In vitro anthelmintic activity against *Haemonchus contortus* adult worms and antioxidant properties of hydroalcoholic extract of fruits of *Piliostigma reticulatum* (DC.) HOCHST (Fabaceae)

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Abstract

Introduction: Low-income populations in developing countries have traditionally used medicinal plants to treat themselves and their animals for gastrointestinal parasitosis. This study aimed to investigate the anthelmintic properties of the fruits of *Piliostigma reticulatum* (DC.) Hochst.

Methods: Hydroalcoholic maceration was performed with 70% ethanol of *Piliostigma reticulatum* fruit powder. Phytochemical screening of the extracts was done using thin-layer chromatography. The content of phenolic compounds and flavonoids in the extract and the antioxidant capacity were determined by DPPH, ABTS and FRAP tests. The *in vitro* anthelmintic activity was evaluated on adult worms of *Haemonchus contortus*. Levamisole is a standard.

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Results: The assay revealed that the extract of the fruits of *P. reticulatum* is rich in phenolic compounds with 187.32 ± 0.07 µg ETG/mg and low in flavonoids to 9.4 ± 0.06 µg EQ/mg. The extract showed intense antiradical activity by ABTS test IC_{50} = 6.36 ± 0.23µg/mL and high iron reducing power 3352.45±24.70 µmol/mL. The anthelmintic activity was moderate with IC_{50} = 10.42 mg/mL against 0.27 for levamisole.
Conclusion: *Piliostigma reticulatum* fruit extract has good antioxidant activity and anthelmintic properties, which explains why it is used in treating intestinal parasites. Its traditional use in the treatment of intestinal parasitosis.

Keywords: *Piliostigma reticulatum*, anthelmintic, antioxidant, *in vitro*, Burkina Faso.

Activité anthelminthique *in vitro* sur les vers adultes *Haemonchus contortus* et propriétés antioxydantes de l'extrait hydroalcoolique des fruits de *Piliostigma reticulatum* (DC.) HOCHST (Fabaceae)

Résumé

Introduction : Les populations à faible revenu des pays en développement utilisent traditionnellement des plantes médicinales pour se soigner et soigner leurs animaux des parasitoses gastro-intestinales. Cette étude a pour objectif d'étudier les propriétés anthelminthiques des fruits de *Piliostigma reticulatum* (DC.) Hochst.
Méthodes : Une macération hydro-éthanolique70 % d'éthanol de la poudre de fruits de *Piliostigma reticulatum* a été réalisée. Le criblage phytochimique des extraits a été effectué en utilisant la chromatographie sur couche mince. La teneur en phénoliques totaux et en flavonoïdes totaux de l'extrait ont été évaluées par méthodes de complexation et la capacité antioxydante ont été déterminées par les méthodes DPPH, ABTS et FRAP. L'activité anthelminthique *in vitro* a été évaluée sur des vers adultes de *Haemonchus contortus*. Le lévamisole a été utilisé comme contrôle positif .
Résultats : L'extrait des fruits de *Piliostigma reticulatum* contient des composés phénoliques (187,32 ± 0,07 µg ETG/mg) et faible en flavonoïdes (9,4 ± 0,06 µg EQ/mg). L'extrait a montré une forte activité antiradicalaire par le test ABTS avec une IC_{50} = 6,36 ± 0,23 µg/mL et un pouvoir de réduction du fer de 3352,45 ± 24,70 µmol/mL. L'activité anthelminthique était modérée avec IC_{50} = 10,42 mg/mL contre 0,27 mg/mL ??? pour le lévamisole.
Conclusion : l'extrait de fruits de *Piliostigma reticulatum* possède une bonne activité antioxydante et des propriétés anthelminthiques, ce qui permet de comprendre son utilisation traditionnelle dans le traitement des parasitoses intestinales.

Mots clés : *Piliostigma reticulatum*, anthelmintique, antioxydant, ,.

