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Anthelmintic Activity *in Vitro* of *Ceratotheca Sesamoides* Endl and *Striga Hermonthica* (Delile) Benth Aqueous Extracts on *Haemonchus Contortus* Adult Worms

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ABSTRACT

In order to contribute for alternatives solutions to the chemical fight against NGIs, we carried out *in vitro* anthelmintic activity test of *C. sesamoïdes*, and of *S. hermonthica* aqueous extracts on adult worms of *H. contortus*. Three concentrations of each extract has be done; 100mg / ml, 50mg / ml and 25mg / ml. A negative control (PBS 1X) and a positive control levamisole at 2.5 mg / ml were constituted. The test was performed in three replicates with three replicas for each concentration per replicate. Adult worms were contacted with each concentration and then incubated in petri dishes (60X15cm) at 27 °C for 20 hours. The results showed a mortality rate of 79.86% at the concentration of 100mg / ml of C. sesamoids and 22.13% at the same concentration of the aqueous extract of S. hermonthica after 20 hours. Statistical analysis shows high significance P (<0,05). The LC (50) obtained were 78.74mg / ml and 212.13mg / ml respectively for C. sesamoides and S. hermonthica

Keywords: Bioactive fodder; C. sesamoides Endl, S. hermonthica (Deli.) Benth; *Haemonhus contortus*

Introduction

Agriculture and livestock employ more than 90% of the population in Burkina Faso [1]. With 26% of export earnings, livestock is the third largest provider of currency after gold and cotton [2]. Livestock provides 38.8% of cash income to households allowing them to access basic social services [3,4]. In 2018, small ruminants breeding represent the second largest activity in the livestock sub-sector in terms of numbers after poultry farming with respectively 15,635,000 number of goats and 10,442,000 number of sheep [5]. Small ruminants is a source of food and nutritional security as well as easily mobilized savings and provides 32% of 30 billion CFA that livestock contribute to the economy of Burkina Faso [1,6]. Despite small ruminant's socioeconomic importance, their productivity is low due to gastrointestinal parasitosis endemicity. Among these parasites, infestations due to gastrointestinal strong

loses are the most dangerous and cause more significant economic losses [1,7]. *H. contortus* represents the most widespread and the most pathogenic due to its feeding method (hematophagous [8]. Synthetic anthelmintics are commonly used as a means of combating digestive parasitosis caused by gastrointestinal nematodes. These molecules remain unavailable or even inaccessible to rural producers who are the most vulnerable to infestations due to gastrointestinal nematodes [9]. Globally, the excessive use of these antiparasitic has generated resistance to all anthelmintic molecules [10]. The objective of our study is to evaluate the *in vitro* anthelmintic activity worms of aqueous extracts of two Sahelian herbaceous on *H. contortus* adult worms.

Material and Methods

Harvest of the Studied Species

Chosen species were collected in the municipality of Dori, capital city of Séno province. These are forage herbs C. sesamoides and S. hermonthica. Both species were harvested between the end of September and mid-October and then dried in the shade at the Regional Direction for Environmental and Agricultural Research (DRREA-Sahel). The plants were identified at the National Herbarium of Burkina Faso (HNBF) respectively under the numbers 8758 and 8759.

Study Environment

The *in vitro* tests were carried out at the Animal Biology and Health Laboratory of the Center for Environmental, Agricultural and Training Research (CREAF) in Ouagadougou/ Kamboinsin.

Extract's Preparation

The samples of each dried plant were ground into powder. Aqueous maceration (100g of powder in 900ml of distilled water) was carried out for 24 hours with mechanical stirring at room temperature. The macerations were then filtered three times with hydrophilic cotton and concentrated in the freezer. The concentrated filtrates were subsequently lyophilized.

Biological Tests

Preparations of Stock Solutions

Stock solutions of each extract were prepared by diluting 3g of extract from each plant in 30ml of PBS (1X) to obtain 100mg / ml concentration for each extract. The dilution was homogenized using a sonicator for 3 minutes. From the stock solution two other solutions of lower concentrations, namely 50mg / ml and 25mg / ml of each extract were produced. Levamisole (standard anthelmintic) 2.5mg / ml and PBS (1X) were used as positive and negative controls, respectively.

Harvests of Adult Worms

The adult worms were collected from abomasum of freshly slaughtered sheep at the Kamboinsin slaughterhouse. The abomasum was incised longitudinally then emptied of their contents. The worms were then collected with forceps and placed in an 80 X 15cm petri dish containing PBS (1X).

Mortality Test for Adult *Haemonchus Contortus* Worms

The adult worm mortality test was carried out according to the modified method of Kaboré. (2009) [9]. The test was carried out using 60X15Cm petri dishes. Three replicates were performed with three replicas for each concentration at each replicate. For each test, 5 adult worms were contacted with 5ml of extract and then incubated for 20 hours. Observations were made after 1h, 2h, 6h, 6h and 20h. After 20 hours, the mortality of the worms was assessed. A worm was pronounced dead if no movement of the head, abdomen or tail was observed after pinching and dipping it in PBS (1X) for 30 minutes. The mortality rate (MR) for each concentration of extract was calculated by the following formula:

MR (%): (Number of adult dead worms / Number of live adult worms placed in the petri dish) X 100.

