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In vitro Anthelmintic Activity of *Icacina trichantha* Leaf Crude Extract and Fractions on *Haemonchus contortus*

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OLAYEMI, O., M. ONAKPA, O. JEGEDE: *In vitro* Anthelmintic Activity of *Icacina trichantha* Leaf Crude Extract and Fractions on *Haemonchus contortus.* Vet. arhiv 92, 331-337, 2022.

ABSTRACT

Haemonchus contortus is an important gastrointestinal parasite of ruminants, which causes anaemia, submandibular oedema, progressive weight loss, weakness and the death of infected animals. This study evaluates the *in vitro* ovicidal and larvicidal activity of *Icacina trichantha* leaf crude extract and fractions against the parasite *H. contortus* eggs and larvae. Phytochemical analysis of the crude extract was carried out using standard procedures to determine the metabolites contained in the plant. The *in vitro* anthelmintic activity of the crude extract and fractions was determined using egg hatch inhibition assay (EHA) and larval development inhibition assay (LDA), while albendazole was the positive control. The extract of *I. trichantha* leaves inhibited hatching of eggs and larval development of *H. contortus* in a dose graded response. The concentrations of 0.78, 1.56, 3.12, 6.25 and 12.5 mg/ml of the crude extract and fractions showed the positive inhibitory effects of the ovicidal and larvicidal on *H. contortus,* and this compared positively with the anthelmintic activity of albendazole. At the concentration of 12.5 mg/ml, the crude extract, fractions and albendazole produced 100% ovicidal and larvicidal inhibitory effects on *H. Contortus*, except for n-butanol and ethyl acetate fractions, which inhibited 98% and 99.8% of egg hatching and larval development, respectively. However, the crude extract and fractions' activity compared statistically with albendazole, with no significant ($p>0.05$) difference. Therefore, the results obtained from this study showed that all the tested extracts possess anthelmintic compounds that warrant further *in vivo* evaluation for safety and toxicity profiles.

Keywords: *Icacina trichantha*; medicinal plant; *Haemonchus contortus;* albendazole; leaf crude extract; fractions

Introduction

Helminthosis is a disease condition caused by parasitic gastrointestinal helminths, which often results in economic losses and reduced production capacity of food producing animals. This clinical condition is usually complicated by the inappropriate use of anthelmintics on the host animal (FERRIERA et al., 2013). In tropical regions, helminthiasis affects ruminants with a high incidence of morbidity and mortality that impedes the growth rate and capacity of livestock

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production (JEGEDE et al., 2006). NWOSU et al. (2007) reported that in North-Eastern Nigeria 60% of sheep and 39% of goats slaughtered and screened were infected with parasitic helminths, and this represents the burden of helminths in ruminants in that region. A previous study carried out by OLUBUKOLA et al. (2014) on cattle slaughtered in South-western Nigeria also reported that the total helminth burdens as result of gastrointestinal infections were high as 71.7% represented by nematodes (41.6%), trematodes (26.5%) and cestodes (2.01%). In Kenya, the annual economic loss due to helminthiasis in livestock production was estimated to be about 11.8% of the total slaughtered cattle, and 46.0% for both sheep and goats (JEGEDE et al., 2006, KIPYEGON, 2017).

Haemonchus contortus is a pathogenic and highly prevalent parasite of both small and large ruminants, particularly found in tropical and humid areas. It is a role model parasite, used to study the development and mechanisms of anthelmintic resistance of gastrointestinal nematodes. Anthelmintic resistance due to inappropriate use of anthelmintics, sub-standard anthelmintic production, and reduced efficacy against the nematodes in ruminants are becoming a threat and concern in some African countries, and this has led to the evaluation of medicinal plants as an alternative source of anthelmintics (DEVI 2014; EGUALE and GIDAY, 2009; KAPLAN and VIDYASHANKAR, 2012; KUMSA and WOSENE, 2006).

