Phytochemical and *in vitro* anthelmintic properties of mesocarp of fruit extracts from *Balanites aegyptiaca* (L.) Delile (Zygophyllaceae)

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ARTICLE INFO	ABSTRACT
	Introduction: *Balanites aegyptiaca* L. (DEL) is a plant used in Burkina Faso in traditional medicine to treat gastrointestinal parasitosis. The purpose of this study was to assess the *in vitro* anthelmintic activity of aqueous and hydro-ethanolic macerates (AM and HEM) of the fruit mesocarp from *Balanites aegyptiaca* against *Haemonchus contortus*. **Methods:** Phytochemical screening of the extracts was done using thin-layer chromatography. The egg hatch inhibition assay (EHIA) at 0.125, 0.15, 0.25, 0.625, 1.25, and 5 mg/mL and Adult worms mortality assay (AWMA) at 0.625, 1.25, 2.5, 5, 10, and 15 mg/mL concentrations were evaluated. Albendazole and levamisole were used as standards, and phosphate-buffered saline was used as a negative control. The antioxidant potential of extracts was evaluated using the ABTS [2,2'-azinobis (3-ethyl benzoin-6-sulphonate)] and DPPH (2,2-diphenyl-picrylhydrazine) methods and Fe^{3+} ion reducing power. The standards used were trolox and ascorbic acid. **Results:** Several secondary metabolites such as sterols, triterpenes, tannins, flavonoids, coumarins, and saponins were identified. For EHIA, the AM was more effective than HEM with 50% inhibitory concentrations (IC_{50}) of 0.35 mg/mL and 0.43 mg/mL, respectively. AM was even more effective for AWMA than HEM, with 50% lethal concentrations (LC_{50}) of 3.15 mg/mL and 8.37 mg/mL, respectively. The DPPH method gave an IC_{50} of 834.55 μg/mL and the capacity to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) was 270.4 μmol/Ascorbic acid equivalent. **Conclusion:** AM and HEM from the mesocarp of *B. aegyptiaca* have anthelmintic properties. AM is more effective than HEM, which justifies their use with priority for AM.

Implication for health policy/practice/research/medical education:
The fruit's mesocarp of *Balanites aegyptiaca* is thought to have anthelmintic properties. So, its use in traditional medicine against helminths is justified and should be encouraged, given its efficacy. It also has an anti-free radical effect, which is of interest in fighting against stress associated with parasitic infections. The mesocarp of the fruit from *B. aegyptiaca* could be an essential source for developing nutraceuticals with anti-parasitic properties.
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Introduction

Helminthiases affect over one billion people worldwide and constitute a public health problem (1). Around 1.5 billion people are infected with géohelminthiasis caused by roundworms, hookworms, and whipworms (2,3). These infections are common among people living in areas with poor access to drinking water, sanitation, and adequate hygiene (3). The burden of these parasitic infections

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is anaemia, delayed development, and reduced mental capacity, especially in children. They also reduce work capacity, leading to severe losses in productivity (4,5). They cause high morbidity and hundreds of thousands of yearly deaths (6). In sub-Saharan Africa, helminthiasis caused around 200 000 deaths in 2004 (7).

In Burkina Faso, according to the Ministry of Health, intestinal parasitosis is the fifth most common cause of outpatient consultations in primary health facilities, with 878 830 cases in 2021, 280 097 for children under five, 179 851 for children aged 5 to 14, and 418 882 for people aged 15 and over (8). Anthelmintic treatment for helminthiasis is sometimes unavailable or inaccessible to rural populations, who are most vulnerable to gastrointestinal nematode infestation (9). Moreover, reductions in efficacy and the development of resistance to these molecules have been reported (10). Eradication of these helminths is a major concern, especially with the emergence of resistance and side effects (11,12).

Traditional medicine can be an alternative for treating parasites. It is essential to human health. Plants such as *Pterocarpus erinaceus* and *Parkia biglobosa* have shown effects on the development cycle of *Haemonchus contortus* (13). Traditional preparations of remedies with the leaves of *Anogeissus leiocarpa* and with the stem bark of *Daniellia oliveri* have shown anthelmintic activity in vitro on the eggs, L_1 larvae, and adult worms of *H. contortus* (9). Concerning *Balanites aegyptiaca*, all plant parts are used in traditional medicine. In Mali, the fruit is used to relieve pain. In Burkina Faso, the pulp is used to treat diabetes. A mixture of dry powder from the seeds of *B. aegyptiaca* and sorghum flour is also used to treat helminthiasis (14,15). *Balanites aegyptiaca* fruit extract has been shown to have an anthelmintic action against the various stages of nematode *Trichinella spiralis* and cestode effect against adult worms of *Dipylidium caninum* (16,17). In Burkina Faso, several studies have been carried out on the *B. aegyptiaca* species, particularly on almonds and root barks (18–20). However, studies on the parasiticidal properties of the fruit mesocarp have yet to be explored. This work aims to exploit the plant's anti-parasitic potential. The general objective is to study in vitro the anthelmintic properties of aqueous and hydroalcoholic macerates of the fruit mesocarp of *B. aegyptiaca* (L.) Delile (Zygophyllaceae). We used a strongyle nematode, *H. contortus*, as a biological model of the parasite. Antioxidant tests were also carried out using extracts from the mesocarp of *B. aegyptiaca* fruit to test the ability of our extracts to trap free radicals. These experiments will help enhance the value of plant products, hence traditional medicine.

