Scientific Research Publishing

Pharmacology & Pharmacy, 2024, 15, 207-221
https://www.scirp.org/journal/pp
ISSN Online: 2157-9431
ISSN Print: 2157-9423

Acute Toxicity and Anthelmintic Effects of *Gomphrena serrata* L. (Amaranthaceae) Extracts on Adult Worms and Eggs of *Haemonchus contortus*

Hippolyte Ouedraogo[1,2*], Mohamed Bonewendé Belemlilga[1,3], Aristide Traore[1], Gilchrist Abdoul Laurent Boly[1], Félicité Moyenga[1,2], Gueswindé Geoffroy Ouedraogo[1,3], Sylvain Ilboudo[1,3], Lazare Belemnaba[1,3], Sylvin Ouedraogo[1]

[1]Laboratoire de Recherche-Développement de Phytomédicaments et Médicaments (LR-D/PM), Institut de Recherche en Sciences de la Santé (IRSS), Centre National de la Recherche Scientifique et Technologique (CNRST), Ouagadougou, Burkina Faso
[2]Laboratoire de Développement du Médicament (LADME), Centre de Formation, deRecherche et d'Expertises en Sciences du Médicament (CEA-CFOREM), École Doctorale Sciences de la Santé (ED2S), Université Joseph KI-ZERBO, Ouagadougou, Burkina Faso
[3]International Research Laboratory-Environnement, Santé, Sociétés (IRL 3189, ESS) CNRS/CNRST/UCAD/UGB/USTTB, Ouagadougou, Burkina Faso
Email: *hippolyteouedraogo226@gmail.com

How to cite this paper: Ouedraogo, H., Belemlilga, M.B., Traore, A., Boly, G.A.L., Moyenga, F., Ouedraogo, G.G., Ilboudo, S., Belemnaba, L. and Ouedraogo, S. (2024) Acute Toxicity and Anthelmintic Effects of *Gomphrena serrata* L. (Amaranthaceae) Extracts on Adult Worms and Eggs of *Haemonchus contortus*. *Pharmacology & Pharmacy*, **15**, 207-221.
https://doi.org/10.4236/pp.2024.155012

Received: March 8, 2024
Accepted: May 28, 2024
Published: May 31, 2024

Abstract

Gomphrena serrata L. (Amaranthaceae) is a medicinal plant widely used traditionally in rural areas to treat many diseases, including gastrointestinal helminthiasis of small ruminants. This work aims to estimate the plant extracts phytochemical potential and study the activities of the aqueous decoction (GS_AD), aqueous macerate (GS_AM), and hydroalcoholic macerate (GS_HEM) of *Gomphrena serrata* extracts on two developmental stages (eggs and adult worms) of *Haemonchus contortus*. Phytochemical screening was carried out. The acute toxicity test was performed according to the Organisation for Economic Co-operation and Development (OECD) test guideline 423. The *Haemonchus contortus* eggs hatching inhibition test was performed left to incubate for 48 hours in the presence of the different plant extracts at increasing concentrations of 0.125; 0.25; 0.5; 0.625; 1; 2; 3 mg/mL. For the motility inhibition test on adult worms, the worms were contacted with the extracts at concentrations of 0.75; 1; 1.25; 1.5; 2; 3; 4 mg/mL and left to incubate for 24 hours. Albendazole and levamisole were used as standard. Phytochemical screening revealed the presence of tannins, saponins, reducing compounds, coumarins and derivatives, anthocyanins, steroids, triterpenes, and flavonoids. An acute toxicity study showed that the oral LD_{50} value of *G.*

serrata was estimated to be 5000 mg/kg. All extracts showed an effect on both stages of parasite development. The hatching inhibition rate was 100% from 3 mg/mL for all three extracts. The rate of inhibition of motility was 100% from 4 mg/mL for each extract. HEM is more efficient on eggs and *Haemonchus contortus* adult worms than other extracts with IC_{50} of 0.457 mg/mL and LC_{50} of 1.329 mg/mL, respectively. These results suggest that *Gomphrena serrata*, a plant used by farmers, has anthelmintic properties.

Keywords

Gomphrena serrata, *Haemonchus contortus*, Anthelmintic, *In Vitro*

1. Introduction

In most developing countries, livestock production is an essential source of income and contributes to food security. Indeed, Livestock farming is an important source of income for those involved in the livestock sector, and also contributes to the fight against malnutrition by providing protein and micronutrients to the population.

However, gastrointestinal strongylosis in ruminants is one of the main diseases that cause substantial economic losses to livestock keepers [1]. Particularly in small ruminants, infestations due to gastrointestinal nematodes constitute a fundamental constraint in breeding [2] [3] because they cause growth retardation and weight loss in these animals [4] [5]. Thus, the parasitic fauna of the digestive tract of small ruminants is dominated by strongyles, particularly *Haemonchus contortus* (*H. contortus*), by its frequency and intensity [6] [7] [8] [9]. Control of these parasites has long relied on the use of synthetic anthelmintics for their efficacy.

In many developing countries, small-scale livestock keepers are poor, so they have difficulty accessing veterinary products due to their high cost [10] [11]. Synthetic anthelmintics administered to animals are eliminated directly into nature in the form of active metabolites, via urine and faeces, constituting a source of environmental contamination that is potentially toxic for biodiversity. Globally, the use of reference anthelmintics is at the root of the development of chemoresistance [12]. To counteract the inaccessibility and chemoresistance associated with reference anthelmintics, emphasis should be placed on the use of plant resources that are available and known to farmers to solve the problems posed by these diseases. Thus, medicinal plants could provide a therapeutic response adapted to populations' financial means and socio-cultural environment.

Medicinal plants are a group of great socio-economic importance because they contain active components for treating various diseases [13]. This is why the middle and poor strata of Africa and the world often resort to certain plants with therapeutic virtues to treat or alleviate certain diseases [14] [15] [16].