Introduction

Parasitic worm infections are one of the most critical global health problems. Worm infections can cause irreparable damage to immunocompromised humans and severe losses to livestock production. As a result, helminth infections cause chronic and

sometimes fatal diseases that have a significant socio-economic impact worldwide [1]. In humans, parasitic worms cause approximately 14 million cases of disease worldwide, also known as neglected tropical diseases (NTDs) [1,2]. Gastrointestinal (GI) nematodes, such as hookworms, whipworms, and roundworms, affect most people under 15 [3]. Approximately 10% of the world's population is infected with nematode worms [4]. Helminthiasis causes digestive disorders (diarrhoea, nausea, vomiting), gastritis, and intestinal obstruction. They are also responsible for nutritional deficiencies, which can have severe consequences for children and girls of childbearing age through stunted physical and cognitive growth [5]. In addition, school failure and loss of work capacity can lead to moral decay and social imbalance (Baird et al., 2011). According to a WHO study, lymphatic filariasis results in a loss of productivity of approximately US$ 1.3 billion [6].

Sub-Saharan Africa remains the region with the highest prevalence [5]. According to a study conducted in rural Côte d'Ivoire, 55.2% of school-age children had helminthiasis [7]. In Burkina Faso, Savadogo et al. [8] reported an 82% prevalence of helminthiasis in the same age group among children living near water reservoirs in three villages near the capital, Ouagadougou. In Burkina Faso, according to the Ministry of Health, intestinal parasitosis is the sixth most common cause of outpatient consultations in primary health facilities, with 860 864 cases in 2023, 253 935 for children under five, 190 265 for children aged 5 to 14, and 416 664 for people aged 15 and over [9].

Several preventive and curative control measures have been implemented to eradicate these helminthiases. The WHO recommends annual anthelmintic chemoprevention and systematic deworming of at-risk populations in endemic regions. Anthelmintic treatment against helminthiasis is sometimes unavailable or inaccessible to rural populations, who are most vulnerable to gastrointestinal nematode infestation [10]. In addition, reduced efficacy and resistance to these molecules have been reported [11]. Eradication of these helminths is a significant concern, especially with the emergence of resistance and side effects [12,13]. Traditional medicine can be an alternative for the treatment of parasites. It is essential for human health. Medicinal and nutritional Plants such as *Saba senegalensis* and *Balanites aegyptiaca* Stem bark of *Annona senegalensis* Pers (Annonaceae), Leaves of *Diospyros mespiliformis* Hochst. ex A. DC. (Ebenaceae) have shown anthelmintic activity *in vitro* against eggs, larvae or adult worms of *Haemonchus contortus (H. contortus)* [14–18].

Piliostigma reticulatum is a leguminous plant of the family Fabaceae. *P. reticulatum* is widespread in Africa and Asia [19,20]. The bark, root, pod, young stem or leaves have been used to treat leprosy, smallpox, cough, ulcer, heart pain, gingivitis, snakebite, dysentery, fever, wounds and a variety of closely related diseases [20–23]. In Burkina Faso, studies have been carried out on *P. reticulatum* to demonstrate the traditional uses of the plant, particularly in the nutritional and medicinal fields [21,24,25]. Although certain laxative, antiseptic, analgesic and anti-bronchitic properties of the fruit are known, studies on its parasiticidal properties have yet to be explored. This work aims to exploit the antiparasitic potential of the plant. The general objective is to study the anthelmintic properties of hydroalcoholic macerates of the fruits of *P. reticulatum* (DC.) Hochst (Fabaceae) *in vitro*. A strongyle nematode, *H. contortus*, was used as a biological model of the parasite. Antioxidant tests were also carried out using extracts from the fruit of *P. reticulatum* to test the ability of our extract to scavenge free radicals. These experiments will contribute to the scientific development of a plant used in sub-Saharan Africa as a laxative in food and in the treatment of digestive parasites.

