of paw edema induced by carrageenan. They

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significantly reduce the recruitment of immune cells to the inflammatory site. *Piliostigma thonningii* extracts work by blocking the formation of prostaglandins, the substances responsible for inflammation. The results of study suggest that *Piliostigma thonningii* possess some antioxidant properties and provide relief against inflammation making it a possible future therapy for inflammation.

Keywords: *Piliostigma Thonningii*, Antioxidant, Anti-Inflammatory, IC_{50}, DPPH

Introduction

On the one hand, an antioxidant is an agent that prevents or slows down oxidation by neutralizing free radicals. In the body, cellular respiration generates reactive oxygen species which can be the source of free radicals (Hortense *et al.*, 2021). On the other hand, inflammation corresponds to a set of reactions generated by the body in response to an attack. This can be external like an injury, infection, trauma, or internal like those observed in autoimmune pathologies (Azab *et al*., 2016).

The West African plant *Piliostigma thonningii*, (Milne-Redhead) belongs to the subfamily Caesalpinioideae in the legume family, Leguminosae/Fabaceae. In African countries *Piliostigma thonningii* is used for various medicinal purposes. The decoction of the leaves and bark is used for the treatment of ulcers, wounds, heart pain, arthritis, malaria, pyrexia, leprosy, sore throat, diarrhea, toothache, gingivitis, cough, and bronchitis. Its roots and twigs are used in the treatment of dysentery, fever, wound infections, cough, and skin diseases (Afolayan *et al*., 2018). The crude extract of *Piliostigma thonningii* was reported to possess antilipidemic, antibacterial, antihelminthic, and anti-inflammatory activities (Ighodaro *et al*., 2012). In view of the ethnomedicinal information and uses of *Piliostigma thonningii*, the aim of this study was to investigate *in vitro* antioxidant activities of the aqueous and ethanolic root extracts of this plant, as a preliminary step towards validation of anti-inflammatory effects.

Materials

Plant Material Collection and Preparing power

The root of *Piliostigma thonningii* was collected in a forest in the village of Abatta in October 2018 (Abidjan, Ivory Coast). It was identified and authenticated by the botanists of the Laboratory of Environmental Sciences and Technologies, University Jean Lorougnon Guédé Daloa, Ivory Coast. First, the fresh root of the plant was cut and broken into small pieces and oven dried. Then it was crushed to obtain a brown powder and finally kept in a jar.

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Preparing the Experimental Animals

Albino rats weighing between 100-150 g of University Jean Lorougnon Guédé were used. Animals were acclimated during one week before the experiments. They were fed and maintained under standard lighting conditions at a temperature of 29ºC ± 1ºC. They were fasted for 24 hours before experiments, while water was given *ad libitum* (Sylla *et al*., 2021).

Methodology

Preparation and Extraction of Plant Material

A modified method as described by Valan & Oladimeji (2021) was used in this part of the study. The preparation of the extracts was done by the standard cold maceration extraction method because the heat destroys the active constituents of the medicinal plant, cold maceration is more suitable than a decoction. Two solvents of different polarity are used, namely distilled water and ethanol. The maceration was then filtered, and the filtrate was dried in a hot air oven set at 80 °C for 24 h and weighed again. The difference in the weight was determined and percent of water-soluble and alcohol soluble extractives was calculated (Hortense *et al*., 2021). The dried extract obtained was kept for experiments. **AEPT** = Aqueous root extract of *Piliostigma thonningii* and **EEPT** = Ethanolic root extract of *Piliostigma thonningii*.

Qualitative Phytochemical Screening of the Extracts

Qualitative phytochemical screening was done on the two extracts of *Piliostigma thonningii* root using standard procedures described by Sinan *et al*., (2021) to determine the phytochemicals present in the plant extract. The extracts (5 mg) were dissolved in 50 mL of the respective solvents used for their extraction. The solution was made ready for qualitative phytochemical analysis by the following methods, Table 1.