In Burkina Faso, some researchers have already shown interest in plants with

anthelmintic properties for veterinary use that could be an alternative to synthetic anthelmintics. These include: *Balanites aegyptiaca* [17], *Saba senegalensis* [18], *Cassia sieberiana*, *Guiera senegalensis* and *Excoecaria grahamii* [19], *Securidaca longepedunculata* [20]. For more efficiency in improving the health of small ruminants, it would be wise to conduct studies on forage plants for their nutritional values and also for their richness in specific secondary metabolites such as tannins, flavonoids, saponins and alkaloids, whose anthelmintic properties and effects on zootechnical performance are known [21] [22] [23]. Burkinabe traditional medicine has several medicinal plants used by the population to treat gastrointestinal parasitosis. These include *Gomphrena serrata* L. (Amaranthaceae) [24]. Research on forage plants with anthelmintic properties will have the advantage of revealing the levels of primary and secondary metabolites with anthelmintic action contained in these plants.

For our part, the study of *Gomphrena serrata* (*G. serrata*) deserves to be conducted in this sense because this plant has the advantage of being a forage plant and containing bioactive substances such as flavonoids, saponins, tannins, alkaloids [25]. This study aims to evaluate, in vitro, the anthelmintic effect of three extracts of *G. serrata* on the inhibition of egg hatching and the inhibition of the motility of adult worms of *Haemonchus contortus*.

2. Materials and Methods

2.1. Animal Material

The biological material consists of animal material consisting of adult worms of *Haemonchus contortus* and.

2.2. Plant Material

Plant material consists of the *G. serrata* plant. The plants were collected in July 2022, near the city of Ouagadougou, in the morning between 6 am and 8 am and identified by the taxonomists of the Herbarium of the UFR/SVT of the Joseph KI-ZERBO University. *G. serrata* L. was already identified in 2017 under the numbers: N˚ ID: 17012; N˚ Echantillon: 6848 du 08/07/2017, of which a sample of the species was kept in the Herbarium of the Plant Biology and Ecology Laboratory of the Department of Plant Biology and Physiology (LaBEV). The collected plants were washed with tap water and dried in the shade at room temperature in the laboratory between 25 and 28°C. Once dried, the plants were ground to powder in an electric grinder. The powder thus obtained was used to prepare three extracts: an extract by aqueous maceration, an extract by aqueous decoction, and an extract by hydroethanolic maceration. These extracts were freeze-dried before being used for the various tests.

2.3. Preparation of Extracts

The method used is the solid-liquid extraction method. Plant powder, representing the solute, is the solid phase; distilled water (or distilled water and ethanol),

representing the solvent, is the liquid phase.

2.3.1. Aqueous Decoction

A 100 g test portion of the *G. serrata* plant powder was placed in a 2000 mL Erlenmeyer flask. A volume of 1000 mL of distilled water was added to the test portion due to the mucilaginous characteristic of the plant powder. After homogenisation with a glass rod, the mixture of plant material and extraction solvent was boiled on a hot plate for 1 h. After cooling, the extract was filtered through a fine mesh nylon cloth. The filtrate obtained was centrifuged at 2000 rpm for 10 min, and the supernatant was collected and frozen at −24°C for freeze-drying. The resulting lyophilisate was weighed to determine the extraction yield, represented by the mass of extracted material in 100 g of powder, and then stored in a refrigerator at 4°C.

2.3.2. Aqueous Maceration

A 100 g test portion of the *G. serrata* plant powder was placed in a 2000 mL Erlenmeyer flask. 500 mL of distilled water was added to the test portion. After homogenisation with a glass rod, the mixture of plant material and solvent extractor was kept at room temperature in the laboratory (30 °C) for 24 h. The extract was stirred from time to time with a glass rod. After a 24 h stay, the extract was well homogenised, filtered through a fine mesh nylon cloth, and pressed. The filtrate obtained was then centrifuged at 2000 rpm for 10 min. The resulting supernatant was collected, frozen at −24°C, and then lyophilised. The resulting lyophilisate was weighed to determine the extraction yield, represented by the mass of extracted material in 100 g of powder, and then stored in a refrigerator at 4°C.

2.3.3. Hydroethanolic Maceration

A 100 g test portion of the *G. serrata* plant powder was placed in a 2000 mL glass jar with a screw lid. To the test portion, 800 mL of a mixture of ethanol and distilled water in the ratio of 70:30; (v/v) was added. The plant material and extractor solvent mixture was homogenised using a glass rod and then kept at the laboratory's ambient temperature (30°C) for 48 h. The extract was then filtered through Wattman No. 5 paper. The residual pomace was depleted with portions of the extracting solvent until a faded filtrate was obtained. The hydroalcoholic extract of the sample was then concentrated to ¾ under reduced pressure in the rotavapor. The resulting concentrated extract was frozen at −24°C and then lyophilised. The resulting lyophilisate was weighed to determine the extraction yield, represented by the mass of extracted material in 100 g of powder, and then stored in a refrigerator at 4°C.

2.4. Phytochemical Screening

The phytochemical screening aimed to determine the groups of bioactive chemicals in the aqueous and hydroalcoholic extracts of *G. serrata*. The characterisation tests of the different groups of substances were carried out in a liquid me-

dium with chemical reagents specific to each group of bioactive substances. The method used was the one described by Ciulei, adapted by the chemistry laboratory of the Research Institute for Health Sciences (IRSS) [26]. The principle of this method is based on the ability of the functional chemical groups of bioactive substances to react with specific chemical reagents to give their characteristic colored products. The $FeCl_3$ test has identified tannins (polyphenols); flavonoids by the Shibata reaction or cyanidin test; saponins by the foam test; steroids and triterpenes by the Liebermann-Burchard test; alkaloids by the Mayer and Dragendorff tests; Anthracenosides by Bornträger's test; Coumarins and derivatives by Feigl's test; Cardenolides by Kéddé's reagent; Reducing compounds by Fehling's test; Anthocyanosides by Bate Smith's reagent.