Statistical Analysis

The data were entered on the Excel Office software16 which was used for the calculation of the means and the Standard deviations. The adults worms mortality rate at different follow-up times was subject of an one factor ANOVA followed by multiple comparison of mean by Tukey contrasts method at 5% significance level. Analyzes was performed using the Rstudio interface 1.4.1717 with Rcmdr version 2.7-1 packages of the R software version 4.1.0.The probit method was used to determine the lethal concentration of each extract which kills 50% of adult worms with SPSS STATISTICA26 software at 5% significance level.

Results

The results show that *C. sesamoides* aqueous extracts caused high mortality of *H. contrortus* adult worm compare to the negative control P (< 0.05). Its showed 79.86% of adult worm mortality at high of 100mg/ml while lower doses recorded low mortality rate. The LC50 of 78.74mg/ml was obtained for C. sesamoides aqueous extracts. However, *S. hermonthica* aqueous extracts recorded low adult worms moratlity rate with an LC50 of 212.13mg/ml (Table 1).

Table 1: Mortality rate of adult H. contortus worms.

Concentration (mg/ml)	Aqueous Extracts	
	***C. sesamoides* Endl**	***S. hermonthica* (Deli.) Benth**
100	79.86 ± 11.54 b	22.22 ± 10.18a
50	6.66 ± 6 a	0 ± 0a
25	0 ± 0 a	0 ± 0a
Lévamisole (2.5 mg/ml)	100 ± 0 b	100 ± 0b
PBS(1X)	0 ± a	0 ± 0a
P	0.00000000143	2e-16

Note:

(a, b) difference between column; P: Probabilty

Discussion

C. sesamoïdes whole plant aqueous extract showed *in vitro* anthelmintic activity on *H. contortus* adult worms. But *S. hermonthica* aqueous extract anthelminthic activity on adult worm has been low and less than 25%. Many authors have identified the species *H. contrortus* as being one of the parasites having a significant impact on the productivity of small ruminants leading to significant economic losses for producers [11,12]. The use of both these two species has no negative impact on the environment as they are annual grasses harvested at their maturity stages and which are naturally consumed by animals. Both species used in this study are widely used in the human and animal pharmacopoeia against several deseases [13,14]. The *in vitro* tests revealed a *H. contortus* adult worms sensitivity after 20h contact with the aqueous extract of C. sesamoïdes with a mortality rate of 79.86% while that of S. hermonthica was less vermicidal with a mortality rate of 22.13%. The LC (50) of C. sesamoids Endl was 78.74mg / ml while that of *S. hermonthica* (Del.) Benth was 212.26mg / ml. Other studies have found results with high doses of aqueous plant extracts to record a high mortality rate of *H. contortus* adult worms. This is the case with the aqueous extracts of *B. aegyptiaca* which had 100% of adult worms dead at the dose of 50 mg/ml and that of Artemisia herba alba which recorded a mortality rate of 90% at the dose of 50mg/ml after 6 hours of contact [15]. The difference in mortality rate of this study with ours could be due to the nature of the species and the parts of the plants used. Indeed, our study focused on herbaceous plants so this study focused on woody plants. On the other hand, the anthelmintic tests of aqueous extracts of Cassia obtusifolia showed a mortality rate of adult worms of 11, 1% and 100% respectively with 25mg / ml and 100mg ml after only 2 hours of contact [16]. The similarity of this study with ours is the fact that it concerns an herbaceous species but the difference in effectiveness would be due to the chemical compounds contained in the different plants and also the parts used for the tests. Indeed, our study looked at the whole plant while the other study only looked at the leaves.

The *in vitro* adult worm's mortality tests of both whole plants aqueous extracts results, would be due to the presence of certain secondary metabolites such as polyphenols and flavonoids groups. Indeed, several studies have shown that the presence of chemicals groups is the main reason for the anthelmintic activity of many plant species such as *Pterocarpus erinanceus*, *Parkia biglobosa*, *Morus mesozygia*, *Albizia adianthifolia*, *Ficus lutea*, *Newbouldia laevis* and *Zanthoxylum* [17,18].

Conclusion

Our study results show that *C. sesamoïdes* aqueous extract anthelmintic activity on *H. contortus* adult worm was higher and *S. hermonthica* aqueous extracts. This result would be due to the presence of polyphenols and flavonoids groups in the aqueous extracts of these plant. The use of these two herbaceous species as an anthelmintic in the traditional way would therefore be proven. However, it is essential to complete this study with hydroalcoholic extract *in vitro* anthelmintic as well as in vivo tests and phytochemical assays in order to determine the main metabolites secondary in origin of this activity.

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