**In vitro** anthelmintic activity of aqueous extract of _Crassocephalum crepidioides_ (Benth.)

S. Moore on _Haemonchus contortus_

Bogning Zangueu Calvin¹², Olounlade Pascal Abiodoun²³, Alowanou Goue Gérancelin², Ngoumo Edwige Laure⁴, Dongmo Alain Bertrand¹, Azebaze Anatole Guy Blaise⁵, Hounzangbe-Adote Sylvie²

**ABSTRACT**

Objectives: Gastrointestinal parasites are recognized as major constraint to livestock production throughout the tropics. The use of plants and plant extracts remain the most serious alternative to modern anthelmintic drugs. This study evaluates the *in vitro* anthelmintic activity of aqueous leaves extract of _Crassocephalum crepidioides_ against _Haemonchus contortus_.

Methods: Aqueous extract at concentrations of 75 to 2400 µg/ml was tested in *vitro* on three development stages of _H. contortus_ using egg hatch assay (EHA), larval migration inhibition assay (LMIA) and adult worms motility inhibition assay (AMIA). Results: EHA showed significant reduction (P < 0.05) on _H. contortus_ egg hatch. The inhibition of egg hatching was concentration dependent with the greatest inhibition (50.52%) at the highest concentration (2400 µg/ml) of the extract. Thiabendazole used as positive control showed significant inhibition (P < 0.001) with rate of 76.68 % at the highest concentration (500µg/ml). On LMIA the extract significantly (P < 0.05) inhibited larval migration of _L3_ in a concentration dependent manner compared to Phosphate Buffered Saline, but less than levamisole (P < 0.001). The highest inhibition rate was 35.30 % at the concentration of 1200 µg/ml. The addition of polyvinylpolyrrolidone to the aqueous extract slightly reduces (4.9%) the inhibition of the effect induced by the extract on larval migration. Effect of extract on AMIA was concentration-dependent with significant increase (P < 0.05) in percentage of inhibition after 12 h. Levamisole (500 µg/ml) kills 100% worms after 18 h post-exposure but by this time the plant exhibited only 16.67% inhibition at the highest concentration (2400 µg/ml). Conclusion: These finding indicate that _C. crepidioides_ leaves have anthelmintic properties against _H. contortus_. This activity may be due to secondary metabolites such as saponins, flavonoids and tannins present in the extract. Further studies are needed to evaluate deeply the anthelmintic potential of this plant.

**KEY WORDS:** _Crassocephalum crepidioides_; _Haemonchus contortus_; Egg hatching; Larval migration; Worm motility; Anthelmintic activity.

**INTRODUCTION**

Breeding of small ruminants is an important prospective activity of agricultural sector in tropical countries [1]. It contributes to promote social, cultural and economical development in rural areas. This activity ensure the financing of agricultural operations, the promotion of human health by supplying animal’s protein in the form of meat and milk in market or agro foods industries, supplying naturals resources for artists, very used and appreciated in Africans societies during religious and familial feast. Livestock especially small ruminants, represents a major asset among resource-poor small holder farmers [2]. This activity has many constraints who impairing his profitability such as food and sanitary conditions.

However, gastro-intestinal parasites are recognized as a major constraint to livestock production throughout the tropics and subtropics [3]. It’s well known that helminthesis, impairs animal health, welfare and productivity since the presence of worms results in increased death rate and poor growth and reproduction [4]. _Haemonchus contortus_ and _Trichostrongylus colubriformis_ are listed among the top ten of most important ruminant’s parasites [5-7]. _Haemonchus contortus_ causes anaemia, haemorrhagic gastroenteritis, hypoproteinemia partly manifested as submandibular oedema ‘bottle jaw’ and sudden death [8].

The usual mode of control of these parasitic diseases relies on the repeated use of chemical anthelmintic drugs. However, these drugs are often so highly priced that they are not accessible to subsistence and small-scale livestock farmers in developing countries [6], in addition the worldwide emergence of drug resistance in gastrointestinal nematode (GIN) populations has motivated investigation into alternative approaches [9-11]. Other alternative control methods of parasitism are also used or still under investigations [12, 13]. Options like biological control, vaccine, improvement of food quality of host and traditional medicinal plants are being examined in different parts of the...
Bogning et al: Anthelmintic activities of medicinal plant extract

world [14]. Phytotherapy based on the use of preparation of medicinal plant, with low cost and efficiency, remains the most serious alternative to modern anthelmintic drugs [15, 16]. The quantitative adjustment of daily diet offers to animal particularly in proteins allow to supply nutritional’s needs due to the presence of worms and ameliorate thus, the response (resistance and resilience) of the animal on parasitism [17].

