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# CISSUS SUCCULENTA AND SCHINUS MOLLE- INVITRO ANTHELMINTHIC ACTIVITY AGAINST HAEMONCHUS CONTORTUS

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## ABSTRACT

The most significant parasite in terms of economic impact in the production of small ruminants is Haemonchus contortus, the cause of haemonchosis. Chemotherapy control has failed since drug-resistant strains have quickly emerged. Alternative leads, particularly those from plants, are continuously sought after. C.succulenta aerial portions and S. molle bark were evaluated for their anti-helmintic properties against H.contortus. A motility test for adults and an egg hatching inhibitory test were used to assess in vitro adulticidal and egg hatching inhibitory effects of the extracts. The adulticidal activity of the extracts at higher doses (10 and 5 mg/ml) was substantially greater than that of albendazole and significantly superior to the negative control (p<0.05). Similarly, S. molle and C. succulenta extracts had relative egg hatch suppression efficacy values of 96% and 88%, respectively, within 48 hours of treatment at 1 mg/ml. A crude methanolic extract of the plants was found to be effective in inhibiting egg hatching and adulticidal effects in H. contortus.

Key words: C.succulenta, Haemonchus contortus, anti-helmintic properties, S. molle

## **INTRODUCTION**

Global cattle output continues to be severely hampered by parasitic diseases [1]. This nematode parasite causes *haemonchosis* in small ruminant animals by feeding on their blood and causing anorexia, anemia, stunted growth, and even death. [1, 2]. Globally, a highly pathogenic parasite of small ruminants, *H. contortus*, stands in the way of profitable sheep and goat production [2, 3].

A combination of synthetic anthelmintic and proper grazing management is typically used to control worms [4]. The downside of synthetic anthelmintics is their resistance. A broad-spectrum as well as a narrowspectrum anthelminthic resistance has been established for *H.contortus* [5, 6]. As of yet, Ethiopia has not conducted a national survey of anthelmintic resistance [7]. It has been found, however, that several areas of Ethiopia are resistant to albendazole, levamisole, tetramisole, and ivermectin [8]. Developing antihelmintics that are less expensive and effective can be achieved by investigating local herbal medicines [9]. It is becoming increasingly popular to evaluate the antihelmintic properties of medicinal herbs. Animal helminth infections have been effectively treated with plant items in numerous publications, mostly from Africa [10–12].

According to studies, *C.succulenta* and *S.molle* are used to treat different helminthic infectations. According to claims, *C. succulenta* was effective in treating helminths, lices as well as tick infestations in livestock. Similar to this, Ethiopian pastoralists and agropastoralists frequently employ *S. molle* to get rid of intestinal parasites [13, 14]. Therefore, it was determined that it was important to assess the anti-helminthic ability of 2 herbs that the pastoralist populations which was frequently utilised. The need of the study was to assess these plants' antihelmintic effectiveness against *H. contortus* in *in vitro* egg cultures.

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# METHODOLOGY

# Plant materials gathering

Fresh *C. succulenta* aerial fragments and *S. molle* barks were gathered from local supplier. Once the obtained plants had been botanically recognised, by XXXX. A lab mortar and pestle were used to pulverize the plants into a fine powder after the plants had been cleaned, allowed to dry in the shade, mechanically processed, and mechanically processed.

A cold maceration technique was used to prepare the crude extracts. As an extraction solvent, methanol was steeped in batches of coarsely powdered plant materials for three days with constant shaking. This was followed by a filtration of the mixture. Following two separate extractions with the same medium (Whattman filter paper, (NO:3)) of the residual maceration residue, then solution of filtrate passes through filter paper (sterile). We concentrated the filtrate using rotating evaporators (Buchi Rota vapor, Switzerland). A hot-air oven was used to dry the extracts, and they were kept in a refrigerator until needed. They were sealed within vials with transparent labels. Upon weighing the dry extracts, *C. succulenta* and *S molle* yielded 15.2 and 5.3 per cent (w/w), respectively.

#### Plant Chemical Analysis

An analysis of the constituents in the extracts of crude *C. succulenta*, *S.molle* was carried out by phytochemical screening. To identify the components specified in the specification, known screening analysis were done using traditional methods, processes, and reagent [15-17].

## Anthelminthic action (*in vitro*) Hatch Inhibition Assay for Eggs (EHIA)

In a recent capture of an adult female *H. contortus*, the eggs were collected, crushed, and sieved. In the next step, the eggs were mixed in phosphate buffer saline. We centrifuged the samples at 300 rpm/ 2 mins, then collected the sediment. A convex meniscus was formed above the test tube by resuspending this silt in a saturated NaCl solution. After the samples were centrifuged, the tubes were covered with coverslips. A new test tube was then gently washed with water after the coverslip had been removed. Afterwards, the sediment and eggs were centrifuged separately. Three rounds of cleaning with distilled water were performed according to the McMaster protocol, followed by a concentration of 100–200 eggs per milliliter [18].

