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IN VITRO ANTHELMINTIC ACTIVITY OF *SECURIDACA LONGEPEDUNCULATA* FRESEN. (POLYGALACEAE) AQUEOUS ROOT BARK EXTRACT AGAINST *ASCARIDIA GALLI* AND *RAILLIETINA ECHINOBOTHRIDA* ADULT WORMS FROM GUINEA-FOWL (*NUMIDA MELEAGRIS*)

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ABSTRACT

Securidaca longepedunculata Fresen. (Polygalaceae) is commonly used in Burkina Faso by traditional herbalists and pastoralists to control gastrointestinal parasites. The aim of this study was to screen the aqueous extract of *Securidaca longepedunculata* for its anthelmintic properties against tapeworms (*Raillietina echinobothrida*) and roundworms (*Ascardia galli*). Various concentrations of the plant extract was tested in this bioassay and different parameters such as determination of time of paralysis and time of death of the worms were recorded. Levamisole and Trenamide were used as reference standard against resp. tapeworms (*Raillietina echinobothrida*) and roundworms (*Ascardia galli*) and NaCl solution (0.9%) as zero control. All the extracts exhibited significant anthelmintic activity at highest concentration of 50 mg/mL. For *Ascaridia galli*, efficacy against adult worms in 12.5 and 25 mg/mL of aqueous extract of *Securidaca longepedunculata* at 24 h was 29% ($p<0.05$) and 61% ($p<0.05$) and at

36h was 39% (p>0.05) and 100% (p < 0.05) respectively, when compared to the control group. No *Ascaridia galli* survived after 24 h in 50 and 100 mg/mL (p<0.001) of aqueous extract of *Securidaca longepedunculata*. The efficacy against *R. echinobothrida*, at 0.625, 1.25 and 2.5 mg/mL of aqueous extract of *Securidaca longepedunculata* at 2 h was 0% (p>0.05), 32% (p< 0.05) and 48% (p< 0.05). After 4 h, it was 12% (p< 0.05), 72% (p< 0.05) and 88% (p< 0.05), respectively, when compared to the control group. No *Raillietina echinobothrida* survived for 2 h in 5 mg/mL of aqueous extract of *Securidaca longepedunculata* (p< 0.01).

KEYWORDS: *Ascaridia galli*, *Raillietina echinobothrida*, *Securidaca longepedunculata*, Root extracts.

INTRODUCTION

In Burkina Faso, the endoparasitisis caused by polyparasitism constitute a major problem which compromise the survival and performances of traditional poultry farming. Among the wide spectrum of worm infections, *Ascaridia galli*, *Raillietina echinobothrida* and the Spirures gender Tetramer are the most important species in terms of prevalence and pathogenicity, particularly in the domestic fowl.[1] They cause stunted growth of young chicken, emaciation of the adult and decreased egg production of the hen.[2-3] Intestinal worm infections of livestock controlled by treatment using pharmaceutically medicines are not only expensive but also affect host health.[4] Some of the important drawbacks associated with the use of chemical drug include quite often resistance, toxicity and the increasing concern over the presence of drug residues in animal products.[5] In addition, the relatively high cost of veterinary drugs and services to farmers of low income led to the need of other alternative of parasites control methods.[6-7] In Africa, traditional medicine plays a significant role in treating health problems in both livestock and humans.[8] Several plants have been reported for prevention and treatment of gastro-intestinal parasitic diseases of animals and humans in Burkina Faso.[9-11] In this context, investigations on medicinal plants might contribute to develop alternative and sustainable methods readily adapted to rural farming communities.[12] One of those plant species commonly used in the treatment of illness is *Securidaca longepedunculata*. This plant is known for its medicinal properties and has been reported to possess antibacterial and antidiarrhoeal activity.[9-10] Its roots protects against snake bites and are very active also against all intestinal parasites.[9-10] However, scientific validation of these practices has been lacking. This study aimed to test the *in vitro* anthelmintic properties of

aqueous roots bark extracts of *Securidaca longepedunculata* to be used against *Ascaridia galli* and *Raillietina echinobothrida* infection in guinea-fowl.

MATERIALS AND METHODS

Plant materials

The roots of *Securidaca longepedunculata* were collected in October 2015 around the city of Dedougou (humid savanna zone, west) in Burkina Faso. The plant was identified at the herbarium of "Centre National de la Recherche Scientifique et Technologique" (CNRST) in Ouagadougou. A specimen of the plant was deposited under the voucher number HNBU 8714.

Immediately after collection, the plant was cleaned and dried at ambient temperature for two weeks. The dried material was powdered using an electrical blender and labelled for easy identification. The powdered material was stored in dark tightly closed glass bottles until used.