Materials and Methods
Materials
Plant material
The plant's raw material consisted of the fruit pulp of *B.* *aegyptiaca*. These fruits were harvested in Manga, in the south-central region of Burkina Faso, 100 km from the capital, Ouagadougou, in March 2021. A plant sample was collected and identified at the Joseph KI-ZERBO University Herbarium under identification number 17928 with herbarium reference number 6916.

Animal material
The animal material used for this *in vitro* study was eggs and adult worms of *H. contortus* from the abomasums of freshly slaughtered sheep in Ouagadougou.

After a longitudinal section of the rennet bag/stomach, the collected adult worms were washed, sorted, and transferred to a Petri dish, which contained Phosphate-Buffered Saline (PBS, pH: 7.2). The egg collection and the adult worms were realized with previously described methods (21). After sieving with stitches (1 mm and 100 μm), the obtained solution was filtered. Then, the freed eggs were allowed to retain by using a sieve with 38 μm stitches. By rinsing the sieve, the eggs were returned.

Reagents and solvents
Levamisole and albendazole were respectively used as a positive controls on adult worms on eggs. Phosphate-buffered saline (PBS) was the negative control. Reagents as well as aluminium trichloride ($AlCl_3$), DPPH (2,2-diphenylpicrylhydrazine), ABTS, FCR 2N (Folin-Ciocalteu reagent), quercetin, tannic acid, PVPP (polyvinylpolypyrrolidone), and Trolox were obtained with Sigma-Aldrich. Ethanol, methanol, and acetic acid were also used.

Methods
Phytochemical study
A quantity of *B. aegyptiaca* fruits corresponding to a mass of pulp of 566 g was macerated in a volume of distilled water of 2 L for 5 hours. The macerated was then kneaded to remove the pulp from the almond altogether. The aqueous macerate obtained was filtered, centrifuged, freeze-dried, and stored in the refrigerator.

Phytochemical screening of the extract was realized by thin-layer chromatography with chromatoplates of type 60 F_{250}, glass support 20 × 20, and Fluka-Silica gel (22). The researched Chemical groups were polyphenols, tannins, flavonoids, and terpenoids.

Total phenolic content
Total phenolic compounds were dosed according to the Singleton et al× method (23). In a test tube, the extract (1 mL, 1 mg/mL), Folin-Ciocalteu reagent (1 mL of, 2N FCR), and sodium carbonate (3 Ml, 20%) solutions were added. After 40 minutes incubation at room temperature, the absorbance was measured with a spectrophotometer at 760 nm. The extract's total phenolic concentration was calculated using the following formula:

$T_{PT} = (C_{Tube}/ C_i) \times D$

T_{PT}: total phenolic content of the extract expressed in tannic acid equivalents (EAT)/g; C_{Tube}: concentration in mg EAT/mL in the test tube; D: dilution factor; and C_i: concentration in the stock solution (mg/mL).

Total flavonoid contents

The total flavonoid was quantified according to the method of Abdel-Hameed (24). In a test tube, 100 µL of 2% $AlCl_3$ in methanol (CH_3OH) was mixed with 100 µL of the extract at 1 mg/mL in water. After adding a drop of acetic acid, the mixture was made up to 5 mL with methanol. A control was also made with distilled water at 760 nm. After incubating the mixture for 40 minutes, the absorbance was recorded at 415 nm using a spectrophotometer (Agilent 8453). Quercetin was used as a control compound. Total flavonoid content was reported as quercetin equivalents (mg.g⁻¹ extract) using the regression equation from the calibration curve as follows:

$T_{Fl} = (C_{tube}/C_i) \times D$

T_{Flav}: total flavonoid of the extract expressed as quercetin equivalent (QE)/g; C_{Tube}: concentration in mg QE/mL in the test tube; D: dilution factor; and C_i: concentration in mg/mL in the stock solution.

Tannins content

The method described by Singleton et al was adopted to quantify the tannins. The amount of tannins was determined according to the Folin-Ciacalteu method, after precipitation of the phenolic with PVPP (polyvinylpolypyrrolidone). 100 mg of PVPP was mixed with 1 mL of the extract (1 mg.mL⁻¹) in a test tube. After 15 minutes at 4°C, the tubes were vortexed before to centrifuged at 3000 g for 10 minutes. Supernatant (2 mL) of each tube was sampled and used to determine phenolic content as described above (method of phenolic content determination). The tannin content was calculated by deducting from the total phenolic content and expressed as tannic acid equivalent (25). The amount of tannins was determined as the difference between total phenolics (containing tannins) and the total phenolics (in absence of tannins) (26).

Antioxidant and lipoxygenase inhibition assay
DPPH free radical scavenging assay

It was performed according to the method previously described with some modifications (25,27). A serial dilution from the extracts and Trolox (positive control) at a concentration of 1 mg/mL was performed. A solution of DPPH (4 mg in 100 mL methanol) was prepared. A sample mixture of 20 µL of AM and HEM extract dilutions and Trolox was then mixed with 200 µL of DPPH solution in a 96-well microtitre plate. The mixture was incubated for 30 minutes with methanol used as white. The absorbance

was then read at 490 nm on a photospectrometer (Agilent 8453). The DPPH radical percentage scavenging inhibition was calculated using the formula:

% inhibition = [(A0-A1)/A0] × 100

which Ao is the control absorbance and A1 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. The antiradical power (ARP) was calculated by the formula:

$ARP = 1/IC_{50}$

ARP: Antiradical power and IC_{50} was expressed in µg/mL of the extract.