2.5. Acute Toxicity Assay

The toxicity assay was carried out following the Organization for Economic Co-operation and Development (OECD) test guideline 423 concerning acute oral toxicity [27]. Before administration of the *G. serrata* extracts, the female rats were divided into batches of 3 per cage and fasted for 4 h without food but with free access to water. For each extract, the test was conducted in two stages. In the first phase, 3 rats each received a single oral dose of 2000 mg/kg b.w of each extract. After administration of the extracts, the animals were observed every 30 min for 2 h, after which water and food were re-introduced. The rats were further observed once a day for up to 14 days following treatment for probable signs of toxicity, such as behavioral or physiological changes and death and the latency of death [28] [29]. All the animals were weighed before the start of the test and then weighed at D1, D2, D3, D7 and D14 during the test period. After 14 days, the animals were anaesthetised with ketamine (150 mg/kg) and sacrificed. Vital organs such as the heart, lungs, kidneys, spleen, and liver were removed, dissected, and weighed. The relative weight of each organ was calculated.

2.6. Anthelmintic Assays

2.6.1. Collection of *H. contortus* Eggs

Adult female worms are collected, placed in PBS, and then slightly diluted in a mortar with a porcelain pestle to release the eggs. The resulting solution is filtered through mesh screens of different sizes (1 mm and 100 µm). Another 38 µm mesh screen is then used to retain the released eggs. The released eggs are recovered by rinsing the sieve with distilled water. The resulting egg suspension was adjusted to approximately 1000 eggs per mL.

2.6.2. Eggs Hatching Essay

100 uL of the egg suspension adjusted to 1000 eggs/mL was distributed to the wells of a 24-well plate. Each well added a volume of 1900 µL of extract of increasing concentration to 0.125; 0.25; 0.5; 0.625; 1; 2; 3 mg/mL. Albendazole was used as a positive control at the concentrations 0.0019; 0.00780; 0.03125; 0.06250; 0.125; 0.25 and 0.5 mg/mL. Distilled water was used as a negative control. The

plate was resealed and incubated at 25°C for 48 hours. After incubation, a drop of formalin was added to each well to stop the evolution of the eggs. The contents of each well were then placed on a microscope slide, and the number of developed larvae was assessed under a light microscope (Gx40). The test was repeated three times at different concentrations for each of the extracts and controls. The inhibitory activity of the plant extracts was determined by calculating the egg hatch inhibition rate (EHI) using the formula described [30]:

$$\mathrm{EHI}(\%) = 100 - \frac{\text{Number of } L_1 \text{ larvae}}{\text{Number of eggs deposited}} \times 100$$

2.6.3. Collection of Adult Worms

After slaughtering sheep or goats naturally infested with *H. contortus*, the abomasums were collected and packed in a cooler. In the parasitology laboratory, each abomasum was incised longitudinally in order to release the reddish coloured adult worms due to their hematophagous feeding mode. The worms were then carefully collected and placed in a petri dish containing a physiological Phosphate Buffer Saline (PBS, pH: 7.02) at 37°C.

2.6.4. Adult Worms Mortality Assay

Test extract solutions are prepared with PBS in six concentrations 0.75; 1; 1.25; 1.5; 2; 3; 4 mg/mL to perform the test [31]. Collected worms with good motility were placed in Petri dishes at a rate of three worms per dish, in a total volume of 8mL of each test extract prepared with the PBS solution and placed in an incubator at 35°C. A negative control (PBS buffer) and a positive control levamisole 0.0625; 0.125; 0.25; 0.5; 1; 2 and 2.5 mg/mL, prepared in PBS are used. The test is repeated three times for each concentration and the controls. The inhibition of adult worm motility in the treatments performed is used as the criterion for anthelmintic activity. After the worms were exposed to the extracts, motility was observed under a magnifying glass every 6, 20, and 24 hour and the number of dead worms was assessed at each observation. Any worm showing continuous total immobility for 10 seconds is suspected to be dead. It is then carefully removed from the well and placed in 10 ml of PBS solution for possible revitalisation. If the worm regains vitality within 30 minutes of removal from the test well, it is considered alive and returned to its well, and observations continue. If not, the worm is declared dead. The mortality rate per concentration and type of extract is calculated using the formula:

$$\mathrm{M}(\%) = \frac{\text{Number of dead worms in wells}}{\text{Number of living adults worms in wells}} \times 100$$

2.7. Statistical Analysis

Excel spreadsheet software obtained means, standard deviations, and illustrative graphs. Comparisons of treatments and analysis of the significance of a dose effect and a solvent effect are performed using GraphPad prism. The effects of the different doses on the motility of adult worms were compared using Student's t-test.

Table 1. The yield of *G. serrata* extracts.

Extracts	Yield
GS_AD	21.03 %
GS_AM	17.42 %
GS_HEM	13.95 %

GS_AD = *G. serrata* Aqueous Decoction; GS_AM = *G. serrata* Aqueous macerate; GS_HEM = *G. serrata* Hydroethanolic macerate

Table 2. Phytochemical groups found in *G. serrata* extracts.

Phytochemical group	Extracts		
	GS_AD	GS_AM	GS_HEM
Coumarins	+	+	+
Tannins	+	+	+
Steroids and Triterpenes	+	+	+
Saponins	+	+	+
Reducing compounds	+	+	+
Anthocyanosides	+	+	-
Flavonoids	+	+	+
Anthraquinones	-	-	-
Alkaloids	-	-	-
Cardenolides	-	-	-

3. Results

3.1. Extraction Yields

The extraction yields varied according to the solvents and the extraction methods (Table 1).

3.2. Phytochemical Screening

Phytochemical analysis (colorimetric reactions) revealed the presence of tannins, saponoside, reducing compounds, coumarins and derivatives, anthocyanosides, steroids, triterpenes, and flavonoids (Table 2).

3.3. Acute Toxicity Assay

In acute oral toxicity assay, after 14 days of observation post-treatment, no behavioral changes such as motor activity, breathlessness, alertness, restlessness, diarrhea, tremor, convulsion and coma were observed at the administered doses of AD, AM and HEM of *G. Serrata*. Also, the rats were physically active, and no death was observed in different experimental animals groups in a single dose of 2000 mg/kg/b.w. of extracts.