I. Materials and methods

I.1. Biological material

The plant material consisted mainly of the fruits powdered of *P. reticulatum* collected in Manga, in the south-central region of Burkina Faso, 100 km from the capital, Ouagadougou, in March 2023 and identified at the botany laboratory of Joseph KI-ZERBO University. After harvesting, *P. reticulatum* fruits were dried in the shade and protected from dust to preserve all their physicochemical properties. They were then ground using a blade mill and the resulting powders were stored in tinted glass vials at room temperature in the laboratory.The description and other taxonomic data were conformed to World Flora Online with id: wfo-0000170391 for *P. reticulatum.*

The animal material consisted of adult *Haemonchus contortus* worms collected from the abomasums of freshly slaughtered sheep naturally infested. The rennet was purchased from the refrigerated slaughterhouse in Ouagadougou, packed in a cool box and transported to the laboratory.

I.2. Chemical solutions and reagents

The following reference products and solutions were required for the various tests: PBS (phosphate buffered saline), methanol, ethanol, levamisole, Trolox, DPPH (2,2-diphenylpicrylhydrazine), ABTS [2,2'-azinobis(3-ethylbenzoline-6-sulfonate)], quercetin, gallic acid, FCR 2N (Folin Ciocalteu reagent). Sigma-Aldrich (Merck) supplied these products.

I.3. Methods

I.3.1. Phytochemistry

I.3.1.1. Extraction

The extract was prepared according to the instructions of the traditional practitioner. 100 g of powder was macerated in 500 ml of 70 % ethanol for 24 hours under mechanical agitation. The hydroethanolic macerate of *P. reticulatum* (HEM_PR) obtained was concentrated with Rotavapors and then dried in an oven. The dry extract was stored in the refrigerator for further testing.

I.3.1.2. HPTLC screening

The principles of high-performance thin-layer chromatography (HPTLC) are the basic principles of chromatography: separation of compounds based on their interaction with two immiscible phases (stationary and mobile phase).

The phytochemical screening of the extract is carried out on HPTLC plates (10 cm × 10 cm) with silica gel 60 (Merck, Darmstadt, Germany). Approximately 10 µL of extract and 3 µL of each standard are applied in an 8 mm strip along the 8 mm baseline from the bottom of the plate using a semi-automatic sample dispenser. The distance between the spots is 3.4 mm. The distance between the plate's first spot and left edge and between the plate's last spot and right edge is 20 mm. After coating, the plate is placed in a vessel containing the eluent (20 cm × 10 cm, saturation time: 30 min). Sterols, triterpenes, flavonoids, and tannins were detected according to the methods described by Wagner and Bladt and adapted in the IRSS chemical laboratory [26,27]. HPTLC profiles are mainly used to detect these chemical families. Depending on the metabolite to be identified, a specific solvent system was used for elution. Subsequently, a defined reagent was used for elution for each metabolite, and the plate was observed. Thus, saponins were eluted using ethyl acetate, methanol and water in the ratio 20/3.3/2.7. The

flavonoids were eluted in ethyl acetate, methanol and water in 20/3.3/2.7. The solvent system toluene/ethyl acetate/glacial acetic acid (5/4/1) was chosen for triterpene elution.

I.3.1.3. Determination of total phenolic compounds

Total phenolic content was determined by the technique of Singleton et al. [28]. A test tube containing 1 ml of extract at 1 mg/mL concentration added 1 mL of FCR 2N (Folin Ciocalteu reagent) and 3 mL of a 20 % sodium carbonate solution. The mixture was mixed in triplicate with a blank of distilled water. After 40 min incubation at room temperature, the absorbance was measured at 760 nm using a spectrophotometer (Agilent 8453). From the standard curve plotted with tannic acid, the concentration of total phenolics in the extract was calculated using the formula:

$$T_{PT} = \frac{C_{Tube}}{C_i} \times D$$

T_{PT}: is the total phenolic content of the extract expressed as tannic acid equivalent (TAE)/g; C Tube: is the concentration in mg TEA/mL in the test tube; D: is the dilution factor; Ci: is the concentration in mg/mL in the stock solution.