Table 1: Usual methods of phytochemical screening

Phytocompounds	Reagent of identification	Indicator (positive reaction)
Anthraquinones	NH_4OH	Yellow color
Anthocyanin	H_2SO_4 and NH_4OH	Black color
Terpenoids	$CHCl_3$, H_2SO_4	Brown color
Polyphenols	$FeCl_3$ (2%)	Dark blue or greenish color
Flavonoids	Hydrochloric alcohol, Magnesium shavings and Iso-amyl alcohol	Pink-orange or purplish color
Catechic tannins	Formalin and HCl	Gelatinous precipitate
Gallic tannins	Sodium acetate and $FeCl_3$	Blue-black color
Free quinones	NH_4OH	Red to purple color
Saponosides	Foam index	Persistent foam
Alkaloids	$HgCl_2$ and KI (Mayer)	Reddish-brown precipitate
	Picric acid (Hager) I_2 and KI (Wagner)	Creamy-white precipitate

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Coumarins	KOH and HCl	Trouble or precipitate
Sterols and polyterpenes	Acetic anhydride acid and H_2SO_4	Color from purple to blue or green
Mucilage	Absolute ethanol	Flocculent precipitate
Volatile oils	NaOH and HCl	Black color
Cardiac glycosides	$CHCl_3$, H_2SO_4	Brown color

In vitro DPPH Free Radical Scavenging Antioxidant Activity

The antioxidant activities of plant extracts were assessed using their ability to scavenge the activity of the free radicals of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH). The method of Owolabi *et al*., (2018) was employed. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) is an oxidant having in its structure an odd electron. Its purple color is reduced to yellow colored diphenylpicrylhydrazine when it is in contact with an antioxidant that can release a hydrogen atom or electron to it. The change in color was measured at 520 nm using a UV/Visible light Spectrophotometer (Shimadzu UV-1280). DPPH solution was made by adding 6 mg of DPPH in 100 mL of methanol and allowing it to dissolve. About 2 mL of DPPH solution (0.1 mM) was added to 1 mL of various concentrations of the extracts (0.020, 0.040, 0.06, 0.080, 0.100 mg/mL). A mixture of methanol and DPPH was used as control. All mixtures were vigorously shaken and made to stand in a dark place for 30 min. After this, solution absorbance was measured at 520 nm using a spectrophotometer. The experiments were performed in triplicates and the percentage scavenging activity of the extracts on DPPH radical was calculated on the basis of the formula below (Owolabi *et al*., 2018)

$$Percentage\ Scavenging\ Activity\ (PSA) = \frac{1 - Absorbance\ of\ the\ control}{Absorbance\ of\ the\ control} \times 10$$

$$Percentage\ inhibition\ (PI) = \frac{Absorbance\ of\ the\ control\ - Absorbance\ of\ the\ sample}{Absorbance\ of\ the\ control} \times 100$$

IC_{50} values were used to express the ability of the extracts to scavenge DPPH. The term "IC_{50}" which connoted the concentration of the extract needed to scavenge 50% of DPPH radical, was calculated using the graph of scavenging activity plotted against sample concentration using Microsoft Excel software (Owolabi *et al*., 2018).

Determination of *In vivo* Anti-inflammatory Activity of the Extracts (Carrageenan-induced paw edema model)

The rats were divided into eight groups (n = 6); Grp 1: negative control (10 mL/kg, Distilled water); Grp 2: positive control (80 mg/kg bw, Diclofenac, reference standard); Grp 3: treated with 50 mg/kg bw of AEPT; Grp 4: treated

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with 80 mg/kg bw of AEPT; Grp 5: treated with 160 mg/kg bw of AEPT; Grp 6: treated with 50 mg/kg bw of EEPT; Grp 7: treated with 80 mg/kg bw of EEPT; Grp 8: treated with 160 mg/kg bw of EEPT; Carrageenan (0.1 mL of 1%) was injected into the subplantar tissue of the right hind-paw of each rat. The volume of the carrageenan injected into the foot was measured at 0, 30, 60, 120, and 180 minutes using a plethysmometer. The method of Meshram *et al*., (2015) was employed. The percentage inhibition (PI) at each time interval was calculated:

$$Percentage\ inhibition\ (PI) = \frac{(Vt - Vo)control - (Vt - Vo)treated}{(Vt - Vo)control} \times 100$$

Where Vt = Volume of the paw edema at particular time interval (t);
Vo = Volume of the paw before induction of inflammation (0 hour);
(Vt-Vo)control = Volume of edema of the control group of rats;
(Vt-Vo)treated = Volume of edema in the group of treated rats.