However, the average body weights of all control animals and those treated with each of the three extracts showed a relative increase over the 14 days (Figure 1). There was a slight upward trend in the body weights of homologous

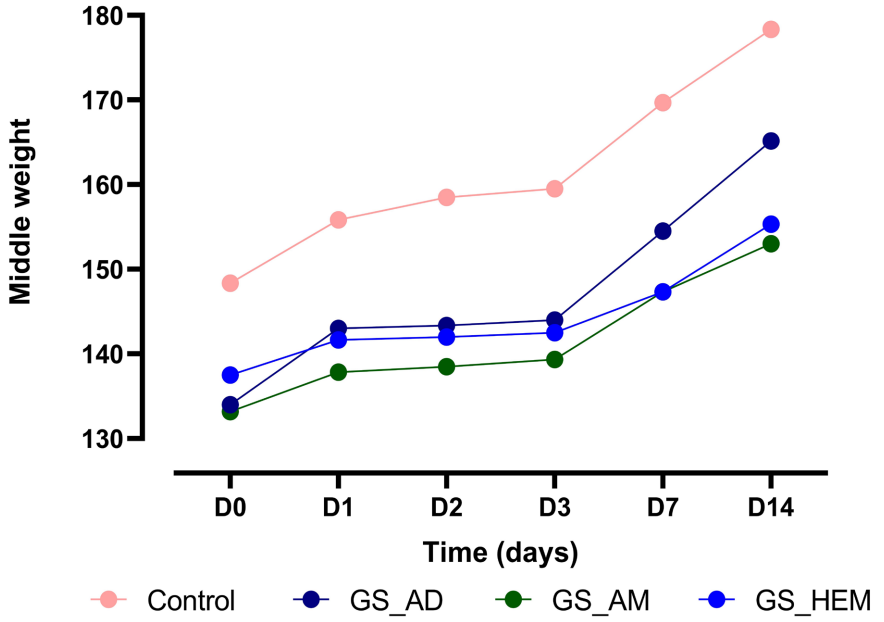

Figure 1. Evolution of the weight of the female rats in the control group in comparison with those in the experimental groups.

Table 3. Changes in the relative weights of female rat organs in the toxicity test.

Organes (g)	Control group	GS_AD	GS_AM	GS_HEM
Heart	0.42 ± 0.04	0.37 ± 0.03	0.43 ± 0.09	0.43 ± 0.03
lungs	0.68 ± 0.14	0.91 ± 0.38	0.71 ± 0.13	0.74 ± 0.18
Liver	0.76 ± 0.07	0.79 ± 0.24	0.68 ± 0.16	0.76 ± 0.14
Kidney	0.35 ± 0.05	0.45 ± 0.25	0.40 ± 0.07	0.38 ± 0.08
Spleen	3.81 ± 0.84	3.87 ± 0.68	3.66 ± 0.98	3.65 ± 0.40

groups of mice treated under the same experimental conditions. These changes in body weight were not statistically significant.

Macroscopic examination of fresh vital organs such as the heart, lungs, liver, kidneys and spleen of control animals and those treated with hydroethanolic macerate, aqueous decoctate and aqueous macerate shows that there was no change in the colour or appearance of these various organs. The relative organ weights of control rats and those treated with 2000 mg/kg bw of the three extracts are given in Table 3.

With n = 6; analysis was made with Two-way ANOVA followed by "Dunnett" multiple comparisons test. No statistically significant difference between the control group and the different extracts ($p > 0.05$).

3.4. Eggs Hatching Inhibition

The egg hatching inhibition *in vitro* of *G. serrata* extracts were compared to the albendazole used as a positive control (Figure 2).

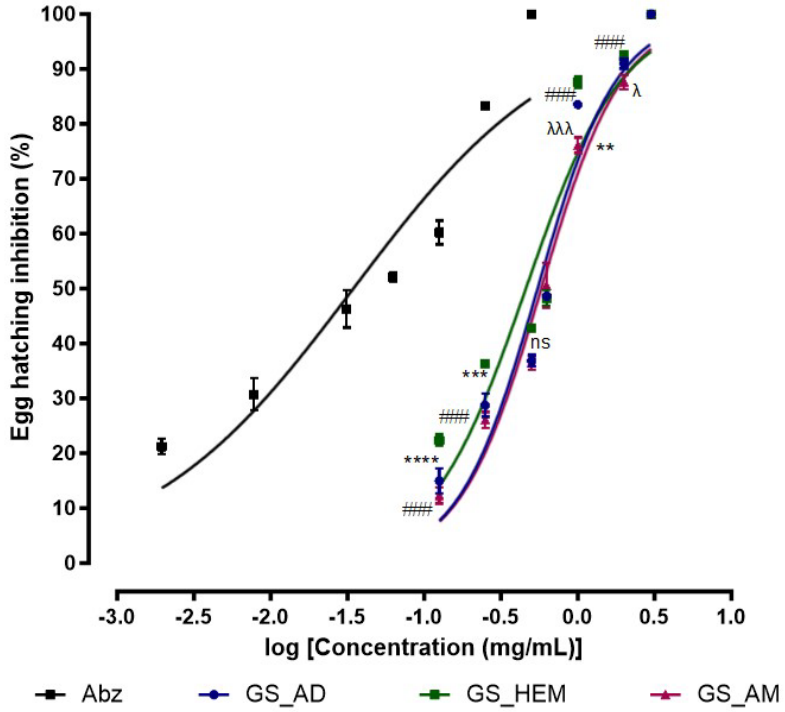

Figure 2. Effects of the different concentrations of the three extracts of *G. serrata* on *H. contortus* eggs hatching.

n = 6 ** $p < 0.01$, *** $p < 0.001$ GS_AD vs. GS_HEM, ### $p < 0.001$ GS_HEM vs. GS_AM, λ $p < 0.1$, λλλ $p < 0.001$ GS_AD vs. GS_AM is considered significant within each row, compare columns. Analysis was made with Two-way ANOVA followed by Tukey's multiple comparisons test. $p < 0.05$ significant between extracts. No significant difference between the different extracts ($p > 0.05$).