Icacina trichantha Oliv. is a small perennial shrub with stems up to 2 metres long from a very large tuber with scandent growth above. It is a traditional herbal medicine in different cultures within Nigeria and West Africa. The Eastern part of Nigeria uses the plant as antiemetic, while the western part of Nigeria uses the plant as remedy for food poisoning, constipation and for malaria treatment (ASUZU and UGWUEZE, 1990; ASUZU and ABUBAKAR, 1995; ONAKPA et al., 2016). The tuber is a good source of nutrients such as starch; it also exhibits a variety of pharmacological activities in animal models (CHE et al., 2016). Recent studies on *I. trichantha* revealed its anti-hyperglycemic effects in alloxan-induced diabetic rats (ONAKPA and ASUZU, 2013) and the *in vitro* and *in vivo* antioxidant effects of these plant extracts were also detected by ONAKPA et al., (2016). This study was carried out to evaluate the anthelmintic effects of crude extract and fractions of the leaves of *I. trichantha* on *H. contortus.*

Material and methods

Plant collection and extraction. Fresh leaves of *Icacina trinchantha* collected from Enugu state, Nigeria were identified by Mr. Alfred Ozioko (a taxonomist). The leaves were washed, air- dried, pulverized and sieved. Three hundred grams of the pulverized plant sample material were weighed and extracted with 80% methanol in Soxhlet apparatus. The extracted sample was concentrated using a rotary evaporator coupled to a thermo-regulator.

*Solvent Partitioning.*The extracted sample was suspended in distilled water and subsequently partitioned with petroleum ether, ethyl acetate and n-butanol, using 150 ml of each solvent. The whole process was repeated in triplicate for each solvent (SIMON et al., 2008; OLAYEMI et al., 2019).

Collection of parasites The collection of parasites was done immediately after evisceration. The abomasums of sheep naturally infected with H. contortus were incised and the content was washed in a clean plastic bucket and then taken to the laboratory. Parasites were recovered by passing the abomasal content through a sieve of 100-mm diameter mesh. Adult female *H. contortus* obtained from the abomasal washings were individually picked with a wire loop under an illuminator (Picker X-ray). The female *H. contortus* were identified and separated from other parasites, and were crushed in a mortar, using a pestle, to obtain the eggs. The collected eggs were further mixed with autoclaved horse feces and incubated at 27 °C for 8 days, after which the larvae (L1) were harvested using modified Baerman's apparatus (SIMON et al., 2008).

In vitro egg hatch assay. The World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines and procedures were used for the egg Hatch Inhibition Assay (EHIA) (COLES et al., 1992). Adult female H. contortus parasites were identified and separated by the method described by TAYLOR et al. (2007) and suspended in distilled water and crushed to liberate the parasite eggs (SIMON et al., 2008). Two hundred (200) H. contortus eggs contained in 0.08ml water were pipetted into 96- flat bottomed micro titre plates with the addition of 0.5 ml at different concentrations (0.78, 1.56, 3.125, 6.25 and 12.5 mg/ml) of the pod extract and the fractions. Similarly, albendazole at (0.5ml in each well) of different concentrations (0.78, 1.56, 3.125, 6.25 and 12.5 mg/ml) and distilled water (0.5 ml in each well) were also tested. The experiment was carried out in triplicate. The eggs were cultured for 48 hours at room temperature, after which a drop of lugol's iodine was added to inhibit further hatching. Thereafter, the contents of each 96-well microtitre plate were examined (OLAYEMI et al., 2019).

Evaluation of the larvicidal activity of the extract. The evaluation of the larvicidal activities of the extract and fractions was conducted according to the methods described by WABO et al. (2011). One hundred (100) larvae of H. contortus contained in 0.1 ml of distilled water were added to each labelled 96 -flat-bottom micro titre plate, and 0.5 ml of the different concentrations (0.78, 1.56, 3.125, 6.25 and 12.5 mg/ml) of the pod extract and fractions were added. Each test was done in triplicate. The plates were covered with foil paper and left at room temperature for 24 hours. Thereafter, the contents of the pod extract, fractions and albendazole in the 96 flat-bottom microtitre plates were examined. The movements or migration of the larva from one point to the other were used to consider the parasites mortality.

Statistical analysis. The mean (±SD) percentage inhibition at different concentrations compared to the controls were determined by one way ANOVA. The Post Hoc statistical significance test employed was Least square difference (LSD), while the difference between the means was considered significant at ($P<0.05$).