ABTS radical reduction test

The free radical scavenging activity of ABTS was evaluated according the method previously described by Re et al (28). A cascade dilution of the hydroalcoholic extract and the reference substance (Trolox) started from 1 mg/mL concentration. Thus, for extract evaluation, 200 µL of radical ABTS⁺ solution, diluted in triplicate, was added to 20 µL of extract in 96-well microplate. The reaction sample mixture was then incubated at 25 °C temperature for 30 minutes in the dark, and the absorbance was read using a spectrophotometer at 415 nm (Epoch, BioTek instruments, USA). Trolox was used as a reference compound. The following formula was used to determine the inhibition percentage:

% inhibition = [(A0-A1)/A0] × 100

A0 = absorbance of the negative control and A1 = absorbance of the sample.
Antiradical power (ARP): = $1/IC_{50}$

Ferric-reducing antioxidant power (FRAP) assay

The extract capacity to reduce the ferric ion was determined according to Benzie and Strain (29). The FRAP solution was prepared by mixing 300mM sodium acetate buffer (pH 3.6) with 10mM TPTZ solution (2,4,6-Tris(2-pyridyl)-s-triazine) and 20 mM ferric chloride (FeCl3) in the proportions of 10:01:01 (v/v). Then, 100 µL of the extract solution of 1 mg/mL concentration was added to 03 mL of FRAP solution. The incubation time was 30 minutes, and then the absorbance was read using a spectrophotometer at 593 nm. A calibration curve with the ascorbic acid solution (A) was previously established (0.025-1 mg/mL). The reducing power of the extract was expressed in equivalent ascorbic acid (EAA).

Inhibition activity of 15-lipoxygenase (LOX)

The test was realized according to the method described by Malterud and Rydland (30).

So, a mixture of 146.25 µL lipoxygenase (400 U/mL) and 3.75 µL extract (16 mg/mL) was realized in the well microplate. The mixture was incubated during 2 minutes at 25 °C. After incubation, 150 µL of the substrate (1.25 mM linoleic acid) was added to the mixture to initiate the reaction. The reaction absorbance was read at 234 nm for 2 minutes with a microplate reader (Epoch, BioTek instruments, USA). Tests were performed in triplicate. The inhibitory capacity of the extract was determined using the following formula:

Inhibition percent = (S-E) /S × 100
E: activity of the enzyme without inhibitor; S: activity of the enzyme in the presence of the test sample.

In vitro anthelmintic activity
Egg hatch inhibition assay (EHIA)
A volume of 100 µL of egg suspension adjusted to approximately 1000 eggs/mL was deposited in the wells of a multi-well plate (24 wells) according to Coles et al method (31). In each well, 1.9 mL of *B. aegyptiaca* pulp extracts of AM and HEM was added at concentrations of 0.125, 0.15, 0.25, 0.625, 1.25, and 5 mg/mL EHIA. The positive control of albendazole was also constituted at the concentrations of 0.00195, 0.0078, 0.03125, 0.125, 0.25, and 5 mg/mL. The plates were incubated for 48 hours at 25 °C. Then, to stop the development of eggs, a drop of formalin was added to each well. The dead or alive larvae were counted under microscope (Olympus BH-2, Japan) in x40. The test was repeated 3 times. The following formula estimated the egg hatch rate (EHR) for each concentration tested (n = 5):

$$EHR(\%) = \frac{Number\ of\ remaining\ eggs}{Number\ of\ eggs\ put\ incubating\ in\ the\ well} \times 100$$

Adult worms mortality assay
It was realized according to the method described by Athanasiadou et al (32). The AM and HEM extracts, dissolved in 0.625, 1.125, 2.5, 5, 10, and 15 mg/mL PBS, were introduced to Petri boxes for a total volume of 3 mL. Then, the adult worms of *H. contortus* were placed in the Petri boxes containing the extract solution at the rate of 3 worms. Levamisole was used as a standard at the concentrations of 0.0625, 0.125, 0.5, 1, 2, and 2.5 mg/mL. The device was incubated at 37 °C during 24 hours. The worms' motility and survival were observed through the optical microscope at 24 hours. After incubation, the worms, which were previously treated with levamisole or the extract were dipped back to PBS (30 minutes) to observe the possible resumption of the motility. After exposure to the extract, the mortality rate (M%) for each concentration of the extract was determined by the following formula.

$$M(\%) = \frac{Number\ of\ dead\ worms}{Number\ of\ alive\ grown - total\ worms\ put\ in\ the\ Petri\ box} \times 100$$

The test was repeated 5 times for each concentration of the extracts.

Statistical analysis
The mortality rates and the inhibition of eggs hatching were analyzed with GraphPad Prism 10.0.2 software. Two-way ANOVA followed by Dunnett multiple comparison test was used to compare the extracts. $P < 0.05$ was considered significantly different. The results were expressed as mean ± standard error of mean (SEM). The variation was considered significant at $P < 0.05$.

Results
This study enabled us to identify several secondary metabolites involved in anthelmintic activity. We were also able to measure total phenolics, tannins, and flavonoids in our extracts. The antioxidant activity of AM and HEM of *B. aegyptiaca* mesocarp extracts was determined by four different methods.