The 50% inhibitory concentrations (IC_{50}) were: GS_AD = 0.5454; GS_HEM = 0.4572; GS_AM = 0.5760 and Abz = 0.03437.

After 48 hours of contact between the eggs of *H. contortus* and the different concentrations of the three extracts of *G. serrata*, the hatching inhibition rate was 100% for the three extracts from 3 mg/mL.

3.5. Adults Worms Mortality

The motility of adult *H. contortus* worms was inhibited *in vitro* by *G. serrata* extracts compared to the positive control, levamisole (**Figure 3**). The HEM has proven to be more effective than the AD, which is, in turn, more effective than the AM.

n = 6 ** $p < 0.01$, *** $p < 0.001$ GS_HEM vs. GS_AM, ## $p < 0.01$ GS_AD vs. GS_AM is considered significant compared each row, to columns. Analysis was made with Two-way ANOVA followed by Tukey's multiple comparisons test. $p < 0.05$ significant between extracts. There is no significant difference between the different extracts ($p > 0.05$). After 24 hours of contact between the adult worms, the mortality rate reaches 100% for the three 4 mg/mL extracts.

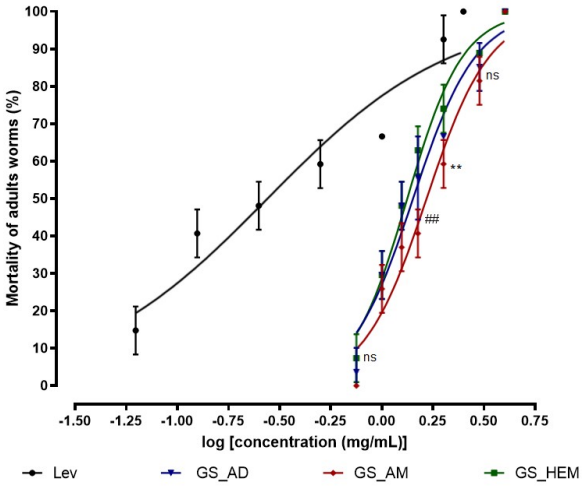

Figure 3. Effects of the different concentrations of the three extracts of *Gomphrena serrata* on the adult worms of *H. contortus*.

The 50% lethal concentrations (LC_{50}) were: GS_AD = 1.418; GS_HEM = 1.329; GS_AM = 1.652 and Lev = 0.2753.

4. Discussion

In the phytochemical study, the highest yields were observed with the aqueous decoction at 21.03%, followed by the aqueous macerate at 17.42%. The lowest extraction yield was with hydroalcoholic macerate: 13.95%. The extraction yield, therefore, depends on the nature and physicochemical characteristics of the solvents used, and in particular their polarity, and also on the solubility of the substances contained in the plant material. The higher yield of decoction may be due to the action of heat, a physical catalyst that dilates cell membranes, promoting the escape of hydrophilic compounds and also certain emulsified hydrophobic compounds.

Phytochemical screening reveals the presence of tannins, saponins, reducing compounds, coumarins and derivatives, steroids and triterpenes, and flavonoids at the level of the three extracts. However, anthocyanosides are only found in the aqueous decoction and maceration. The absence of anthocyanosides in the hydroalcoholic macerated could be explained by a lack of solubility and affinity with the majority extracting solvents, ethanol. Indeed, the absence of anthocyanins in the ethanolic extract of the leaves of *Myrtus communis* and *Rhamnus alaternus* [32]. Cardenolides and alkaloids are absent in all three extracts. Identifying tannins, saponins, steroids and triterpenes as flavonoids in *G. serrata* agrees with the work of Prasanna *et al.* [33]. He also highlighted the alkaloids. Our extracts have the advantage of containing coumarins which other publications on *G. serrata* have not highlighted. The plant development stage could explain phytochemical composition differences at harvest and climatic conditions, edaphic conditions, and genetic factors [34] [35].

The acute toxicity study tests the harmful effects of an agent on the organism

after a single or short-term exposure [36] [37]. Assessment of acute systemic toxicity in rats given a lethal dose of 2000 mg per kg body weight of each extract resulted in no deaths or signs of toxicity after 14 days of follow-up. According to the acute toxicity class method of OECD test guideline 423 and the Global Harmonized System, AD, AM, and HEM extracts of *G. Serrata* can be classified to the 5th toxicity class [27] [38]. Therefore, the LD_{50} value was estimated at 5000 mg/kg body weight, and the extracts have a relatively low acute oral toxicity.