Results

The phyto-chemical studies carried out on the crude extract of *Icacina trichantha* leaves showed that tannins, flavonoids, saponins, alkaloids, steroids and other chemical metabolites were present (Table 1). It was observed that the crude extracts and fractions significantly ($P<0.05$) inhibited the hatching of eggs and larval development of *H. contortus* in a concentration-graded response. There was a positive correlation between the concentrations of the crude extract, fraction, albendazole and the rates of egg hatch inhibition, whereby as the drug concentration increased, the ovicidal and the larvicidal inhibitory effects also increased (Figures 1, 2, 3 and 4), although there were variations in the concentrations (mg/ml) required for each of the extracts and fractions to show individual anthelmintic activity and efficacy. At the concentration of 12.5 mg/ml, the crude extracts, fractions and albendazole tested in this study produced 100% ovicidal and larvicidal activity of the parasite, while n-butanol and ethyl acetate fractions inhibited 98% and 99.8% ovicidal and larvicidal activity of the parasite, respectively.

Table 1. Qualitative phytochemical analysis

Phytochemical analysis	Plant constituents	*I. trichanta* extract
Froting test	Saponin	+
Dragendoff test	Alkaloid	+
Molish test	Carbonhydrate	+
LeibermanBucchard test	Steroid	+
LeibermanBucchard test	Triterpine	+
Keller kiliani test	Cardiac glycoside	+
Ferric chloride test	Tannin	+
Sodium hydroxide	Flavonoid	+
Bontrager's test	Anthraquinone	+
Ferric chloride test	Phenol	+

(+) indicates the presence of the phytochemical compounds in the extract. The plant extract had all the listed secondary metabolites.

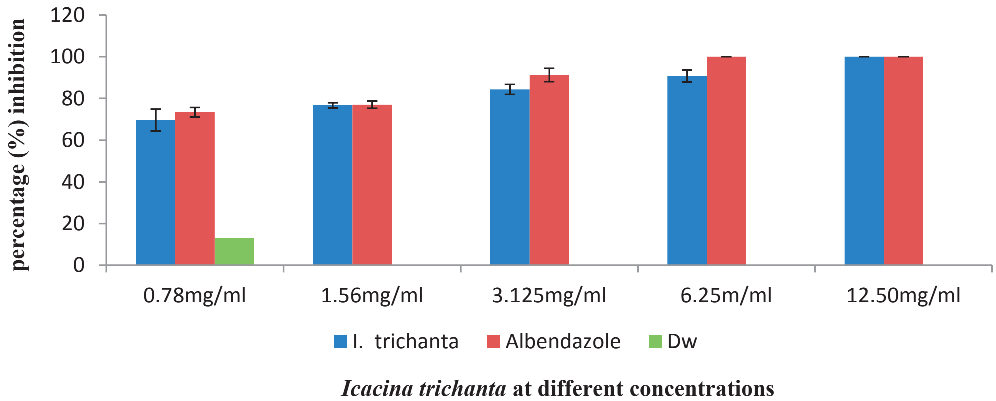

Fig. 1. Showing the Percentage inhibition of larval development of *Haemonchus contortus* at different concentrations of *I. trichanta* extract (mg/ml) and Albendazole (mg/ml).

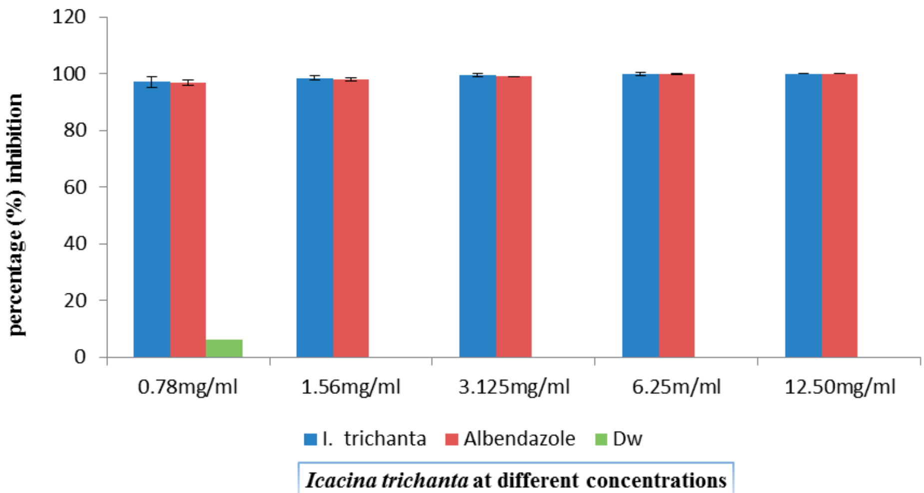

Fig. 2. Showing the Percentage inhibition of egg hatch of H. contortus at different concentrations of I. trichanta extracts (mg/ml) and Albendazole (mg/ml)