Phytochemical results
Phytochemical groups identified
Phytochemical analysis using the High-performance thin-layer chromatography method revealed the presence of phytochemical groups of interest represented in Table 1.

Total phenolic and flavonoid content
The total phenolic, tannins, and flavonoid contents of the extract and fractions are shown in Table 2.

Anthelmintic activities
Egg hatch inhibition assay
After 48 hours of incubation, unhatched eggs noticed in each well were counted. The number of not hatched eggs increased as the extract concentration increased (Figure 1).

Adult worms mortality assay
The microscope observation showed immobile and dead worms at 24 hours. The inhibitory effect on motility and lethality was proportional to the concentrations. The results are presented in Figure 2.

Table 1. Secondary metabolites identified in aqueous macerate (AM) and hydroethanolic macerate (HEM) from the mesocarp of *B. aegyptiaca* fruit

	Phenols and tannins	Sterols and triterpenes	Coumarins	Flavonoids	Saponins
AM	-	+	+	+	+
HEM	-	+	+	+	+

+ : present ; - : absent.

Table 2. Total phenolic, tannin, and flavonoid contents in aqueous macerate (AM) and hydroethanolic macerate (HEM) from the mesocarp of *B. aegyptiaca* fruit

Treatment	Total phenolics (µg TAE/mg extract)	Total tannins (µg TAE/mg extract)	Total flavonoids (µg QE/mg extract)
Aqueous macerate	13.7 ± 0.13*	11.78 ± 0.02[ns]	4.68 ± 0.39
Hydroethanolic macerate	13.9 ± 0.17[ns]	11.34 ± 0.18	5.00 ± 1.15[ns]

TAE: tannic acid equivalent; QE: quercetin equivalent. ns: not significant. Values are mean ± SEM for triplicate; *$P < 0.05$ significant compared to flavonoid (One-way ANOVA analysis followed by Dunnett's test).

Table 3. Summary of antioxidant activity results 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)

Treatment	ABTS	DPPH	FRAPP	LOX
	IC_{50} (µg/mL)	IC_{50} (µg/mL)	EAA mmol/g	%Inh
Aqueous macerate	88.00 ± 1.31***	834.55 ± 8.92***	270.4 ± 0.00	14.10 ± 0.93
Hydroethanolic macerate	93.52 ± 1.81***	$CI_{50} > 900$	<2	nd
Trolox	2.89 ± 0.14	6.39 ± 0.21	nd	nd

EAA: Equivalent ascorbic acid (n= 3); nd: not determined. ***$P < 0.001$ significant compared to Trolox.

Antioxidant result

The antioxidant effect of the AM and HEM of the mesocarp extracts of *B. aegyptiaca* was determined using the ABTS, DPPH, FRAP methods, and lipid peroxidation inhibition. The antioxidant activity results are shown in Table 3.

Discussion

Phytochemical screening of this study revealed the presence of secondary metabolites such as flavonoids, sterols, triterpenes, coumarins, and saponins. These results are comparable to those of other authors who have used this fruit (15,33). They used the high-performance liquid chromatography method to determine the different components of the mesocarp. These constituents are at the origin of many pharmacological properties, such as anthelminthic activity (33). Chemical analysis of the mesocarp and kernel of ripe *B. aegyptiaca* fruits has allowed the characterization of a wide range of phytochemical groups in both parts of the plant. In particular, the authors noted a high concentration of saponins in the almond extract and a lower concentration of saponins in the whole
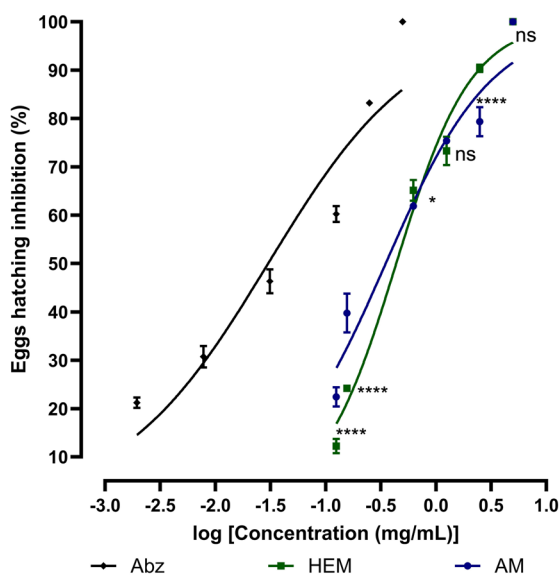

Figure 1. Effect of aqueous macerate (AM) and hydroethanolic macerate (HEM) of *B. aegyptiaca* and albendazole (Abz) on the eggs hatching of *H. contortus*, after 48 h of incubation. For egg hatch inhibition assay Abz: $IC_{50} = 0.030$ mg/mL; AM: $IC_{50} = 0.35$ mg/mL; HEM: $IC_{50} = 0.44$ mg/mL. Emax is 100% for all three products (n= 6). **** $P < 0.0001$ significant compared to the control group (Two-way ANOVA followed by Dunnett multiple comparison test. *$P < 0.05$ significant compared to standard.
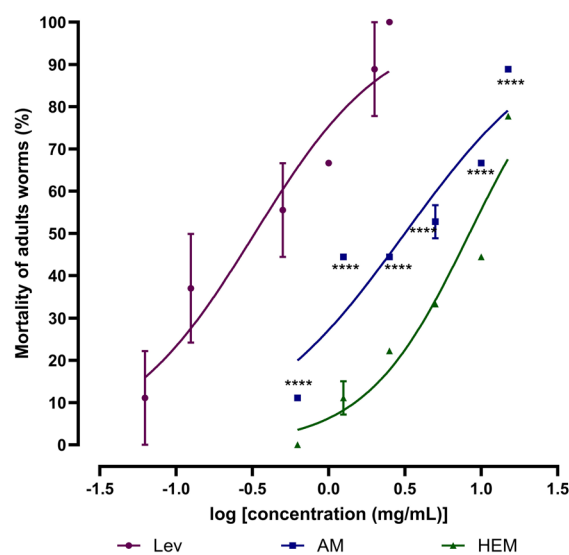