Regarding the *in vitro* inhibition of eggs hatching, our results show an inhibition rate of 100% from 3 mg/mL for the three types of extracts. The mortality rate of adult worms is 100% for three extracts: AD, AM and HEM, at a concentration of 4 mg/mL in 24 hours. This indicates that the three extracts effectively inhibit the motility of adult worms. However, our extracts are less effective in inhibiting adult worms' motility than levamisole, which gives a mortality rate of 100% in 24 hours with a 2.5 mg/mL concentration. Our three extracts are less effective in the inhibition of egg hatching than albendazole, which gives a rate of 100% in 48 hours with a concentration of 0.5 mg/mL. HEM is more efficient on eggs and *Haemonchus contortus* adult worms compared to others with IC_{50} of 0.457 mg/mL and LC_{50} of 1.329 mg/mL, respectively. Our results are comparable to those obtained by Houngnimassoun *et al.* [23], which is 100% from 64 mg/mL of *Ficus exasperata*, and those obtained by Belemlilga *et al.*, who showed that the extract of Saba senegalensis gave a 97% hatch inhibition rate with 15 mg/mL [31]. *G. serrata*, through its three extracts, proved effective against the two parasitic stages considered in this study. However, the HEM extract was more effective than the AD and AM extracts. This suggests that the mode of extraction by HEM of *G. serrata* should be favored for effective anthelmintic action because of its ability to effectively extract polar and apolar compounds. The anthelmintic activity of *G serrata* would be due to secondary metabolites such as flavonoids, tannins, and saponins [39] [40] [41]. Indeed, tannins interfere with oxidative phosphorylation by blocking ATP synthesis in *H. contortus* [42]. Condensed tannins are the main compounds responsible for the anthelmintic effect [21] [41]. Inhibitions of worm motility and egg hatch are due to condensed tannins [43], which would diffuse to the membrane surface of eggs and larvae to bind to free membrane proteins, thus inhibiting egg hatch [44] [45]. Also, the anthelmintic effect of saponins would be explained by the destabilization of the membranes, hence an increase in the permeability of the cells, which will cause the turgidity of the cells and their bursting [39]. Today's challenge in the fight against helmintiasis is to use natural anthelmintics, to reduce the selection pressure of helminths by favouring the use of forage plants rich in flavonoids, tanninsand saponins, and by eliminating helminth-infected animals. The effectiveness of the effect observed in the anthelmintic effect would be due to a synergistic action of secondary metabolites such as flavonoids, tannins, and saponins. The *in vivo* anthelmintic properties of *Gomphrena serrata* need to be studied in order to confirm the efficacy of plant extracts on the different stages of *Haemochus contortus* development.

5. Conclusion

Our study first confirmed the richness of *Gomphrena serrata* in metabolites such as tannins, saponins, reducing compounds, coumarins and coumarin derivatives, anthocyanins, steroids, triterpenes, and flavonoids. Then, it showed that *G. serrata* inhibits the hatching of eggs and the motility of adult worms of *Haemochus contortus*. The HEM of *Gomphrena serrata* showed more efficacy than the AD and the AM, respectively, on the inhibition of egg hatching and the inhibition of the motility of adult *Haemochus contortus* worms. The traditional use of the leaves of *Gomphrena serrata* as an anthelmintic then seems to be justified. However, it would be necessary to carry out *in vivo* parasitological studies to consider the extracts' metabolism in the animal's digestive tract. The results of our study reveal the scientific basis of *Gomphrena serrata* anthelmintic activity, and will also enable us to enhance the value of this neglected plant.

Acknowledgements

The authors would like to thank the Laboratory for Research and Development of Phytomedicines and Medicines (LR-D/PM) of the Institut de Recherche en Sciences de la Santé (IRSS/CNRST), Ouagadougou, Burkina Faso.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Morel, P.-C., Itard, J. and Chartier, C. (2000) Précis de parasitologie vétérinaire tropicale. Lavoisier Tech & Doc (Collection Universités francophones), Paris, 796 p.

[2] Roeber, F., Jex, A.R. and Gasser, R.B. (2013) Impact of Gastrointestinal Parasitic Nematodes of Sheep, and the Role of Advanced Molecular Tools for Exploring Epidemiology and Drug Resistance—An Australian Perspective. *Parasites & Vectors*, **6**, Article No. 153. https://doi.org/10.1186/1756-3305-6-153

[3] Hoste, H., Meza-Ocampos, G., Marchand, S., Sotiraki, S., Sarasti, K., Blomstrand, B.M., *et al*. (2022) Use of Agro-Industrial by-Products Containing Tannins for the Integrated Control of Gastrointestinal Nematodes in Ruminants. *Parasite*, **29**, Article No. 10. https://doi.org/10.1051/parasite/2022010

[4] Laha, R., Das, M. and Goswami, A. (2013) Gastrointestinal Parasitic Infections in Organized Cattle Farms of Meghalaya. *Veterinary World*, **6**, 109-112. https://doi.org/10.5455/vetworld.2013.109-112

[5] Renwal, K.K., Gupta, A., Kumar, N., Pilania, P.K. and Manohar, G.S. (2017) Prevalence and Risk Assessment of Gastrointestinal Helminthoses in Dairy Animals of Bikaner, Rajasthan. *Journal of Parasitic Diseases*, **41**, 557-561. https://doi.org/10.1007/s12639-016-0850-x

[6] Chiejina, S.N., Behnke, J.M., Musongong, G.A., Nnadi, P.A. and Ngongeh, L.A. (2010) Resistance and Resilience of West African Dwarf Goats of the Nigerian Savanna Zone Exposed to Experimental Escalating Primary and Challenge Infections with *Haemonchus contortus*. *Veterinary Parasitology*, **171**, 81-90. https://doi.org/10.1016/j.vetpar.2010.03.007

[7] Attindehou, S. and Salifou, S. (2012) Epidemiology of Cestodes Infections in Sheep and Goats in Benin. *Veterinary Research*, **5**, 59-62.

[8] Djawe, P.B., Salhine, R., Bamia, A., Saotoing, P. and Maiwore, J. (2019) Caractérisation des helminthes gastro-intestinaux de bovins dans le département de la Vina. *International Journal of Advanced Research in Biological Sciences*, **6**, 44-53.

[9] Sheferaw, D., Mohammed, A. and Degefu, A. (2021) Distribution and Prevalence of Gastrointestinal Tract Nematodes of Sheep at Highland and Midland Areas, Ethiopia. *Journal of Parasitic Diseases*, **45**, 995-1001. https://doi.org/10.1007/s12639-021-01397-8

[10] Lalchhandama, K. (2010) *In vitro* Effects of Albendazole on *Raillietina echinobothrida*, the Cestode of Chicken, *Gallus domesticus*. *Journal of Young Pharmacists*, **2**, 374-378. https://doi.org/10.4103/0975-1483.71630

[11] Tariq, K.A. and Tantry, M.A. (2012) Preliminary Studies on Plants with Anthelmintic Properties in Kashmir—The North-West Temperate Himalayan Region of India. *Chinese Medicine*, **3**, 106-112. https://doi.org/10.4236/cm.2012.32017