I.3.1.4. Determination of total flavonoid compounds

The method described by Abdel-Hameed was used to determine total flavonoids[29]. In a test tube, 100 µL of extract at a concentration of 1 mg/mL in water was mixed with 100 µL of 2 % aluminium trichloride ($AlCl_3$) in methanol. The volume of the mixture was made up to 5 mL with methanol after the addition of a drop of acetic acid (CH_3COOH). A blank control was run in parallel with the 1 mL of methanol and 1 mL of $AlCl_3$ free extract. The mixtures were incubated for 40 min before measuring the absorbance at 415 nm using a spectrophotometer (Agilent 8453). A standard curve was plotted using quercetin as the reference compound. The total flavonoid content of the extract was determined as quercetin equivalent (QE) using the following formula.

$$T_{Flav} = \frac{Ctube}{Ci} \text{ x D}$$

TFlav: is the total flavonoid content of the extract expressed as quercetin equivalent (QE)/g; CTube: is the concentration in mg QE/mL in the assay tube; D: is the dilution factor; Ci: is the concentration in mg/mL in the stock solution.

I.3.2. *In vitro* antioxidant assays

I.3.2.1. DPPH free radical scavenging activity assay

The DPPH free radical scavenging activity assay was performed according to the method described by Kim et al. [30]. A cascade dilution of extracts and Trolox (positive control) at 1 mg/mL concentration was performed. A solution of DPPH (4 mg in 100 mL methanol) was prepared. A reaction mixture of 20 µL of each dilution of extracts and Trolox with 200 µL of DPPH solution was then prepared in a 96-well microplate. The mixture was incubated for 30 minutes with methanol used as a blank. Absorbance was then measured at 490 nm using a spectrometer (Agilent 8453). Percentage inhibition was calculated using the formula:

$$\%\text{Inhibition} = \frac{(A_0 - A_1)}{A_0} \text{x } 100$$

which A_0 is the control absorbance and A_1 is the test/standard absorbance. The median inhibitory concentration (IC_{50}) was determined from regression analysis of a graphical plot of percentage scavenging potentials against concentration.

I.3.2.2. ABTS radical-scavenging assay

Using the method described by Re et al., a cascade dilution of the extracts and Trolox (positive control) at a concentration of 1 mg/mL was performed [31]. A reaction mixture was prepared on a 96-well microplate with 20 µL of each dilution and 200 µL of ABTS-+ solution diluted in triplicate. The reaction mixture was incubated for 30 minutes at room temperature in the dark. The activity was monitored using a photo spectrometer (Agilent 8453) at a wavelength of 432 nm. The following formula was used to determine the percentage of inhibition:

$$\%\text{Inhibition} = \frac{(A_0 - A_1)}{A_0} \text{x } 100$$

A_0 = absorbance of the negative control and A1 = absorbance of the sample. The IC_{50} of ABTS is determined.

I.3.2.3. Ferric reducing antioxidant power (FRAP) assay

The chelating capacity of metals, exclusively iron, has been determined using the FRAP method. It is based on reducing ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The ability of the extract to reduce Fe^{3+} to Fe^{2+} by donating electrons was referred to as its reducing potential. The Benzie

and Strain technique was used to determine the ability of extracts to reduce ferric ions. [32].

The FRAP solution was prepared by mixing 300 mM sodium acetate buffer and adjusted to pH 3.6. Ten (10) mM TPTZ (2,4,6-Tris (2-pyridyl)-s-triazine) solution and 20 mM ferric chloride $FeCl_3$ in the ratio 10:01:01. To 100 µL of extract solutions, 03 mL of FRAP solution was added. The absorbance of the mixture was measured at 593 nm after 30 min of reaction. The increase in absorbance in the reaction medium was proportional to the rise in iron reduction. A calibration curve (0.025-1 mg/mL) was first established by preparing a series of ascorbic acid (AA) solutions used as a reference. All preparations and analyses were performed in triplicate.

I.3.3. Adult worms mortality assay

Adult worms were collected after a longitudinal incision of the abomasum. They were carefully sorted and washed successively with distilled water to remove faecal matter. The worms were then placed in a Petri dish containing a solution of phosphate-buffered saline (pH 7.2) and immediately used for the biological test.