Figure 2. Effect of aqueous macerate (AM) and hydroethanolic macerate from the mesocarp of *B. aegyptiaca* and levamisole (Lev) on the adult worms of *H. contortus* after 24 h of incubation (n = 6). For adult worms mortality assay Lev: $LC_{50} = 0.32$ mg/mL; AM: $LC_{50} = 3.16$ mg/mL; HEM: $LC_{50} = 8.37$ mg/mL.****$P < 0.0001$ significant compared to the control group (Two-way ANOVA followed by Dunnett multiple comparison test.

mesocarp and its extract. The sterols and triterpenes are moderately concentrated in the whole pulp and low in the mesocarp extracts.

Molecules belonging to 4 families of chemical compounds have been isolated from the plant: 4 coumarins, 12 saponosides, 5 flavonosides and 2 alkaloids. Three sapogenins, balanitins 1, 2, and 3 were isolated from methanolic extracts of roots and barks (34). Balanitins 4, 5, 6, and 7 have been isolated from its fruits. Balanitosides, 6-methyldiosgenin (sapogenol), balanitins B1 and B2, and balagyptin have been isolated from the mesocarp of the fruit (35–37). The fruit also contains balanitoside (furastanol glucoside), diosgenin, diosgenin, and 6-methyldiosgenin, while the seeds contain deltonins and isodeltonins (spirostanol glucosides) (38,39). Our assay revealed a better content of phenolic compounds in the aqueous extract of *B. aegyptiaca*. But, the content of flavonoids in the extract was low.

High values of the inhibitory concentrations with radicals DPPH and ABTS showed an average antiradical activity of the aqueous extract of the mesocarp of the fruit of *B. aegyptiaca*. Methanolic extract of the mesocarp had IC_{50} values of around 40 μg/mL and 125.85 μg/mL, respectively (DPPH and ABTS methods) (40). This result confirms the average antioxidant activity of the pulp of *B. aegyptiaca* with an IC_{50} of 88 μg/mL for AM, which is more effective than HEM with an IC_{50} of 93.53 μg/mL. Moreover, the root bark of *B. aegyptiaca* also revealed a weak antiradical activity (41).

The aqueous extract reduced ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) around 270 μmol/EAA. These results indicate that the AM of the fruit pulp of *B. aegyptiaca* has iron-reducing antioxidant activity. The aqueous extract could act by chelation of metal ions, including ferric ion (Fe^{3+}). However, the antioxidant activity generally remained average.

The results obtained from anthelmintic activity indicate a vermicidal and ovicidal effect of the two extracts from the mesocarp of the fruits of *B. aegyptiaca*. The 50% lethal concentration (LC_{50}) of adult worms was 3.16 mg/mL for AM and 8.37 mg/mL for HEM, indicating effective vermicidal effects. However, the LC_{50} of the extract was higher than that of levamisole (0.33 mg/mL). This means levamisole is more potent than the extract on adult *H. contortus* worms.

The level of 88% dead worms is comparable to that of the trunk bark of the *Acacia nilotica* var adansonii plant, which was 88.89% (42). The leaves of *Saba senegalensis* also has vermicidal effects (97.77% of dead worms), similar to that of the pulp of *B. aegyptiaca* (43). The bioactive compounds present in the mesocarp of the fruit of *B. aegyptiaca* and secondary plant metabolites, such as saponins, triterpenes, and flavonoids, could interact on the motility of worms (44,45). Saponins can inhibit parasite development. This will likely result in free radicals that induce membrane

damage by nematode peroxidation (46). The extract from the pulp of *B. aegyptiaca* could destroy the wall or cuticle of adult *H. contortus* worms (16).

B. aegyptiaca mesocarp extracts inhibited egg hatching with a 50% inhibition concentration (IC_{50}) at 0.35 mg/mL for AM and 0.44 mg/mL for HEM. This indicates a strong ovicidal activity of the extract, and AM is more potent than HEM. However, albendazole showed 100% of dead worms, an effective and powerful action at only 2.5 mg/mL with IC_{50} = 0.030 mg/mL compared to the extracts. The strong ovicidal activity of the pulp of *B. aegyptiaca* is comparable to the activity of the essential oil of *Ocinum gratissimum*, which inhibited 100% eggs hatching of *H. contortus* at 2.5 mg/mL (47). It also had better activity than the ethanolic extract of *Mangifera indica*, which inhibited 91% of the hatching of *H. contortus* eggs at a high concentration of 10 mg/mL (48). The mesocarp extract of *B. aegyptiaca* contains saponins, flavonoids whose anthelmintic properties on the adult worm of *H. contortus* have been demonstrated by more authors. Flavonoids have shown anthelmintic effects on L_3 larvae of *H. contortus* (49). The extract might be acted by perturbing the cuticle integrity of the parasite or by attaching to proteins used by the worms for their nutritional functions.