[12] Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., Von Samson-Himmelstjerna, G., Silvestre, A., *et al*. (2006) The Detection of Anthelmintic Resistance in Nematodes of Veterinary Importance. *Veterinary Parasitology*, **136**, 167-185. https://doi.org/10.1016/j.vetpar.2005.11.019

[13] Khamesipour, F., Kheyri, P., Shojaat, S., Chelgerdi Dehkordi, B., Basirpour, B., Afzal, S.S. *et al*. (2021) A Review of the Effect of Medicinal Plant on Helminthic Infections. *Infectious Diseases and Herbal Medicine*, **2**, Article 127. https://doi.org/10.4081/idhm.2021.127

[14] Githiori, J.B., Athanasiadou, S. and Thamsborg, S.M. (2006) Use of Plants in Novel Approaches for Control of Gastrointestinal Helminths in Livestock with Emphasis on Small Ruminants. *Veterinary Parasitology*, **139**, 308-320. https://doi.org/10.1016/j.vetpar.2006.04.021

[15] Santa Rosa, S.S., Santos, F.O., Lima, H.G., Reis, I.M.A., Cassiano, D.S.A., Vieira, I.J.C., *et al*. (2018) *In vitro* Anthelmintic and Cytotoxic Activities of Extracts of *Persea willdenovii* Kosterm (Lauraceae). *Journal of Helminthology*, **92**, 674-680. https://doi.org/10.1017/S0022149X17000979

[16] Toklo, P.M., Ladekan, E.Y., Linden, A., Hounzangbe-Adote, S., Kouam, S.F. and Gbenou, J.D. (2021) Anthelmintic Flavonoids and Other Com-Pounds from *Combretum glutinosum* Perr. ex DC (Combretaceae) Leaves. *Acta Crystallographica Section C*: *Structural Chemistry*, **77**, 505-512. https://doi.org/10.1107/S2053229621007841

[17] Gnoula, C., Guissou, P., Duez, P., Frédérich, M. and and Dubois, J. (2007) Nematocidal Compounds from the Seeds of *Balanites aegyptiaca* Isolation and Structure Elucidation. *International Journal of Pharmacology*, **3**, 280-284. https://doi.org/10.3923/ijp.2007.280.284

[18] Belemlilga, M.B., Traoré, T.K., Boly, G.A.L., Ouédraogo, N., Traoré, A., Lompo, M., *et al*. (2019) Evaluation of Antioxidant, Anti-Inflammatory and Analgesic Activities of Leaves of *Saba senegalensis* (A.DC) Pichon (Apocynaceae). *European Journal of Medicinal Plants*, **27**, 1-12. https://doi.org/10.9734/ejmp/2019/v27i330116

[19] Traore, A., Ouédraogo, S., Belemlilga, M.B., Kaboré, A., Guissou, I.P., Belemlilga, B.M., *et al*. (2017) Phytochemical Analysis and Ovicidal Activity of Cassia Sieberiana, *Guiera senegalensis* and *Excoecaria grahamii* Extracts. *African Journal of Pharmacy and Pharmacology*, **11**, 554-560. https://doi.org/10.5897/AJPP2017.4837

[20] Tianhoun, D.F., Meda, N.-T.R., Konate, A., Kabore, A., Tamboura, H.H. and Belem,

A.M.G. (2020) Phytochemical Screening and *In vitro* Anthelmintic Activity of *Cassia alata* (L) Roxb. on *Haemonchus contortus* of Small Ruminants in Burkina Faso. *The Journal of Advances in Parasitology*, **7**, 14-19. https://doi.org/10.17582/journal.jap/2020/7.3.14.19

[21] Hoste, H., Martinez-Ortiz-De-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., Fourquaux, I., *et al*. (2012) Direct and Indirect Effects of Bioactive Tannin-Rich Tropical and Temperate Legumes against Nematode Infections. *Veterinary Parasitology*, **186**, 18-27. https://doi.org/10.1016/j.vetpar.2011.11.042

[22] Gomes, D.C., de Lima, H.G., Vaz, A.V., Santos, N.S., Santos, F.O., Dias, Ê.R., *et al*. (2016) *In vitro* Anthelmintic Activity of the *Zizyphus joazeiro* Bark against Gastrointestinal Nematodes of Goats and Its Cytotoxicity on Vero Cells. *Veterinary Parasitology*, **226**, 10-16. https://doi.org/10.1016/j.vetpar.2016.06.004

[23] Houngnimassou, H.M.A., Attindehou, S., Salifou, S., Koumodji, K.D. and Salifou, S. (2017) Effets Strongylicides *in vitro* de l'extrait aqueux de feuilles de *Ficus exasperata* Valh. 1805 (Moraceae). *International Journal of Biological and Chemical Sciences*, **11**, 1012-1020. https://doi.org/10.4314/ijbcs.v11i3.6

[24] Nacoulma, O.G. (1996) Plantes médicinales et Pratiques médicales Traditionnelles: Cas du plateau central. These d'Etat, Université de Ouagadougou, Ouagadougou.

[25] Nandini, K., Palaksha, M. and Gnanasekaran, D. (2018) A Review of *Gomphrena serrata*. *International Journal of Science and Research Methodology*, **11**, 105-110.

[26] Ciulei, I. (1982) Pratical Manuals on the Indistrial Utilization of Chemical and Aromatic Plants. Methodology for Analysis of Vegetable Drugs, Ministry of Chemical Industry B.