Microtiter plates with 48 wells were filled with 100 eggs and 200 liters of water each [19]. There were 200 liters of each plant extract used per test well, totaling 400 liters. There were four concentrations of plant extracts tested: 0.1, 0.25, 0.5, as well as 1 milligram per ml. The positive control and the untreated control were distilled water and 0.25 mg/mL albendazole, a conventional drug. A total of three tests were run for each test. After incubating

the plate for 48 hours at  $37^{\circ}$ C in a humid incubator, it was cooled down to  $37^{\circ}$ C. Then the eggs react with Lugol's solution for protection from hatching further. In each well, unhatched eggs and larvae from the first instar were counted. As shown below, the procedure used to calculate the per cent of Hatching of egg was used [19].

Percent inhibition (%) = 
$$100 \left( 1 - \frac{P_{\text{test}}}{P_{\text{non-treated}}} \right)$$

#### Adult motility assessment (AMA)

The Sharma et al method was used to perform AMA on adult *H. contortus* worms [20]. The test was conducted using a glass Petri dish with a diameter of 5 cm. In all, the study used about 368 mature parasites. There were four distinct concentrations of each plant extract. Each treatment was given to 10 worms in triplicate at room temperature (25-30 °C) in three different Petri plates.

#### The four groups were as follows:

**Group A**: The methanolic extract of *C. succulenta* (1.25,2.5, 5 and 10 mg per ml in PBS)

**Group B**: The methanolic extract of *S. molle* (1.25, 2.5, 5, and 10 mg/ml)

Group C: Albendazole as Positive control (0.25 mg/ml).

Group D: Phosphate Buffer Saline as negative Control.

Inhibited worm movement was used as a marker for worm death or paralysis. The worms' motility was assessed up to 7 hours after treatment, and the quantity of motile worms was counted periodically. The worms were selected and transferred in luke-warm phosphate buffer saline for 10 mins. The worms were classified as living if their motility recovered; otherwise, they were classified as dead.

Analysis of the data was done using SPSS Version 20 to organise, edit, and analyse the information. The one-way ANOVA was performed to evaluate the data from both studies using the Tukey HSD multiple comparison test. Statistical significance was defined as a p value of 0.05 at a 95% confidence interval.

#### Results

#### Screening for phytochemicals

A phytochemical examination revealed the presence of Alkaloids and Tannins in both extract, as well as Flavonoids as well as Phenols in the Methanolic extract of *C. succulenta*.

#### Action as an anthelmintic

According to both *in vitro* assays, both plants extracts have possible adulticidal and egg hatching inhibiting properties. It showed a dose-dependent antihelmintic activity in both AMA and EHIA.

# Motility test (Adult)

This investigation demonstrated that the anthelmintic activity of both extracts and the commonly used anthelmintic, albendazole, was about similar. The activity increased over time and with effort. After being exposed to different plant extract concentrations for seven hours, adult *H. contortus* from both plants had a significance (p<0.05) as well as reduction in dose dependence manner (Table 1).

Adult *H. contorts* died at rates of 95% and 100% at the maximum concentration (10 mg/mL) from the plants after being exposed to the extracts for 7 hrs. At a dose of 0.25 mg/mL, albendazole killed adult worms within 4 hours of exposure; at a dose of 0.25 mg/mL, all adult worms were gone. The extracts' adulticidal efficacy profile can be summarized as follows based on the percentage of

adult parasites eliminated at the conclusion of the observation period: The equivalent concentrations for *C. succulenta* and *S. molle* were 100 and 95% at 10 mg/ml, 97.5 and 92.6% at 5 mg/ml, 95.08 and 91.4% at 2.5 mg/ml, and 91.4 and 89.4% at 1.25 mg/mL.

## Egg hatchability test

The results of an EHIA on *C. succulenta* and *S. molle* extracts are shown in table 2. The results demonstrated that when coupled with albendazole, both extracts had a comparable inhibitory effect on egg hatching. The methanolic extract of *S. molle* barks required a concentration of 1 mg/ml to reduce egg hatching by 96%, whereas *C. succulenta* required 88% at the same concentration.