The test was performed according to the method described by Ademola et al. [33]. Adult *Haemonchus contortus* worms were placed in Petri dishes containing PBS at a rate of 3 worms per dish. Extract dissolved in PBS at increasing concentrations of 5, 7, 9, 11, 13; and 15 mg/mL were added to the Petri dishes to 3 mL. The PBS solution and a levamisole solution (1% w:v) prepared with the PBS solution were used as blank and reference controls, respectively. The parasites were incubated at 37°C for 24 hours. Worm motility and survival observations were made under a light microscope 24 hours after exposure. After 24 h incubation, worms treated with the plant extract were plunged back into the PBS solution for 30 min to observe any resumption of motility. The number of dead worms after exposure to the extract was assessed at 24 h, and the worm mortality rate (TM) for each extract concentration was calculated using the following formula (n=6):

$$\mathrm{TM}(\%) = \frac{\text{Nombre de vers morts}}{\text{Nombre de vers introduits}} \times 100$$

I.3.4. Statistical Analysis

Statistical data on mean percentages of mortality (TM) and egg hatch inhibition (EHI) rates were analyzed using Prism 5.0 software. The Student's t-test was used to compare the extract with the reference. Differences are considered significant when P (p-value) < 0.05 compared to the standard. The results were expressed as mean ± mean standard error (m ± SEM.).

II. Results

Phytochemical TLC analysis revealed the presence of flavonoids, saponins, sterols and triterpenes in Figure 2 below.

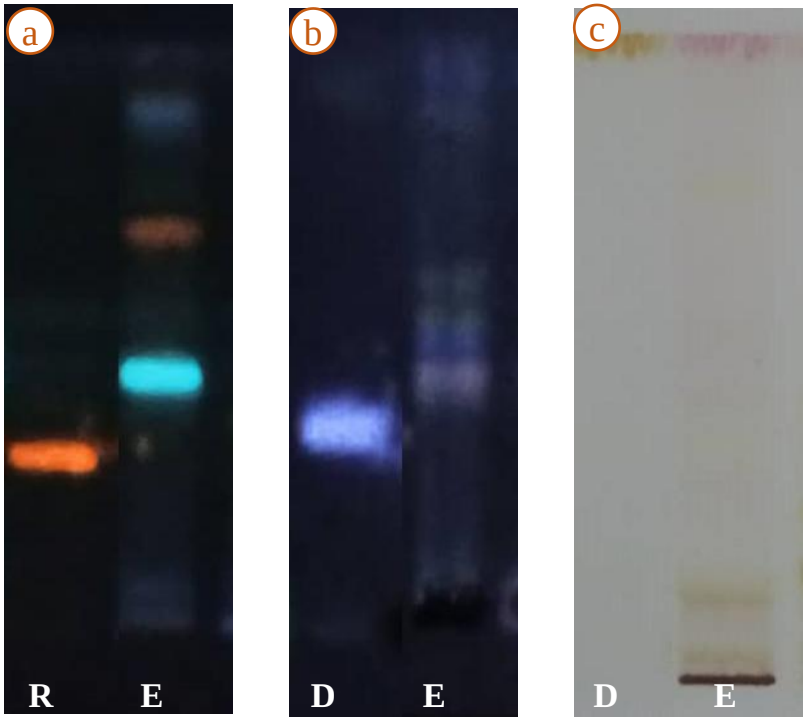

Figure 2: Chromatographic profile of hydroethanolic macerate of fruits of *P. reticulatum*.

(a) Flavonoids (at 366 nm) ; (b) Sterols and triterpenes (at 366 nm) ; (c) Saponins (at visible).
R: rutin, D: diosgenin, E: extract (HEM_PR).

II.1. Phenolic compound content

The total phenolics and flavonoids content is recorded in Table 1 below.

Table I: Flavonoid and phenolic compound content

	Total phenolic (µg TAE/mg)	Total flavonoid (µg QE/mg)
HEM_PR	187,32 ± 0,07	9,24 ± 0,06

Values are expressed as mean ± SEM.