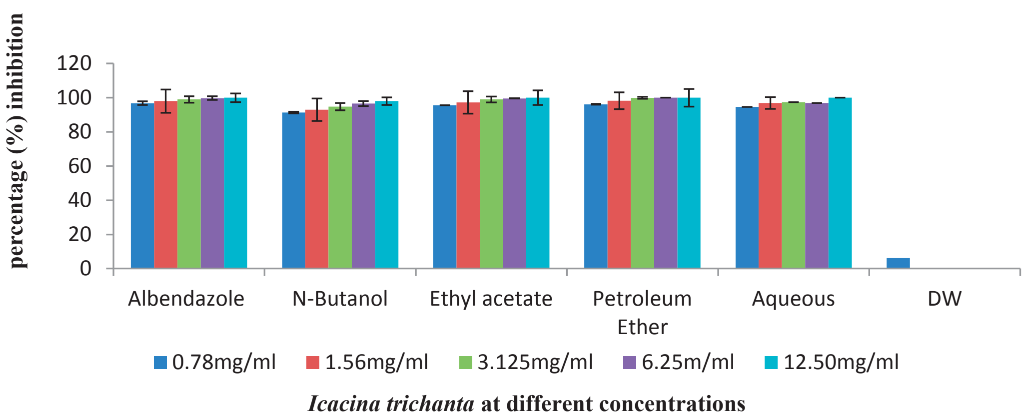

Fig. 3. Showing the Percentage inhibition of egg hatch of *H. contortus* at different concentrations of *I. trichanta* fraction (mg/ml) and Albendazole (mg/ml).

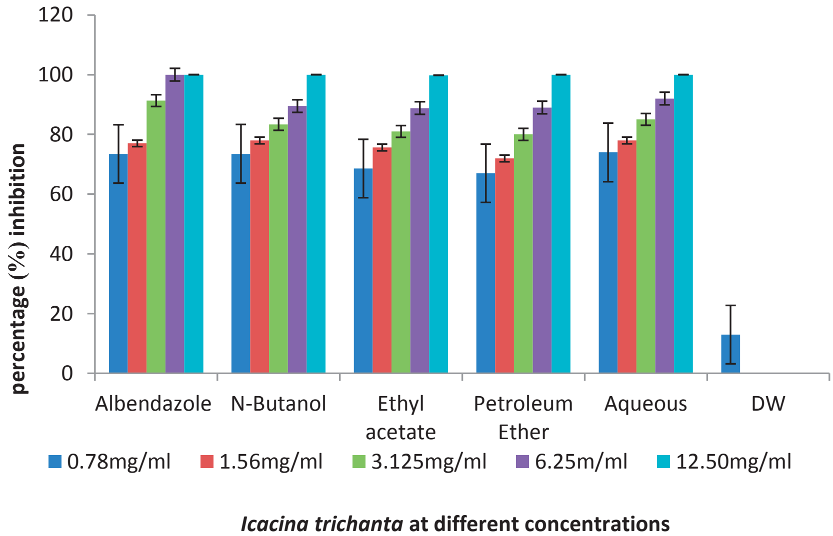

Fig. 4. Showing the Percentage inhibition of larval development of *H. contortus* at different concentrations of *I. trichanta* fraction (mg/ml) and Albendazole (mg/ml)

Discussion

The leaf extract and fractions of *I. trichantha* showed a significant (P<0.05) inhibitory ovicidal effect on *H. contortus*. There was no significant difference (P>0.05) in the effects observed on the leaf extract, fractions and albendazole on the hatching of *H. contortus* eggs. Previous studies had shown that the plant extract produces concentration–dependent effects when tested on helminth eggs (ENEJOH et al., 2015; KOLLINS et al., 2012). It was observed that the crude extracts had more inhibitory ovicidal effects compared to the fractions and this may be attributed to the synergistic effects of the different chemical constituents present in the crude extract that interacted in complex ways to produce an effect greater than the individual components. It could also be attributed to the penetration of the active phyto-constituents into the helminth's egg shell which interfered with the segmentation process, and inhibited the ovicidal activity (COCK 2011; KOLLINS et al., 2012).