Extraction

Aqueous extraction was performed by soaking a weighed amount of dried powder (500g) in distilled water and shaking for 24 hours with electric shaker. The suspension was filtered using Whatman No.1 filter paper. The filtrate was kept in deep freezer for 24 hours and then, lyophilized. The lyophilized dried powder was stored in dark tightly closed glass bottles and kept in a desiccator to avoid absorption of water until used.

In vitro anthelmintic assays

Worms's collection

Adult worms (*Ascaridia galli* and *Raillietina echinobothrida*) were taken from the gut of naturally infected *Numida meleagris*. Guts were bought at the local slaughterhouse of Ouagadougou (Burkina Faso), conditioned in an icebox and forwarded to the laboratory. These organs were incised longitudinally with scissors to release the worms. The parasites were then washed and kept in physiological solution (NaCl, 0.9%).

In vitro screening with adult worms

The anthelmintic assay was carried by Ajaiyeoba et al method of with minor modifications.[13]

Motility Assay (*Ascaridia galli*)

Different concentrations (100, 50, 25, 12.5, 6.25 and 3.125 mg/mL) of the plant extract were prepared by dissolving with physiological solution and plotted in separate Petri dishes. A batch of five live adult worms was introduced into each concentration. Similar treatment was performed for different doses of levamisole used as standard anthelmintic drug, tannic acid and gallic acid. One group of worms was maintained as control in a medium containing only physiological solution. Three replications per each concentration were used and each test was done in three repetitions. The number of motile (alive) and immotile (dead) worms were counted under magnifying glass and recorded for each concentration. Paralysis was defined as complete loss of spontaneous activity after physical stimulation of the worms. Death of worms was ascertained by absence of motility for an observation period of 5 seconds.

Motility Assay (*Raillietina echinobothrida*)

Different concentrations (5, 2.5, 1.25, 0.625 and 0.312 mg/mL) of the plant extract were prepared with physiological solution and plotted in separate Petri dishes. A batch of five live worms was introduced into each concentration. Similar treatment was performed for different doses of Trenamide (standard anthelmintic drug), tannic acid, quercetin and gallic acid. One group of worms was maintained as control in a medium containing only physiological solution. Three replications per each concentration were used and each test was done in three repetitions. The number of motile (alive) and immotile (dead) worms were counted under magnifying glass and recorded for each concentration. Paralysis was defined as complete loss of spontaneous activity after physical stimulation of the worms. Death of worms was ascertained by absence of motility for an observation period of 5 seconds.

Statistical analysis

Statistical evaluation of data was done using Graph Pad Prism Version 3.0 for Windows (Graph Pad Software, San Diego, California). One-way ANOVA was used followed by Dunnett's Test for parametric multiple comparisons between the control and the treatment groups. Differences were considered significant when the p value was less than 0.05 ($p < 0.05$).

RESULTS

In vitro* effects on *Ascaridia galli

Observations on the efficacy of the plant extract and the drug in terms of survivability of the parasites are shown in Fig. 1 to 4. For the negative control (NaCl, 0.9%), percentage of

motility inhibition for *Ascaridia galli* in the different assays was 0% up to 36 hours. The aqueous extract at 6.25 and 12.5 mg/mL showed no significant effects on *Ascaridia galli* inhibition motility compared to the negative control ($p > 0.5$) up to 36 hours. The aqueous extract at 25 mg/ml showed significant effects on *Ascaridia galli* motility inhibition compared to the NaCl negative control ($p< 0.5$) up to 36 hours. The extract concentrations of 50 and 100 mg/mL showed higher ($p<0.01$) inhibition rate compared to the physiological solution (negative control). The aqueous extract of root had significant effect between 24 and 36 hours of incubation. No *Ascaridia galli* survived after 24 h in 50 and 100 mg/ml ($p<0.01$) of aqueous extract of *Securidaca longepedunculata*. After 6 hours, Levamisole screened as positive control killed the parasites at 0.5mg/ml, when compared to the control group ($p<0.001$).

The aqueous extract of *Securidaca longepedunculata* was slightly more effective compared to gallic acid on adult worms (*Ascaridia galli*). No *Ascaridia galli* survived for 24 h in 6.25, 12.5, 25 and 50 mg/mL of gallic acid. On the other hand, all *Ascaridia galli* survived for 36 h in 6.25, 12.5, 25 and 50 mg/mL of tannic acid (Fig 2.).