II.2. Antioxidant activities

The results of the DPPH and ABTS anti-free radical tests and the FRAP reducing power test are shown in Table 2 below.

Table II: Antioxidant activity of 2,2'-azinobis (3-ethyl benzoin-6-sulphonate) (ABTS), 2,2-diphenyl-picrylhydrazine (DPPH), Ferric reducing antioxidant power (FRAP) and 15-lipoxygenases inhibition (LOX)

	ABTS	DPPH	FRAP
	IC_{50} (µg/mL)	IC_{50} (µg/mL)	AAE µmol/mL
MEM_PR	6,36± 0,23***	1277,9 ± 0.06***	3352,45 ± 24,70
Trolox	2,89 ± 0,14	6,39 ± 0,21	nd

Values are expressed as mean ± SEM, *** $p < 0.001$ is considered significant compared with the Trolox control.

II.3. Anthelmintic activity against *Haemonchus contortus* adult worms

At the highest concentrations, mortality of worms was observed after 24 hours of incubation. The results are shown in the tables below.

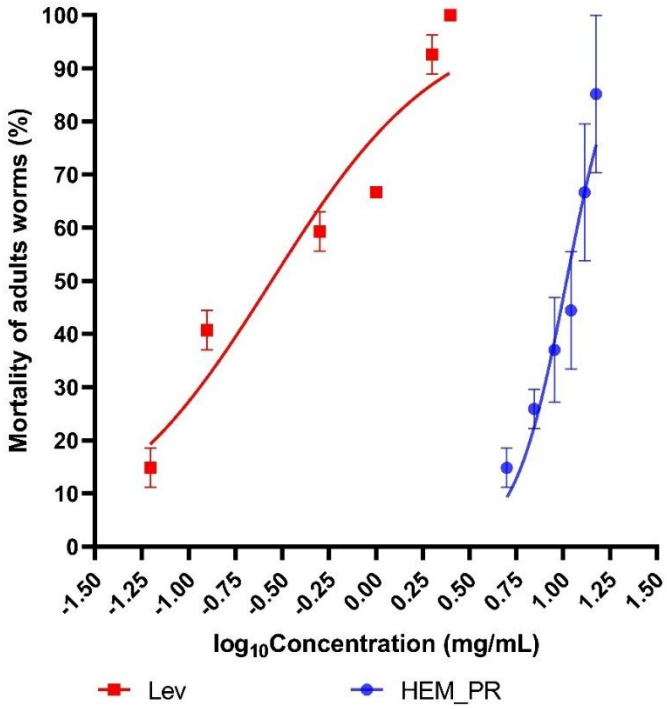

Figure 3: Effect of hydroethanolic macerate from the fruits of *P. reticulatum* and levamisole (Lev) on the adult worms of *H. contortus* after 24 h of incubation (n = 6).

For the adult worms mortality assay, a lethal concentration of 50% of worms is given as follows. Lev: LC_{50} = 0.27 mg/mL; HEM_PR: LC_{50} = 10.42 mg/mL with P value = 0.3457 t-test (and no parametric test). Values expressed as mean ± SEM.

III. Discussion

Phytochemical analysis revealed the presence of chemical groups of interest, such as flavonoids, sterols triterpenes, and saponins in the extract (Figure 2). Studies on the *P. tonningii* variant also show the presence of phenolic compounds with a notable absence of tannins in the leaves (Table 1) [34]. The results of the analyses indicate that the extract of *P. reticulatum* fruits is rich in phenolic compounds (187.32 ± 0.07 µg TEA/mg) but very low in flavonoids. This low flavonoid content in the fruit was also reported by Boualam et al. for the methanolic extract of the leaves at 13 µg QE/mg [35]. The phenolic compound content of 44.49 ± 0.32 µg EAA/mg obtained by Sergine et

al. with the hydroethanolic extract of *P. reticulatum* bark was lower than in the present study. Edaphic factors could explain the difference in content, the part of the plant and the proportion of alcohol in the hydroalcoholic extraction solvent.