Crassocephalum crepidioides (Benth.) S. Moore is an african leafy vegetable that belongs to the Asteraceae’s family. The tender succulent leaves and stems of this plant are mucilaginous and used in the preparation of soups and stews, especially in West and Central Africa [18]. The leaves of Crassocephalum crepidioides are also used for their medicinal value against some diseases including indigestion, headache, upset stomach, liver complaints, fresh wounds and intestinal worms [19-21]. According to Dairo and Adanlawo [22], this plant is a good source of protein in human and animal nutrition. It also possesses antioxidant, and cyto-protective properties [23]. However, there is no report on the anthelmintic activity of this plant.

The present study was undertaken to evaluate the in vitro anthelmintic potentials of the aqueous extract of C. crepidioides leaves against the gastro-intestinal nematode Haemonchus contortus.

MATERIALS AND METHODS

Plant collection

The leaves of Crassocephalum crepidioides (Benth.) S. Moore (Asteraceae) were collected in July 2014 at Famleng in West Region of Cameroon. The plant was authenticated at the National Herbarium Yaounde-Cameroon, where a voucher specimen is deposited under the number N° 24250/SRF Cam. The leaves were subsequently sun-dried 4 hours/day during 14 days and powdered using a laboratory grinder (Bender and Hobein 8042 Zurich.) and kept at room temperature until used.

Plant extraction

The extract was prepared by decoction using 300 g of powdered leaves of Crassocephalum crepidioides in 3 L of distilled water during 30 minutes. After cold the solution was filtered, and the resulting filtrate was lyophilized to obtain 55.5 g of crude residue extract. The yield of extract was 18.5% w/w. The extract was stored at 4°C, and used for different biological assays.

Phytochemical analysis

Qualitative phytochemical screening of Crassocephalum crepidioides leaves was carried out using the standardly employed precipitation and coloration reactions as described by Odebiyi and Sofowora [24]. Some secondary metabolites were essayed by different methods.

In vitro anthelmintic assays

The anthelmintic activity of the aqueous extract of C. crepidioides leaves was tested on the different life-cycle stages of Haemonchus contortus (eggs, third stage larvae and adult worms) obtained from donor lambs experimentally infected with oral administration of a pure aqueous suspension of 3,000 H. contortus third stage larvae (L3). The tests were performed using three different procedures: egg hatch assay (EHA), larval migration inhibition assay (LMA) and adult worms motility inhibition assay (AMIA).

Bioassays on eggs of Haemonchus contortus

Prior authorization for the use of laboratory animals in this study was obtained from the Institutional Ethical Committee of the University of Douala (Ref. N° CEI-2015/01954).

Collection of eggs: Eggs were collected from the feces which were analyzed using the conventional method of Mc-Master modified by Raynaud [25]. Fecal matter of lamb infected with a strain of H. contortus were freshly collected and weighed. First of all, 1g of feces was analyzed using the method described above to calculate the number (N1) of eggs in the sample (OPG). The initial amount of eggs (N) contained in the fecal mass was then determined as the product of N1 by the total weight (P). After N1 and N were determined, the fecal mass was stirred continuously for dissoluation in five times its volume. The mixture was then poured through a series of sieves decreasing mesh (200 µm, 125 µm, and 40 µm). The residue on the final sieve was collected in a small volume of water and centrifuged. The pellet was added to a saturated NaCl solution and then centrifuged again. The supernatant was then filtered and the residue was washed three times followed by centrifugation. The resultant pellet was added to a known volume of water.