According to Paolini et al. the extracts can also act by functioning perturbation of the genital tract of female worms (50). Thus, the hatching activity of AM and HEM from the mesocarp of *B. aegyptiaca* may be explained by the possible ability of plant extracts to bind directly to the lipoproteins of the egg membrane. Saponins could generate ions and cause lipid peroxidation of egg membranes, which induces better permeability leading to their hatching (51-53).

Conclusion

This study was undertaken to determine the phytochemical groups, anthelmintic efficacy and antioxidant power of extracts from the fruit mesocarp of *B. aegyptiaca*. It revealed that the anthelmintic activity on eggs and adult worms of *H. contortus* of AM was higher than HEM extract of the pulp of *B. aegyptiaca*. The presence of the main compounds, such as sterols, triterpenes, flavonoids, and saponosides with anti-parasitic properties could explain this result. The *in vitro* anthelmintic effects observed on adult worms and eggs of *Haemonchus contortus* seem to justify its use in traditional medicine against gastrointestinal parasites. However, carrying out acute toxicity and *in vivo* parasitological studies would be necessary.

Authors' Contribution

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Conflict of interests

The authors declare that they have no conflicts of interest

Ethical considerations

International standardized protocols were carried out for the experimental protocols [guidelines established by the European Union on protecting the environment]. Experiments involving laboratory animals were conducted with the approval of the Institutional Ethics Committee for Health Sciences Research of the Research Institut for Health Sciences (IRSS) (Ethics N/Ref: A015-2022/CEIRES). Every effort was made to minimise the pain and suffering of the animals.

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References

1. World Health Organization (WHO). Investing to Overcome the Global Impact of Neglected Tropical Diseases: Third WHO Report on Neglected Tropical Diseases. WHO 2015. p. 191. Available from: http://apps.who.int/iris/bitstream/10665/152781/1/9789241564861_eng.pdf.

2. Organisation Mondiale de la Santé (OMS). Intégrer les maladies tropicales négligées dans l'action pour la santé mondiale et le développement. OMS; 2017. p. 1-6. Available from: http://apps.who.int/iris/bitstream/10665/255015/1/WHO-HTM-NTD-2017.02-fre.pdf.

3. World Health Organization, Organisation Mondiale de la Santé. African programme for onchocerciasis control: progress report, 2014-2015. Wkly Epidemiol Rec. 2015;90(49):661-74.

4. Bartsch SM, Lopman BA, Ozawa S, Hall AJ, Lee BY. Global economic burden of norovirus gastroenteritis. PLoS One. 2016;11(4):e0151219. doi: 10.1371/journal.pone.0151219.

5. de Vlas SJ, Stolk WA, le Rutte EA, Hontelez JA, Bakker R, Blok DJ, et al. Concerted efforts to control or eliminate neglected tropical diseases: how much health will be gained? PLoS Negl Trop Dis. 2016;10(2):e0004386. doi: 10.1371/journal.pntd.0004386.

6. Stepek G, Buttle DJ, Duce IR, Behnke JM. Human gastrointestinal nematode infections: are new control methods required? Int J Exp Pathol. 2006;87(5):325-41. doi: 10.1111/j.1365-2613.2006.00495.x.

7. Savioli L, Albonico M, Engels D, Montresor A. Progress in the prevention and control of schistosomiasis and soil-transmitted helminthiasis. Parasitol Int. 2004;53(2):103-13. doi: 10.1016/j.parint.2004.01.001.

8. Ministère de la Santé et de l'Hygiène Publique Burkina Faso. Annuaire Statistiaue 2021. 2022. p. 389.

9. Kabore A, Belem AMG, Tamboura HH, Traore A, Sawadogo L. In vitro anthelmintic effect of two medicinal plants (*Anogeissus leiocarpus* and *Daniellia oliveri*) on *Haemonchus contortus*, an abosomal nematode of sheep in Burkina Faso. Afr J Biotechnol. 2009;8(18):4690-5.

10. Chandra S, Prasad A, Yadav N, Latchumikanthan A, Rakesh RL, Praveen K, et al. Status of benzimidazole resistance in *Haemonchus contortus* of goats from different geographic regions of Uttar Pradesh, India. Vet Parasitol. 2015;208(3-4):263-7. doi: 10.1016/j.vetpar.2015.01.005.

11. Yeates KO, Bigler ED, Dennis M, Gerhardt CA, Rubin KH, Stancin T, et al. Social outcomes in childhood brain disorder: a heuristic integration of social neuroscience and developmental psychology. Psychol Bull. 2007;133(3):535-56. doi: 10.1037/0033-2909.133.3.535.

12. Gasbarre LC, Smith LL, Hoberg E, Pilitt PA. Further characterization of a cattle nematode population with demonstrated resistance to current anthelmintics. Vet Parasitol. 2009;166(3-4):275-80. doi: 10.1016/j.vetpar.2009.08.019.

13. Dedehou VF, Olounladé PA, Adenilé AD, Azando EV, Alowanou GG, Daga FD, ET AL. Effets in vitro des feuilles de *Pterocarpus erinaceus* et des cosses de fruits de *Parkia biglobosa* sur deux stades du cycle de développement de *Haemonchus contortus* nématode parasite gastro-intestinal de petits ruminants. J AnimPlant Sci. 2014;22(1):3368-78.