TREATMENT	CONCENTRAT	NUMBER OF PARASITES DEAD (POST-TREATMENTS IN HOUR					
	ION	1 hour	2 hour	4hour	6 hour	8 hour	
	Mg per ml						
C. succulenta	1.25	$0.66 \pm 0.32$	1.65±0.33	3.65±0.31	5.31±0.25	5.31±0.29	
	2.5	0.66±0.32	2.00±0.59	4.65±0.31	7.01±0.57	7.33±0.31	
	5	2.31±0.31	3.65±0.35	5.01±0.55	7.31±0.35	8.65±0.31	
	10	4.01±0.57	5.35±0.31	8.65±0.31	$10.01 \pm 0.01$	10.01±0.00	
S. molle	1.25	0.00±0.00	0.65±0.31	3.03±0.55	3.65±0.31	4.33±0.00	
	2.5	0.31±0.35	1.35±0.31	4.52±0.31	5.33±0.01	5.35±0.01	
	5	0.65±0.33	1.37±0.32	3.58±0.31	5.65±0.35	6.01±0.00	
	10	2.01±0.57	3.05±0.57	6.04±0.57	$7.05 \pm 0.57$	7.35±0.31	
Albendazole	0.25	5.35±0.32	8.35±0.31	10.01±0.0	$10 \pm 0.00$	10±0.00	
PBS	1ml	$0.00 \pm 0.00$	$0.00 \pm 0.00$	0.61±0.31	1.31±0.35	2.65±0.31	

#### Table 2:

Table 1:

TREATMENT	CONCENTRATION	NO OF	NO OF L1	% INHIBITION
	Mg per ml	UNHATCHED	LARVAE	
		EGGS		
C. succulenta	0.1	54.5 ±33.05	44.62±3.11	41.65±1.54
	0.25	65.0±1.89	36.98±1.98	51.59±4.29
	0.5	75.0±2.54	22.95±2.26	69.85±3.98
	1	88.0±3.59	10.95±3.58	87.59±4.98
S. molle	0.1	69.23 ±3.89	29.98±3.87	39.23±2.14
	0.25	55.59±1.01	43.89±1.01	56.87±3.47
	0.5	42.98±2.98	56.78±2.89	73.87±3.14
	1	96.89±1.98	2.98±1.89	95.54±1.78
Albendazole	0.25	99.01±4.78	1.59±5.21	99.54±5.54
Distilled water		93.45±1.87	5.88±1.88	7.14±2.04

## DISCUSSION

Concerns about toxicity, anthelmintic resistance, and drug residues in animal products have generated a rise in interest in adopting plant-based medicines. The plant components used in the current inquiry were discovered by Ethiopian traditional healers to work as anthelmintic agents from a range of sources. Utilizing free-living stages of parasitic nematodes, new plant compounds' anthelmintic activity can be evaluated in vitro [21]. *In vitro* methods are preferred to *in vivo* ones because they are less expensive, simpler to use, and have a faster turnaround time [22]. We found a statistically significant correlation between graded extract concentrations, the exposure test interval, and adult parasite mortality in the current study.

A traditional system of medicine claims C. succulenta's entire plant has therapeutic properties based

on ethnobotanical research conducted by ethnobotanists [23]. A study conducted by Luseba *et al.* [24] found that ethanol extracts and dichloromethane extract from *C. succulenta* stems could inhibit the growth of bacteria. The greatest efficacy value for the plant extract in the current study, 10 mg/ml, was 100% effective against the parasite and equivalent to the widely used anthelmintic albendazole. When a dose of 1 mg/ml was used, this plant extract inhibited egg hatching by 88%.

Various herbal remedies have been purportedly used to cure a wide range of human and animal health issues using *S.molle*, a medicinal plant that has undergone significant research in folk medicine worldwide [25–27]. In Ethiopia's Somali Regional State, pastoralists and agropastoralists successfully use *S. molle* against endoparasites. *S. molle* barks have reportedly been used to treat illnesses caused by several parasites [26]. The current study, which discovered that mature *H. contortus* parasites at a concentration of 10 mg/ml in methanolic extracts of *S. molle* were 95% lethal, serves as evidence for this. *S. molle* exhibited a 96% inhibition. As the concentration of plant extract was increased, the inhibition of egg hatching increased, demonstrating a dose-dependent impact.

The anthelmintic effect of *S. molle* is due to the constituents like Alkaloids and Tannins, which were investigated in the phytochemical test. A phytochemical screening revealed that *C. succulenta* contains constituents

such as Alkaloids, Flavonoids, Tannins, and Phenols. There is evidence that a range of medicinal plants contain secondary plant metabolites that may have therapeutic effects [28]. There are studies on tannins, alkaloids, and flavonoids' anthelmintic properties [29, 30]. It is possible that these phytochemicals may be responsible for the anthelmintic activity of plant extracts observed in this study. Furthermore, tannins interfere with linked oxidative phosphorylation, which prevents these parasites from synthesizing ATP [31]. A variety of plant extracts can also be tested in vitro for their potential anthelmintic properties with in vitro methods. In vivo results were not generalizable from results obtained in vitro because of biotransformation, food interactions, and differences in absorption. Therefore, the findings should be validated through in vivo testing.

# CONCLUSION

The current experiment showed that methanolic extracts of *C. succulenta* aerial parts and *S. molle* barks had *in vitro* anti-helmintic efficacy against adult and oval stages of *H. contortus*. But compared to *S. molle*, *C. succulenta* was more effective as an anthelmintic. The crude extracts must be fractionated, and more study is required to determine how well anti-helmintics work at different stages of parasite development.

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