Egg hatch assay (EHA): This test was performed according to the procedure described by Coles et al. [26]. Eggs suspension was adjusted to 1000 eggs/ml and distributed in 24-multiwell plates (1 ml/well). For the treatment, 1ml of aqueous extract prepared with PBS at different concentrations (75, 150, 300, 600, 1200 and 2400 µg/ml) were added. PBS was used as negative control and a thiabendazole (125, 250 and 500 µg/ml in PBS) was used as positive control. The mixture was then incubated at 27 ℃. After 48 hours, egg hatching was stopped by adding two drops of formaldehyde solution (10%) per well. Thereafter the number of hatched eggs was counted using an optical microscope. The test was repeated five times. The percentage inhibition of hatching (IEO) for each concentration was calculated using the modified formula of Coles et al. [27]: % IEO = 100 (1- X1 / X2), where X1 was the number of eggs hatches in contact with the extracts and X2 the number of eggs hatches with the control.

Bioassays on infective larvae of Haemonchus contortus

Collection of larvae: Infective larvae of H. contortus (L3) were obtained by fecal culture collected from an
experimentally infected lamb at room temperature. After egg hatching, infective stage was reached after 10 days. The L3 were then collected by sedimentation using Baermann's devices.

Larval migration inhibition assay (LMIA): The larval migration inhibition assay was tested as described by Rabel et al. [28], adapted for plant extract [29]. This test is based on measurement of the rate of migration of larvae through a membrane after contact with the plant extracts. A known quantity of L3 larvae (1000/ml) was brought into contact with aqueous extract at different concentrations (1200, 600, 300, 150 and 75 µg/ml) and incubated for three hours at 20 °C. The assay was replicated three times for each extract concentration and for the controls. Then, the larval suspension was washed and centrifuged three times with PBS buffer. Each suspension was allowed to migrate through a 20 microns diameter mesh for three hours at 23°C. PBS and levamisole (250 µg/ml) were used as negative and positive control respectively. After 3 h of incubation, the inserts were removed and larvae that migrated were included in a volume adjusted to 1.5 ml by adding PBS. After counting the larvae under a magnifying glass, the percentage of LMI was calculated using the following formula: % LMI = [(T – M)/ T×100], where T is the total number of larvae L3 that were in contact with PBS and M is the number of larvae L3 in contact with the extracts.

Involvement of the tannins in the anthelmintic activity: Polystyrene polypyrrolidone (PVPP) forms complexes with tannins and polyphenols and thus blocks their potential biological activity [30]. PVPP was added to the aqueous extract at concentration of 1200 and 600 µg/mL and kept overnight in a 1:50 ratio [31]. These solutions were then centrifuged (4500 RPM, 5 min, 20° C), and the supernatant was used to incubate infective larvae (L3). Thereafter, the LMI assay was performed as described previously.

Adult worm's bioassays

Collection of adult worms: After the slaughter of experimentally infected lamb, the abomasum was removed, opened and the contents placed in 9 % saline solution at 37 °C. The mobile worms were rapidly collected, washed, recovered and placed in saline at 27 °C.

Adult worms motility inhibition assay (AMIA): The anthelmintic effect of aqueous extract of plant on adult worm motility was performed according to Houzhangbe-Adote et al. [6]. Solutions of aqueous extract were prepared with PBS at six different concentrations (75, 150, 300, 600, 1200, and 2400 µg/ml), and 1 ml of each of these solutions were deposited in titration plate wells. One actively moving adult worm was then placed into each well. PBS and a levamisole (125, 250 and 500 µg/ml in PBS) were also prepared and were used as negative and positive controls. The test was repeated six times for each concentration and for controls. Inhibition of motility of adult worms was used as the criterion for anthelmintic activity. After exposing worms to the aqueous extract, motility was observed every 6 hours using a magnifying glass. Adult worms’ motility inhibition was evaluated as the ratio: number of immotile worms divided by the total number of worms for each concentration or control. The death of the worms was determined by the absence of motility for five seconds. The observations ended when all the worms in PBS died.

Data analysis

The results were summarized as means ± standard error of means, while differences between means were analyzed at the 5 % level of significance using the one-way analysis of variance (ANOVA) followed by Dunnett’s post hoc test on Graph Pad Prism Version 5.03 software.

RESULTS

Phytochemical analysis

The phytochemical screening of the leaves aqueous extracts of *Crassocephalum crepidioides* revealed the presence of several bioactive components such as saponins, phenols, flavonoids, tannins and sterols. Anthraquinones, terpenes, reducing compounds and glycosides were not detected in the extract (Table 1).