Statistical Analysis

The analysis of variance was used to compare the averages between more than two groups. Values with $p < 0.05$ were considered statistically significant. Graphs were obtained using the Microsoft Excel 2016 spreadsheet. Statistical analyzes were performed in GraphPad Prism for Windows.

Results

Yield Extraction

The percentage yield of ethanolic and aqueous extracts of *Piliostigma thonningii* is presented in Table 2.

Table 2: Percentage yield of EEPT and AEPT

Extract	Mass (g)	Yield (%)
EEPT	4.75	9.50
AEPT	4.05	8.10

Phytochemicals Qualitative Aqueous and Ethanolic extracts of *Piliostigma thonningii*

Phytochemical screening test results of *Piliostigma thonningii* (EEPT and AEPT) are presented in Table 3. The most important types of phytochemicals found in this species are Cardiac glycosides, Sterols and polyterpenes, Terpenoids, Alkaloids, Anthocyanins, Catechic tannins, Gallic tannins, Free quinones, Saponins, Polyphenols and Flavonoids.

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Table 3: Results of phytochemicals analysis

Secondary metabolites	EEPT	AEPT
Cardiac glycosides	+	+
Coumarins	-	-
Mucilages	-	-
Volatile oils	-	-
Anthraquinones	-	-
Sterols and polyterpenes	+	+
Terpenoids	+	+
Alkaloids	+	+
Anthocyanins	+	+
Catechic tannins	+	+
Gallic tannins	+	+
Free quinones	+	+
Saponins	+	+
Polyphenols	+	+
Flavonoids	+	+
+ = Positive means present, - = Negative means absent.		

The free radical scavenging ability of extracts on DDPH

Antioxidant potential is inversely proportional to inhibitory concentration (IC_{50}) value which was calculated from the linear regression of the percentage inhibition versus extract concentration. The results of the inhibition study are presented in Figure 1 and Table 4. The results in Table 4 presents the 50% Inhibitory Concentration (IC_{50}) values and DPPH radical scavenging activities at 1.00 mg/mL of ethanolic and aqueous extracts of *Piliostigma thonningii*. The result shows that ethanol extract has IC_{50} value (0.052 mg/mL) and aqueous (0.063 mg/mL) compared with vitamin C which had an IC_{50} of 0.047 mg/mL.

Table 4: DPPH Inhibitory Concentration (IC_{50}) of the extracts of *Piliostigma thonningii*

Extract	IC_{50} values (mg/ml)	Scavenging activity at 0.10 mg/ml (%)
EEPT	0.052	80.49 ± 2.44
AEPT	0.063	67.48 ± 3.12
Vitamin C	0.047	90.24 ± 2.44

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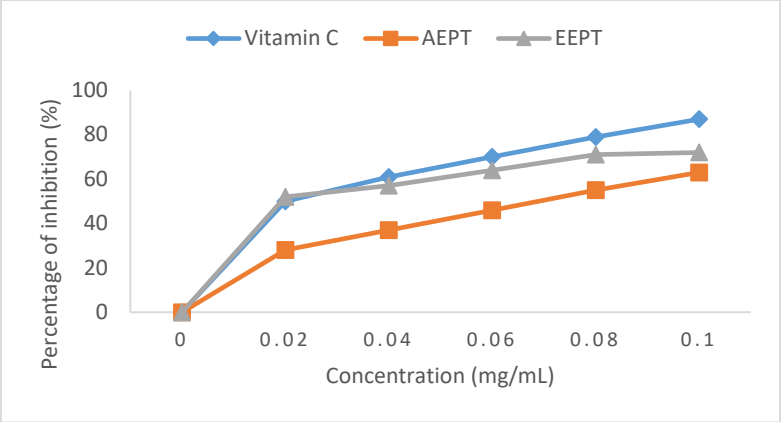

Figure 1: DPPH radical scavenging activities of AEPT and EEPT

Anti-inflammatory Activity of *Piliostigma thonningii* Extracts (Carrageenan-induced paw edema model)

The evaluation of anti-inflammatory activity of aqueous and ethanolic extracts of the roots of *Piliostigma thonningii* (50, 80, 160 mg/kg, p.o.) showed a dose-dependent, significant inhibition of carrageenan-induced rat paw edema from 30 minutes to 180 minutes following drug administration, compared to the control group. The maximum PI of paw edema by the aqueous extract was observed as 66.66, 68.96 and 71.26 at the doses of 50, 100, 200 mg/kg p.o., respectively. The maximum PI of paw edema by the ethanolic extract was observed as 68.96, 70.11 and 72.41 at the doses of 50, 80, 160 mg/kg p.o., respectively. Diclofenac 80 mg/kg p.o. showed a maximum PI of 64.37% at 180 minutes after its administration (Table 5).