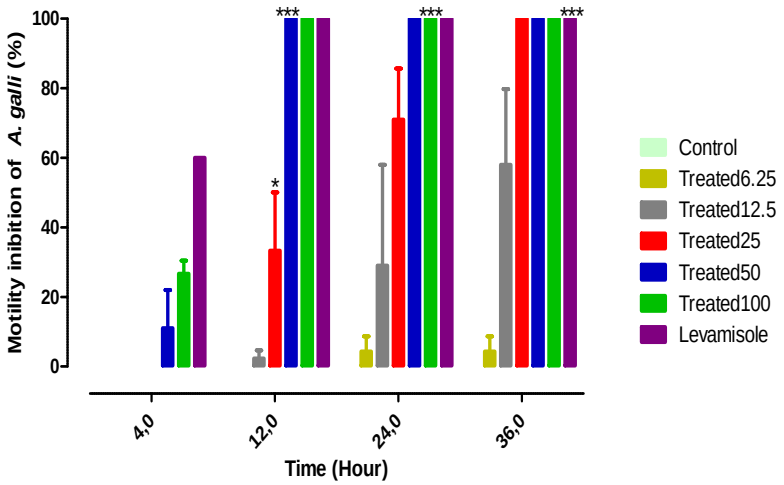

Fig. 1: Effect of the plant extract at different concentrations during 36 hours post administration

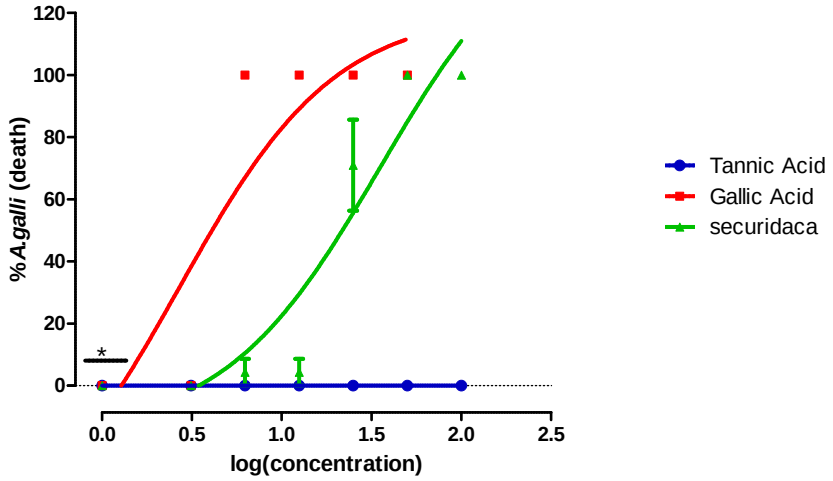

Fig. 2: Comparaison of tannic acid, gallic acid and Plant extract effect on *A. galli* at different concentrations after 36 hours

In vitro* effects on *Raillietina echinobothrida

For the negative control (NaCl, 0.9%), the percentage of *Raillietina echinobothrida* motility inhibition in the different assays was 0% up to 6 hours. The aqueous extract of 0.625 mg/mL showed no significant effects on *Raillietina echinobothrida* motility inhibition compared to the negative control (NaCl; 0.9%) ($P > 0.5$) up to 6 hours. The extract concentrations of 1.25 and 2.5 mg/ml showed significant ($p<0.05$) effects on *Raillietina echinobothrida* motility inhibition rate compared to the physiological solution (negative control). The extract concentration of 5 mg/ml showed higher ($p<0.01$) inhibition rate compared to the physiological solution (negative control). No *Raillietina echinobothrida* survived for 2 h in 5 mg/mL of aqueous extract of *Securidaca longepedunculata* ($p< 0.01$). After 2hours, Trenamide screened as positive control killed the parasites at 0.5mg/mL (Fig. 2), when compared to the control group.

Comparison between effect of gallic acid, tannic acid, quercetin and aqueous extract of *Securidaca longepedunculata* revealed that *R. echinobothrida* was more susceptible to gallic acide than aqueous extract of this plant at small concentrations. All *R. echinobothrida* survived for 6 h in 0.625, 1.25, 2.5 and 5 mg/mL gallic acid, tannic acid, quercetin (Fig 3.).