[27] OECD (2002) Test No. 423: Acute Oral Toxicity—Acute Toxic Class Method. Oecd Guideline for Testing of Chemicals Section 4, OECD, 1-14. https://doi.org/10.1787/9789264071001-en

[28] Ouedraogo, G.G., Ouedraogo, M., Lamien-Sanou, A., Lompo, M., Goumbri-Lompo, O.M. and Guissou, P.I. (2013) Acute and Subchronic Toxicity Studies of Roots Barks Extracts of *Calotropis procera* (Ait.) R. Br Used in the Treatment of Sickle Cell Disease in Burkina Faso. *British Journal of Pharmacology and Toxicology*, **5**, 194-200. https://doi.org/10.19026/bjpt.4.5401

[29] Magnini, R.D., Nitiéma, M., Ouédraogo, G.G., Ilboudo, S., Bancé, A., Millogo-Koné, H., *et al*. (2021) Toxicity and Bacterial Anti-Motility Activities of the Hydroethanolic Extract of *Acacia senegal* (L.) Willd (Fabaceae) Leaves. *BMC Complementary Medicine and Therapies*, **21**, Article No. 178. https://doi.org/10.1186/s12906-021-03348-5

[30] Angelo, B.F.D., Wabo, P.J., Yondo, J., Komtangi, C.M., Vittori, S. and Mpoame, M. (2014) Evaluation of Ovicidal and Larvicidal Activities of Methylene Chloride-Methanol Extract of *Annona senegalensis* (Annonaceae) Stem Bark on *Heligmosomoides bakeri* (Nematoda, Heligmosomatidae). *Global Journal of Science Frontier Research*: *C Biological Science*, **14**, 29-39.

[31] Belemlilga, M.B., Traoré, A., Ouédraogo, S., Kaboré, A., Tamboura, H.H. and Guissou, I.P. (2016) Anthelmintic Activity of *Saba senegalensis* (A.DC.) Pichon (Apocynaceae) Extract against Adult Worms and Eggs of *Haemonchus contortus*. *Asian Pacific Journal of Tropical Biomedicine*, **6**, 945-949. https://doi.org/10.1016/j.apjtb.2016.07.015

[32] Ouahiba, B., Karima, Y., Narimen, B., Razika, L., Souheila, T., Haddad Djillali, E., *et al*. (2020) Criblage phytochimique et évaluation du pouvoir antioxydant des feuilles de *Myrtus communis* L. et *Rhamnus alaternus* L. phytochemical Screening and

Evaluation of the Antioxidant Power of Leaves of *Myrtus communis* L. and *Rhamnus alaternus* L. *Revue Agrobiologia*, **10**, 1749-1761.

[33] Prasanna, M.M., Priyanka, M. and Neelothpala, N. (2017) Physicochemical Evaluation of Leaf of *Gomphrena serrata*. *Advanced Herbal Medicine*, **3**, 16-25.

[34] Sangwan, N.S., Farooqi, A.H.A., Shabih, F. and Sangwan, R.S. (2001) Regulation of Essential Oil Production in Plants. *Plant Growth Regulation*, **34**, 3-21. https://doi.org/10.1023/A:1013386921596

[35] Paolini, J., El Ouariachi, E.M., Bouyanzer, A., Hammouti, B., Desjobert, J.M., Costa, J., *et al*. (2010) Chemical Variability of Artemisia Herba-Alba Asso Essential Oils from East Morocco. *Chemical Papers*, **64**, 550-556. https://doi.org/10.2478/s11696-010-0051-5

[36] Krishnaraju, A.V., Rao, T.V.N., Sundararaju, D., Vanisree, M., Tsay, H.-S. and Subbaraju, G.V. (2005) Assessment of Bioactivity of Some Sudanese Medicinal Plants Using Brine Shrimp (*Artemia salina*) Lethality Assay. *International Journal of Applied Science and Engineering*, **3**, 125-134.

[37] Kharchoufa, L., Bouhrim, M., Bencheikh, N., El Assri, S., Amirou, A., Yamani, A., *et al*. (2020) Acute and Subacute Toxicity Studies of the Aqueous Extract from *Haloxylon scoparium* Pomel (*Hammada scoparia* (Pomel)) by Oral Administration in Rodents. *BioMed Research International*, **2020**, Article ID: 4020647. https://doi.org/10.1155/2020/4020647

[38] United Nations (2023) Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Tenth Revised Edition United. United Nations, New York and Geneva.

[39] Ademola, I.O. and Eloff, J.N. (2010) *In vitro* Anthelmintic Activity of *Combretum molle* (R. Br. ex G. Don) (Combretaceae) against *Haemonchus contortus* Ova and Larvae. *Veterinary Parasitology*, **169**, 198-203. https://doi.org/10.1016/j.vetpar.2009.12.036

[40] Leicach, S.R. and Chludil, H.D. (2014) Plant Secondary Metabolites: Structure-Activity Relationships in Human Health Prevention and Treatment of Common Diseases. *Studies in Natural Products Chemistry*, **42**, 267-304. https://doi.org/10.1016/B978-0-444-63281-4.00009-4

[41] Spiegler, V., Liebau, E. and Hensel, A. (2017) Medicinal Plant Extracts and Plant-Derived Polyphenols with Anthelmintic Activity against Intestinal Nematodes. *Natural Product Reports*, **34**, 627-643. https://doi.org/10.1039/C6NP00126B

[42] Martin, R.J. (1997) Modes of Action of Anthelmintic Drugs. *The Veterinary Journal*, **154**, 11-34. https://doi.org/10.1016/S1090-0233(05)80005-X

[43] Molan, A.-L. (2014) Effect of Purified Condensed Tannins from Pine Bark on Larval Motility, Egg Hatching and Larval Development of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Nematoda: Trichostrongylidae). *Folia Parasitologica*, **61**, 371-376. https://doi.org/10.14411/fp.2014.036

[44] Brunet, S., Montellano, C.M.O. de Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Aguilar-Caballero, A.J., Capetillo-Leal, C., *et al*. (2008) Effect of the Consumption of *Lysiloma latisiliquum* on the Larval Establishment of Gastrointestinal Nematodes in Goats. *Veterinary Parasitology*, **157**, 81-88. https://doi.org/10.1016/j.vetpar.2008.07.013

[45] Zabré, G., Kaboré, A., Bayala, B., Katiki, L.M., Costa-Júnior, L.M., Tamboura, H.H., *et al*. (2017) Comparison of the *in Vitro* Anthelmintic Effects of *Acacia nilotica* and *Acacia raddiana*. *Parasite*, **24**, Article No. 44. https://doi.org/10.1051/parasite/2017044