The aqueous and ethanolic extracts (160 mg/kg) of *Piliostigma thonningii* inhibits acute inflammation induced by carrageenan (Figure 2). This inhibition is much more pronounced 30 minutes after plantar injection of carrageenan with maximum values observed 3 hours after administration (Figure 2). However, these effects are more important than those observed with diclofenac, a non-steroidal anti-inflammatory used in the study as a reference drug. Indeed, carrageenan is a mucosaccharide whose administration in the intraplantar way to rats causes acute inflammation that induce edema, all under the influence of vasoactive mediators. The AEPT and EEPT (160 mg/kg) inhibits the progression of edema to varying degrees. This suggests that it interferes with the effects which inhibit the release of mediators involved in these phases of inflammation.

Table 5: Effect of the AEPT and EEPT with carrageenan-induced paw edema in rats

Paw volume (mL) PI = Percentage of inhibition (%)						
Groups	**Before**	**0 min**	**30 min**	**60 min**	**120 min**	**180 min**

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Distilled water (10mL/kg)	0.99 ± 0.01	1.03 ± 0.02	1.72 ± 0.02	1.90 ± 0.01	2.53 ± 0.03	1.96 ± 0.01
Diclofenac (80 mg/kg)	1.00 ± 0.01	1.02 ± 0.01	1.48 ± 0.03 (33.33)	1.5O ± 0.03 (44.83)	1.60 ± 0.02 (61.59)	1.36 ± 0.01 (64.37)
AEPT (50 mg/kg)	0.99 ± 0.02	1.01 ± 0.03	1.62 ± 0.03 (11.60)	1.71 ± 0.01 (19.54)	1.85 ± 0.03 (44.37)	1.30 ± 0.03 (66.66)
AEPT (80 mg/kg)	0.99 ± 0.02	1.02 ± 0.03	1.55 ± 0.02 (23.19)	1.61 ± 0.03 (32.18)	1.75 ± 0.03 (51.65)	1.29 ± 0.01 (68.96)
AEPT (160 mg/kg)	0.99 ± 0.01	1.03 ± 0.02	1.65 ± 0.03 (10.14)	1.63 ± 0.02 (31.03)	1.84 ± 0.02 (47.68)	1.32 ± 0.01 (71.26)
EEPT (50 mg/kg)	0.99 ± 0.01	1.02 ± 0.03	1.54 ± 0.01 (24.64)	1.61 ± 0.03 (32.18)	1.71 ± 0.03 (54.30)	1.29 ± 0.01 (68.96)
EEPT (80 mg/kg)	0.99 ± 0.01	1.02 ± 0.01	1.63 ± 0.02 (11.60)	1.63 ± 0.03 (29.88)	1.74 ± 0.03 (52.32)	1.28 ± 0.01 (70.11)
EEPT (160 mg/kg)	0.99 ± 0.01	1.03 ± 0.02	1.61 ± 0.03 (15.94)	1.62 ± 0.02 (32.18)	1.76 ± 0.01 (51.65)	1.27 ± 0.01 (72.41)