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Assays</th>
<th>C. crepidioides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>Test index foam</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>HCl-NaOH test with Fehling’s solution</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>Test with ether and ammonia</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>Ferric chloride test</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>Liebermann-Buchard test</td>
<td>+</td>
</tr>
<tr>
<td>Terpens</td>
<td>Liebermann-Buchard test</td>
<td>-</td>
</tr>
<tr>
<td>Reducing compounds</td>
<td>Test with Fehling’s solution</td>
<td>-</td>
</tr>
</tbody>
</table>

Egg hatch assay

The results of the egg hatch assay using the aqueous extract of *Crassocephalum crepidioides* are presented in Fig. 1. Compared to the control, this extract showed significant reduction (P < 0.05) in *H. contortus* egg hatch. The extract inhibited egg hatching in a concentration-dependent manner with a maximum inhibition of 50.32% at the highest concentration (2400 µg/ml). Thiabendazole used as positive control significantly (P < 0.001) inhibited egg hatching with an inhibition rate of 76.68% at the
concentration of 500 µg/ml.

Larval migration inhibition assay

The leaves aqueous extracts of *C. crepidioides* significantly (P < 0.05) inhibited larval migration compared to PBS (Fig. 2), but less than levamisole (P < 0.001). This effect was concentration dependent, with the greatest inhibition (35.30%) at the highest concentration (1200 µg µl) of the extract used. Adding polyvinylpyrrolidone (PVPP) in the aqueous leaves extract slightly reduces the inhibition the effect induced by the extract on larval migration from 35.30% to 33.57% inhibition at the highest concentration (Fig. 3).

Adult worms' motility inhibition assay

Effect of aqueous extract of *C. crepidioides* on survival of *Haemonchus contortus* was concentration-dependent and 18 h post-exposure, a significant increase (P < 0.05) in percentage of inhibition of live worms was observed on the group treated at the concentration of 1200 and 2400 µg/ml, except PBS (control treatment). *C. crepidioides* showed slow onset of activity as compared to levamisole (Table 2). All the worms were found dead in levamisole (500 µg/ml) treated group after 18 h post-exposure but by this time the plant failed to kill 100% worms and exhibited only 16.67% inhibition at the highest concentration (2400 µg/ml).

DISCUSSION

The aerial parts of *Crassocephalum crepidioides* are commonly used as vegetable, natural pasture and considered as a good source of protein for human and animal feeding. The leaves are used in Cameroon against intestinal worms. This work was performed to assess the efficacy of a commonly grown Cameroonian aqueous leaves extract of *C. crepidioides* against the gastro-intestinal nematode *Haemonchus contortus*.

Several methods are commonly used for testing anthelmintic activity of plant extracts. Scientific validation of anthelmintic activity has mainly been through in vitro studies. The in vitro methods provide a means to screen rapidly for potential anthelmintic activities of different plant extracts and to analyze the possible mechanisms involved in the interactions between active compounds and parasites [31]. The aqueous extract of *C. crepidioides* was assayed in vitro on three development cycle stages of *Haemonchus contortus*.

The results obtained from EHA indicated that the aqueous extract of *C. crepidioides* have in vitro ovicidal activity. Concentration dependent effects on egg hatching were found with the extract of the plant. The plant extract induced inhibition of hatching from 22.02 to 50.51% at the concentration of 75 to 2400 µg/ml. Similar results have been reported on *H. contortus* eggs by Hounzangbe-Adote et al. [6] with alcoholic extracts from four plants of South of Benin (*Morinda lucida, Carica papaya, Newbouldia laevis* and *Zanthoxylum zanthoxyloides*) which the inhibition rates...
were ranged between 40 and 60%. Egg hatching inhibition effect of *C. crepidioides* suggests that the plant extract have bioactive molecules that could affect the biology of parasitic eggs when sprayed. This activity may be due to presence of saponins in the leaf of the plant as previously report by Lukhoba et al. [32] and Adamu et al. [33]. According to Price et al. [34], saponins are known to destabilize cell membranes hence increase cell permeability by combining with membranes associated sterols. These molecules are known to stop the *H. contortus* from egg hatching [14, 35]. The result of this study may be significant as the inhibition of egg hatch is possibly an important method of reducing pasture contamination by the animals during grazing helping in the helminthes control.