14. Arbonnier M. Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest [Internet]. 2nd ed. Vol 2. Versailles: Ed. Quae, MNHN; 2002. p. 1-576. Available from: http://publications.cirad.fr/une_notice.php?dk=548725. Accessed October 21, 2018.

15. Yaméogo S. Etude chez lapin de l'effet du macéré hydroalcoolique du fruit de *Balanites aegyptiaca* Del. (Balanitaceae) sur les paramètres biologiques témoins du diabète sucré [dissertation]. Université de Ouagadougou; 2003. p. 123.

16. Shalaby MA, Moghazy FM, Shalaby HA, Nasr SM. Effect of methanolic extract of *Balanites aegyptiaca* fruits on enteral and parenteral stages of *Trichinella spiralis* in rats. Parasitol Res. 2010;107(1):17-25. doi: 10.1007/s00436-010-1827-9.

17. Shalaby HA, Kandil OM, Hassan NM, Helal MA. Cestocidal effects of *Balanites aegyptiaca* fruits' methanolic extract on the adult *Dipylidium caninum*. Bull Natl Res Cent. 2020;44(1):52. doi: 10.1186/s42269-020-00312-0.

18. Zerbo P, Millogo-Rasolodimey J, Nacoulma-Ouerdraogo OG, Van Damme P. Contribution à la connaissance des plantes médicinales utilisées dans les soins infantiles en pays San, au Burkina Faso. Int J Biol Chem Sci. 2007;1(3):262-74. doi: 10.4314/ijbcs.v1i3.39704.

19. de Sousa Araújo TA, Alencar NL, de Amorim EL, de Albuquerque UP. A new approach to study medicinal

plants with tannins and flavonoids contents from the local knowledge. J Ethnopharmacol. 2008;120(1):72-80. doi: 10.1016/j.jep.2008.07.032.

20. El Aziz MM, Ashour AS, Melad A. A review on saponins from medicinal plants: chemistry, isolation, and determination. J Nanomed Res. 2019;7(4):282-8. doi: 10.15406/jnmr.2019.07.00199.

21. Jabbar A, Iqbal Z, Khan MN. In vitro anthelmintic activity of *Trachyspermum ammi* seeds. Pharmacogn Mag. 2006;2(6):126-9.

22. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. Springer; 1996. p. 373.

23. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152-78. doi: 10.1016/s0076-6879(99)99017-1.

24. Abdel-Hameed E-SS. Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples. Food Chem. 2009;114(4):1271-7. doi: 10.1016/j.foodchem.2008.11.005.

25. Tibiri A, Rakotonandrasana O, Nacoulma GO, Banzouzi JT. Radical scavenging activity, phenolic content and cytotoxicity of bark and leaves extracts of *Entada africana* Guill. and Perr. (Mimosaceae). J Biol Sci. 2007;7(6):959-63. doi: 10.3923/jbs.2007.959.963.

26. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3):144-58.

27. Kim KS, Lee S, Lee YS, Jung SH, Park Y, Shin KH, et al. Anti-oxidant activities of the extracts from the herbs of *Artemisia apiacea*. J Ethnopharmacol. 2003;85(1):69-72. doi: 10.1016/s0378-8741(02)00338-0.

28. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9-10):1231-7. doi: 10.1016/s0891-5849(98)00315-3.

29. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-6. doi: 10.1006/abio.1996.0292.

30. Malterud KE, Rydland KM. Inhibitors of 15-lipoxygenase from orange peel. J Agric Food Chem. 2000;48(11):5576-80. doi: 10.1021/jf000613v.

31. Coles GC, Jackson F, Pomroy WE, Prichard RK, von Samson-Himmelstjerna G, Silvestre A, et al. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol. 2006;136(3-4):167-85. doi: 10.1016/j.vetpar.2005.11.019.

32. Athanasiadou S, Kyriazakis I, Jackson F, Coop RL. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. Vet Parasitol. 2001;99(3):205-19. doi: 10.1016/s0304-4017(01)00467-8.

33. Sunil Kumar KN, Sangeetha B, Suchitra P, Ravishankar B, Yashovarma B. Pharmacognosy and quality characterization of *Balanites aegyptiaca* (L.) Delile fruits. Indian J Nat Prod Resour. 2016;7(1):40-50. doi: 10.56042/ijnpr.v7i1.9792.

34. Liu HW, Nakanishi K. The structures of balanitins, potent molluscicides isolated from *Balanites aegyptiaca*.

35. Hosny M, Khalifa T, Çališ I, Wright AD, Sticher O. Balanitoside, a furostanol glycoside, and 6-methyldiosgenin from *Balanites aegyptiaca*. Phytochemistry. 1992;31(10):3565-9. doi: 10.1016/0031-9422(92)83728-h.

36. Kamel MS, Koskinen A. Pregnane glycosides from fruits of *Balanites aegyptiaca*. Phytochemistry. 1995;40(6):1773-5. doi: 10.1016/0031-9422(95)00551-h.

37. Speroni E, Cervellati R, Innocenti G, Costa S, Guerra MC, Dall' Acqua S, et al. Anti-inflammatory, anti-nociceptive and antioxidant activities of *Balanites aegyptiaca* (L.) Delile. J Ethnopharmacol. 2005;98(1-2):117-25. doi: 10.1016/j.jep.2005.01.007.