The antioxidant activity of the *P. reticulatum* extract was better on the ABTS radical with an IC_{50} = 6.36 ± 0.23µg/mL than on the DPPH radical with an IC_{50} = 1277.9 ± 0.06µg/mL (Table 2). This high antiradical activity on the ABTS radical indicates the extract is rich in hydrogen proton donating chemical groups [36]. Similar values of antiradical activity on the ABTS radical (4.90 ± 0.07 µg/mL) were obtained by Ouedraogo et al. with the methanolic extract of *P. reticulatum* bark. The antiradical activity of the hydroalcoholic extract on the ABTS radical appeared better than that of the methanolic extract of *P. reticulatum* leaves, which was 13.47 ± 2.623 µg/mL [37]. However, for the DPPH radical, the methanolic extract (8.88 ± 0.114 µg/ml) showed a better radical scavenging activity than the h µmol/mg hydroalcoholic extract [35].

The *P. reticulatum* extract showed a high iron-reducing capacity (3352.45 ± 24.70 µmol/mL) compared to the reference compound Trolox. During a parasitic infection, parasites can cause damage to the mucosa, triggering an anti-inflammatory response with the release of free radicals. The antioxidant activity of the plant could help combat this oxidative stress. This reducing power of the extract is due to the presence of reducing molecules which act on free radical chains by donating hydrogen atoms, causing them to break down [38]. The high content of phenolic compounds may explain the enhanced antioxidant activity of *P. reticulatum* extract [39].

The anthelmintic activity of the extract was moderate, with an LC_{50} = 10.42 mg/mL. This may indicate a low level of anthelmintic activity. However, the anthelmintic activity of the *P. reticulatum* extract remains weak compared to the extracts of *Acacia nilotica* (LC_{50} = 2.63 mg/mL) et *Saba senegalensis* (LC_{50} = 6.79 mg/mL) [16,40]. As the fruits of *P. reticulatum* are preferentially used as food, the strong antiradical activity of ABTS combined with the antiparasitic effect could be a good option for the formulation of drugs for human and animal use against oxidative stress associated with parasitosis.

Phytochemical screening of the *P. reticulatum* plant extract revealed the presence of saponins, triterpenes and flavonoids, which may contribute

to the anthelmintic activity [41,42] [with an independent or synergistic effect [43,44].

The fruit extract of *P. reticulatum* contains saponins and flavonoids whose anthelmintic properties on the adult worm of *H. contortus* have been demonstrated by several authors. Polyphenols and terpenoids are the most reported groups of medicinal plants, extracts, and compounds, and different chemical groups have been studied for their anthelmintic potential [45–47]. Flavonoids are anthelmintic against L_3 larvae of *H. contortus* [48]. The extract may disrupt the integrity of the parasite's cuticle or bind to proteins used by the worms for their nutritional functions.

The motility of adult worms may have been prevented by the terpenoid compounds present in the extract [49]. According to Ademola and Eloff , saponosides have an anthelmintic effect by destabilising membranes and increasing cell permeability [48].

Conclusion

This study showed that the hydroalcoholic extract of *P. reticulatum* is rich in phenolic compounds and has antioxidant properties. It also has anthelmintic activity at very high concentrations. The fruits of *P. reticulatum* could be used to form medicines to combat oxidative stress and have antiparasitic effects. Its use in traditional medicine for the treatment of gastrointestinal parasitosis seems justified.

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Conflicts of interest

The authors declare no competing interests.

Author contributions

Mohamed B. BELEMLILGA: conceptualization, methodology, statistical analysis and writing and original draft preparation. T. Jonas COULIBAL and Abdoul G. L. BOLY: experimental studies, writing and original draft preparation and visualization. Boukaré KABORE: phytochemical investigation. D. Jacques KONAN, Alimata BANCE and Dofini René MAGNINI: methodology. Souleymane COMPAORE,

Almamy KONATE and Moumouni KOALA: manuscript review, Aristide TRAORE: supervision, review and editing.

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