To evaluate effect of aqueous extract of *C. crepidioides* on infective larvae stage, LMI assay was performed. The aqueous extract of *C. crepidioides* inhibited significantly larval migration of L3 compared to negative control. The mean percentage of inhibition of larval migration was 28.64%. Aqueous and methanolic extracts of seeds *C. crepidioides* showed significant effect and Leva'misole very significant effect on adult worms' from 12 h to 36h post-exposure, *p*<0.05, **p**<0.01 and ***p*<0.001 when compared with PBS. PBS=Phosphate Buffered Saline; LEVA = Levamisole; Extract = aqueous extract *C. crepidioides*; *C. crepidioides* = Crassocephalum crepidioides; Conc. = Concentration.

In order to specify the contribution of some bioactive compound responsible of this activity, PVPP was added to the extract and LMI assay was performed. The result showed in that presence of PVPP, the extract weakly inhibits (4.9%) the larval migration of *H. contortus*. These results suggest that inhibition of larval migration is partially due to the action of tannins. Moreover, this extract contains others major metabolites disturbing the migration of L3 larvae of *H. contortus*. The larval migration might also have been inhibited either by saponins [32] and other triterpens, or by flavonoids and flavonols glycosides [31, 43, 44]. Furthermore, Ayers et al. [45] showed the contribution of phenols and flavonoids with anthelmintic activity of *Struthiola argentea*. Thus flavonoids and saponins present in the aqueous extract of *C. crepidioides* leaves could be actively associated to anti parasitic activity observed.

Perturbation induced by anthelmintic plants on adult worms survival or their prolificacy that constitute the pathogenic stage could be an important element in parasites struggle. Aqueous extract (75-2400 µg/ml) was used in
comparison with positive control levamisole (125-500 µg/ml) on mature live *Haemonchus contortus* of sheep. The inhibition of motility and/or mortality of the worms were used as the criterion for anthelmintic activity. The result of this study showed that exposure of adult worms with the highest concentration of the extract (2400µg/ml) during 12 h lead to 16.67% of inhibition of the parasite motility and 100% after 30 h of incubation. In a similar study Marie-Magdeleine et al. [36] observed with the aqueous extract of seeds of *Cucurbita moschata*, that the effect on motility of *H. contortus* worms was apparent 24 h after with 30.4%, that the effect on motility, developmental stages activities of the plant need to be confirmed leaves have 100% adult worms mobility after 36 h of incubation.

Several hypothesis can be suggest to explain the action of secondary metabolite on gastro-intestinal strongle in relation with different consequences observed on worms biology: the fixation of phenolic compounds identified in this plant extract on the cuticle of adult worms or on the sheath of infective larvae L3, that can cause a spastic paralysis of susceptible nematode by selectively gating acetylcholine receptor ion channels on nerve and muscle, an alteration of feeding or enzymatic process [47]. The extract could also act on adult worms, larvae or eggs by inhibiting the synthesis of proteins or leading metabolic disruptions, the extract anthelmintic activity in part, may be due to bioenergetic disruptions resulting from transmembrane proton discharge [48].

**CONCLUSION**

Given the overall results, *C. crepidiioides* leaves have *in vitro* anthelmintic properties on *H. contortus*. More over the anthelmintic activities of plant were observed for each of the *H. contortus* developmental stages. The active principles responsible for these activities could be secondary metabolites such as saponins, flavonoids and tannins present in the extract.

The *in vitro* activities of the plant need to be confirmed *in vivo*. These results also underlined the need to further perform a bioguided biochemical analysis of the various fractions to identify precisely which are the active compounds.

**ACKNOWLEDGEMENTS**

We wish to express our sincere thanks to the Alexander von Humboldt Foundation for the award of equipment grant to M. Alain DONGMO (AVH Alumni) which I used for these studies.

**COMPETING INTERESTS**

The authors of this manuscript declare that they have no competing interests

---

**REFERENCES**


Bogning et al: Anthelmintic activities of medicinal plant extract