There was an increase in the mean larval mortality rates with the increase in the concentration of all the extracts tested, which means that an increase in concentration represents the supplementary input of the different active compounds. The extracts showed significant (P<0.05) larva mortality rates across the concentrations (WABO et al., 2011). The larvicidal effect of the crude and fractionated extracts was not significantly different (P>0.05) from the effect produced by albendazole. The larvicidal effect produced by these extracts was probably due to the penetration of the active chemical constituents of the extract across the cuticle of the larvae into their circulatory system when the larvae came into contact with the extracts (PAYNE et al., 2013).

At the concentration of 12.5 mg/ml, the crude extracts, fractions and albendazole inhibited 100% ovicidal and larvicidal activity on *H. contortus*. This study showed that *I. trichantha* leaves possess a good amount of active metabolites, such as alkaloids, saponin, flavonoids, phenols and tannins, although the anthelmintic activity of *I. trichantha* leaves is not known. However, tannins have the ability to bind the free proteins available for the parasite and cause their mortality (KATIKI et al., 2011; ATHANASIADOU et al., 2007; PAOLINI and DORCHIES, 2003).

The present study showed that all the tested extracts had ovicidal and larvicidal inhibitory effects on the eggs and larval stage of *H. Contortus*, because the *I. trichantha* leaf contains active and potential anthelmintic compounds that compare with albendazole (a standard drug). In conclusion, the investigation of the phytochemical compounds from medicinal plants is of importance for the development of novel drugs, and may offer an alternative source for the control of gastrointestinal nematodes in ruminants. However, more detailed studies (*in vivo*) are required to identify and evaluate the crude extract and fractions of active constituents, the mechanism of action, and toxicity profile.

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SAŽETAK

*Hemonchus contortu*s je važan gastrointestinalni parazit preživača koji uzrokuje anemiju, submandibularni edem, progresivni gubitak tjelesne mase, slabost i smrt zaraženih životinja. U ovom se istraživanju ocjenjuje *in vitro* ovicidna i larvicidna aktivnost sirovog ekstrakta lista i frakcija biljke *Icacina trichantha* protiv jajašaca i ličinki parazita *H. contortus*. Fitokemijska analiza sirovog ekstrakta provedena je standardnim postupcima za određivanje metabolita sadržanih u biljci. Antelmintička aktivnost sirovog ekstrakta i frakcija in vitro određena je pomoću testa inhibicije leženja jajašaca (EHA) i testa inhibicije razvoja ličinki (LDA), dok je albendazol bio pozitivna kontrola. Ekstrakt listova *I. trihantha* inhibirao je leženje jajašaca i razvoj ličinki *H. contortus* ovisno o stupnjevanoj dozi. Koncentracije 0,78, 1,56, 3,12, 6,25 i 12,5 mg/ml sirovog ekstrakta i frakcija pokazale su pozitivne ovicidne i larvicidne inhibitorne učinke na *H. contortus*, a to se pozitivno usporedilo s antelmintičkom aktivnošću albendazola. Pri koncentraciji od 12,5 mg/ml sirovi ekstrakt, frakcije i albendazol proizvodili su 100% ovicidnih i larvicidnih inhibitornih učinaka na *H. contortus*, osim za frakcije n-butanola i etil acetata, koje su inhibirale 98% leženja jajašaca i 99,8% razvoja ličinki. Ipak, razlike između aktivnosti sirovog ekstrakta te frakcija *I. trihantha* i albendazola nije bila statistički znakovita (P>0,05). Stoga su rezultati dobiveni ovim istraživanjem pokazali da svi testirani ekstrakti imaju anthelmintičke spojeve za koje je potrebno provesti daljnju *in vivo* procjenu profila povezanih sa sigurnošću i toksičnošću.

Ključne riječi: *Icacina trichantha*; medicinska biljka; *Haemonchus contortus*; albendazol; sirovi ekstrakt lišća; frakcije

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