38. Wiesman Z, Chapagain BP. Larvicidal activity of saponin containing extracts and fractions of fruit mesocarp of *Balanites aegyptiaca*. Fitoterapia. 2006;77(6):420-4. doi: 10.1016/j.fitote.2006.05.012.

39. Yadav JP, Panghal M. *Balanites aegyptiaca* (L.) Del. (Hingot): a review of its traditional uses, phytochemistry and pharmacological properties. Int J Green Pharm. 2010;4(3):140-6. doi: 10.4103/0973-8258.69158.

40. Murthy HN, Yadav GG, Dewir YH, Ibrahim A. Phytochemicals and biological activity of desert date (*Balanites aegyptiaca* (L.) Delile). Plants (Basel). 2020;10(1):32. doi: 10.3390/plants10010032.

41. Traoré KT, Ouédraogo N, Belemnaba L, Boly AGL, Atchadé CB, Belemlilga MB, et al. Anti-inflammatory and analgesic activities of extracts from *Balanites aegyptiaca* L. Delile (Balanitaceae) root bark: plant used against liver diseases in Bukina Faso. Afr J Pharm Pharmacol. 2019;13(18):322-9. doi: 10.5897/ajpp2019.5083.

42. Boly AGL, Belemlilga MB, Traore A, Ouedraogo S, Guissou ET. Phytochemical study and in vitro anthelminthic properties studies of the trunk barks aqueous extract from *Acacia nilotica* var. *adansonii* (Guill and Perr). O Ktze (Mimosaceae). Int J Pharmacogn Phytochem Res. 2018;10(01):5-11. doi: 10.25258/phyto.v10i01.11924.

43. Belemlilga MB, Traoré A, Ouédraogo S, Kaboré A, Tamboura HH, Guissou IP. Anthelmintic activity of *Saba senegalensis* (A.DC.) Pichon (Apocynaceae) extract against adult worms and eggs of *Haemonchus contortus*. Asian Pac J Trop Biomed. 2016;6(11):945-9. doi: 10.1016/j.apjtb.2016.07.015.

44. Quijada J, Fryganas C, Ropiak HM, Ramsay A, Mueller-Harvey I, Hoste H. Anthelmintic activities against *Haemonchus contortus* or *Trichostrongylus colubriformis* from small ruminants are influenced by structural features of condensed tannins. J Agric Food Chem. 2015;63(28):6346-54. doi: 10.1021/acs.jafc.5b00831.

45. Khamesipour F, Kheyri P, Shojaat S, Chelgerdi Dehkordi B, Basirpour B, Afzal SS, et al. A review of the effect of medicinal plant on helminthic infections. Infect Dis Herb Med. 2021;2(1):127. doi: 10.4081/idhm.2021.127.

46. Zabré G, Kaboré A, Bayala B, Katiki LM, Costa-Júnior LM, Tamboura HH, et al. Comparison of the in vitro anthelmintic effects of *Acacia nilotica* and *Acacia raddiana*. Parasite. 2017;24:44. doi: 10.1051/parasite/2017044.

47. Pessoa LM, Morais SM, Bevilaqua CM, Luciano JH. Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against *Haemonchus*

Tetrahedron. 1982;38(4):513-9. doi: 10.1016/0040-4020(82)80095-1.

contortus. Vet Parasitol. 2002;109(1-2):59-63. doi: 10.1016/s0304-4017(02)00253-4.

48. Costa CT, Bevilaqua CM, Maciel MV, Camurça-Vasconcelos AL, Morais SM, Monteiro MV, et al. Anthelmintic activity of *Azadirachta indica* A. Juss against sheep gastrointestinal nematodes. Vet Parasitol. 2006;137(3-4):306-10. doi: 10.1016/j.vetpar.2006.01.002.

49. Ademola IO, Eloff JN. In vitro anthelmintic activity of *Combretum molle* (R. Br. ex G. Don) (Combretaceae) against *Haemonchus contortus* ova and larvae. Vet Parasitol. 2010;169(1-2):198-203. doi: 10.1016/j.vetpar.2009.12.036.

50. Paolini V, De La Farge F, Prevot F, Dorchies P, Hoste H. Effects of the repeated distribution of sainfoin hay on the resistance and the resilience of goats naturally infected with gastrointestinal nematodes. Vet Parasitol. 2005;127(3-4):277-83. doi: 10.1016/j.vetpar.2004.10.015.

51. Hoste H, Martinez-Ortiz-De-Montellano C, Manolaraki F, Brunet S, Ojeda-Robertos N, Fourquaux I, et al. Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections. Vet Parasitol. 2012;186(1-2):18-27. doi: 10.1016/j.vetpar.2011.11.042.

52. Tibe O, Sutherland IA, Lesperance L, Harding DR. The effect of purified condensed tannins of forage plants from Botswana on the free-living stages of gastrointestinal nematode parasites of livestock. Vet Parasitol. 2013;197(1-2):160-7. doi: 10.1016/j.vetpar.2013.07.004.

53. Nandi B, Roy S, Bhattacharya S, Sinha Babu SP. Free radicals mediated membrane damage by the saponins acaciaside A and acaciaside B. Phytother Res. 2004;18(3):191-4. doi: 10.1002/ptr.1387.