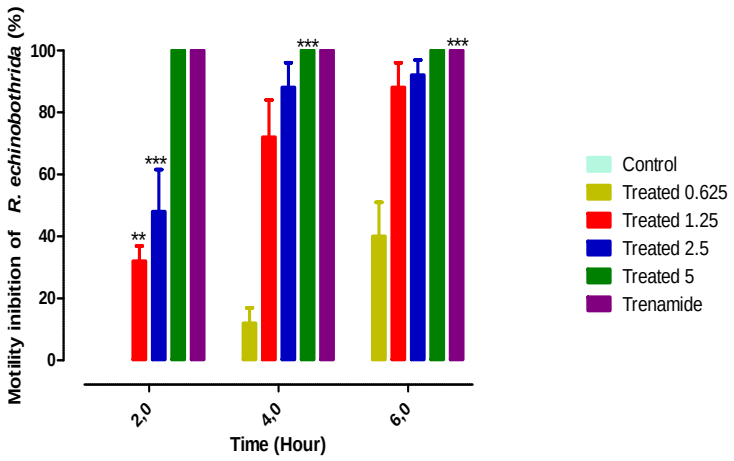

Fig. 3: Effect of the plant extract at different concentrations for 6 hours

***In vitro* effects on adult parasites**

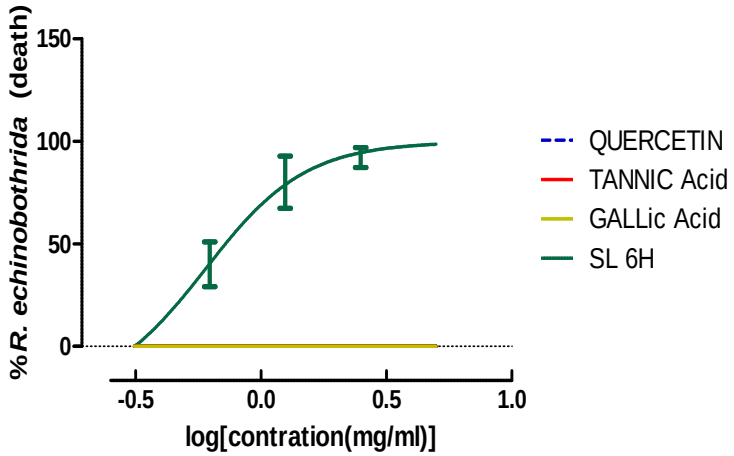

Fig. 4: Comparison of tannic acid, gallic acid and Plant extract effect on *R. echinobothrida* at different concentrations After 6 hours

DISCUSSION

In the present study we evaluated *in vitro* at various concentration levels the efficacy of *S. longepedunculata* against adult worms of gastro intestinal parasites of fowl. The extract caused inhibition of spontaneous motility and the paralysis of worms after 6 and 36 hours of treatment respectivily for *Raillietina echinobothrida* and *Ascaridia galli*. The complete cessation of motility and mortality of worm is a concentration-dependent effect. The extract of *S. longepedunculata* have shown promising *in vitro* anthelmintic activity against adult *Raillietina echinobothrida* and *Ascaridia galli*, which supports the traditional use as anthelmintics. Several groups of plant secondary metabolites are considered the sources of chemicals responsible for wide therapeutic activities.[14-15] This observation can be explained

by the active principles in these classes of chemicals. The extract of the plant extract was shown to have strong antioxidant effect attributed to polyphenol compound. This could be responsible for enhancing the anthelmintic action of the extract. The presence of secondary metabolites in *Securidaca longepedunculata* might also justify anthelmintic properties observed. Host et al. (2007) showed that extracts of plants rich in tannins, affects the kinetics of baring helminths in vitro and in vivo.[16] However, molecules responsible for this plant anthelmintic activity are still poorly known and yet to be identified.[16] In addition, the mechanism of active compounds of *Securidaca longepedunculata* on adult worm is not yet clear but these compounds may act singly or in synergy to produce the observed effect. Anthelmintic effect of secondary metabolites would act directly on the worms. These secondary metabolites could disrupt the integrity of the cuticle of the parasite.[17] The parasite tegument has been ascertained as the principal target site of different classes of synthetic drugs and natural anthelmintic products.[18] Drugs like levamisole and its related compound are known to bind to nicotinic acetylcholine receptors by mimicking the action of acetylcholine. This binding induces a change in the postsynaptic membrane permeability causing muscle contraction, spastic paralysis and eventual death of worms.[19] The *in vitro* methods allow a rapid evaluation of anthelmintic activities of plant extract.[20] In addition, it measured the effect of the plant without interfering the internal physiological functions of the host.[11] The main advantages of using *in vitro* assays are useful and affordable ways to evaluate the anthelmintic activities.[11] However, these effects do not always correspond to *in vivo* conditions and results obtained by *in vitro* study could not be extrapolated for *in vivo* activity.

CONCLUSION

The study confirmed the using of *securidaca longepedouculata* by smallholder farmers to treat intestinal worm infections. The *In vitro* provided a rationale for the traditional use of this plant as anthelmintic. It can, therefore, be used as anthelmintic dosage in poultry feeds, but there is a need to establish the innocuousness and the tolerance in order to valorize this remedy to smallholder farmers.

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