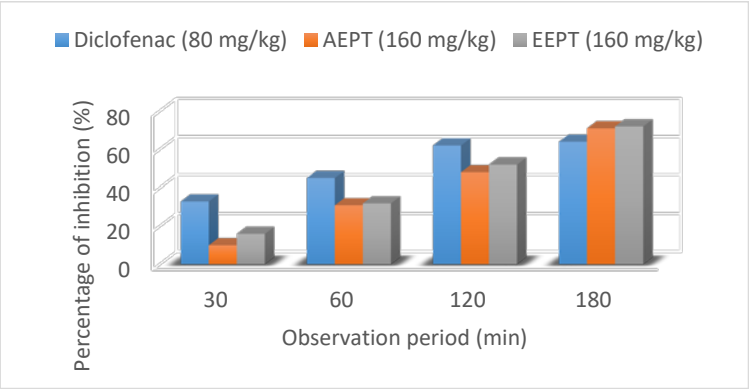

Figure 2: Effect of AEPT and EEPT on inflammation induced by carrageenan in rats

Discussion

The phytochemical screening of *Piliostigma thonningii* revealed the presence of saponins, triterpenes, tannins, flavonoids, cardiac glycosides and steroids in both types of extract. These compounds could be responsible for the obvious anti-inflammatory activities of the extracts of the plant. This is in line with the report of Ahmadiani *et al*., (2000) who stated that flavonoids as well as tannins possess anti-inflammatory effects. Phytochemical principles are responsible for the biological activity of plants. The presence of flavanoids, tannins and saponins in the aqueous and ethanolic extracts of *Piliostigma thonningii* root shows that the root may have an array of biological activities. Alkaloids are used as analgesics, antimalarials, antiseptics and antibacterial agents, saponins exhibit natural antibiotics effect by attacking bacteria and fungi (Okwu & Emenike, 2006). Alkaloids help to defend the plant against herbivores and pathogens (Tion *et al*., 2018). The bioactivities of tannins include cardioprotective activity, histamine release inhibition,

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cytotoxic activity, antidiabetic and antiobesity bioactivities (Beretta *et al*., 2009).

Many plant antioxidant potentials are related to their therapeutic potentials (Eleazu *et al*., 2011). A higher DPPH radical-scavenging activity is associated with a lower IC_{50} value. Therefore, the ethanolic extract had the highest DPPH reducing activity based on its relatively low IC_{50} values which was comparable with vitamin C, difference was observed between their IC_{50} (EEPT, 0.052; AEPT, 0.063 and Vitamin C, 0.047). A positive result by the aqueous and ethanolic extracts in this test indicates that they contain antioxidants that can scavenge free radicals (Pattanayak *et al*., 2012). Therefore, it can be used as a source of natural antioxidants and used in drug formulations for treatment of diseases resulting from oxidative stress (Azazahemad *et al*., 2020)

The carrageenan-induced paw edema model is used to screen the anti-inflammatory activity of a drug in the acute phase of inflammation. Edema induced by carrageenan is believed to be biphasic. (Morais *et al*., 2020). The first phase (1 hour) involves the release of serotonin and histamine and the second phase (> 1 hour) is mediated by cyclooxygenase products. Continuity between the two phases is provided by kinin (Graczyk *et al*., 2021). The AEPT and EEPT significantly inhibited the edema formation in both the first and second phases. The anti-edematous activity of *Piliostigma thonningii* in the first phase could be due to the possible suppression of histamine signaling by the mast cell stabilizing effect, and direct inhibition of histamine H_1 receptor and histidine decarboxylase gene transcriptions. Another possible explanation could be the corticotrophic action of *Piliostigma thonningii* as evidenced by a raise in plasma cortisol levels, which antagonizes nuclear factor-kappa-beta (NF-kß) (Li *et al*., 2020). In the present study, the anti-edematous activity of the AEPT and EEPT persisted in the second phase with the maximal effect observed at 3 hours. This could be explained by the possible inhibition of the release and/or action of kinin and prostaglandin by *Piliostigma thonningii* (Liu *et al*., 2020).

Conclusion

The aqueous and ethanolic root extracts of *Piliostigma thonningii* have *in vitro* DPPH radical scavenging. Therefore, it can be used as a source of natural antioxidants and used in drug formulations for treatment of diseases resulting from oxidative stress. The roots of *Piliostigma thonningii* possess anti-inflammatory activity thus validating the ethnopharmacological claims. This knowledge could be tapped to formulate new agents to treat inflammation.

Ethical Approval

The experimental protocols were conducted in accordance with the guidelines on the care and use of laboratory animals (Ivory Coast National Ethical Committee for Health Research in 2021).

Data Availability

All data are available within the manuscript, and additional data are available from the corresponding authors on request.

Conflicts of interest

The authors declare that no conflicts of interest exist regarding this publication.

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