Research Article

In Vitro Anthelmintic Activity of Crude Extracts of Aerial Parts of Cissus quadrangularis L. and Leaves of Schinus molle L. against Haemonchus contortus

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1. Background

Parasitic infections remain a major constraint to livestock production globally [1]. Haemonchus contortus, the causative agent of Haemonchosis, is a nematode parasite that feeds on blood of small ruminant animals and causes anaemia, anorexia, reduced growth, and eventual death of host animals [1, 2]. H. contortus is highly pathogenic parasite of small ruminants and it is the primary constraint to profitable production of sheep and goats worldwide [2, 3].

Control is generally achieved by use of synthetic anthelmintics in combination with grazing management [4]. Synthetic anthelmintics have several drawbacks including resistance. H. contortus has been documented to be resistant to broad and narrow spectrum families of anthelmintics [5, 6]. Country-wide surveys for anthelmintic resistance have not yet been carried out in Ethiopia [7]. However, studies showed that resistance was detected in different parts of Ethiopia against albendazole, levamisole, tetramisole, and ivermectin [8].

One practical way of developing cheaper and effective anthelmintics is to study indigenous herbal remedies [9]. Evaluation of the activities of medicinal plants claimed for anthelmintic property is getting attention these days. There have been many reports, mainly from Africa, indicating the effectiveness of plant products against helminth infections in animals [10–12]. Studies reported that Cissus quadrangularis and Schinus molle are used against various helminth infections in Ethiopia. C. quadrangularis is claimed to be used in livestock against helminthiasis, tick and lice infestation, and leach infestation. Similarly, S. molle is widely used by pastoralists and agropastoralists of Ethiopia to eradicate intestinal parasites [13, 14]. Therefore, it was found necessary to evaluate the anthelmintic potential of two of the commonly used herbs by the pastoralist communities of Ethiopia. Thus, the present
work was aimed at evaluating the in vitro egg antihelmintic efficacy of these plants against *H. contortus*.

### 2. Methods

#### 2.1. Plant Collection and Extracts Preparation

Fresh aerial parts of *C. quadrangularis* and leaves of *S. molle* were collected from their natural habitat around Jigjiga, eastern Ethiopia. After botanical identification of the collected plants, voucher specimens, SZ01 for *C. quadrangularis* and SZ02 for *S. molle*, were deposited at the National Herbarium of Addis Ababa University, College of Natural Sciences. Plants were then cleaned, shade-dried, mechanically grinded, and coarsely powdered using laboratory mortar and pestle.

The crude extracts were prepared by cold maceration technique. Coarsely powdered plant materials were separately soaked in extraction solvent (methanol) followed by shaking periodically for three days and then filtered. The residue left after maceration was successively extracted twice with the same medium separately and the filtrate was passed through sterile filter paper (Whatman No. 3, Whatman Ltd., England). The filtrate was concentrated with rotary evaporator (Buchi Rota vapor, Switzerland). The extracts were then dried in hot air oven and transferred into well labeled vials and kept in a refrigerator until required for use. The resulting dry extract was weighed and provided a percentage yield of 5.3% (w/w) and 15.2% (w/w) for *C. quadrangularis* and *S. molle*, respectively.

#### 2.2. Phytochemical Screening

Phytochemical screening was carried out to assess the qualitative chemical composition of crude methanolic extracts of *C. quadrangularis* and *S. molle*. Standard screening tests using conventional protocol, procedure, and reagents were conducted using standard procedures to identify the constituents as described in [15–17].

#### 2.3. Collection of Parasites

Adult *H. contortus* were collected from the abomasum of sheep obtained from Jigjiga municipal abattoir. Then, the abomasum was washed with water and the parasites were kept in phosphate buffer saline (PBS) until the in vitro evaluation was started.

#### 2.4. In Vitro Anthelmintic Activity Evaluation

##### 2.4.1. Egg Hatch Inhibition Assay (EHIA)

Freshly collected adult female *H. contortus* were picked, crushed, and sieved to obtain the eggs, which were then triturated in PBS. The suspensions were centrifuged for 2 minutes at 300 rpm and sediment was retained. This sediment was resuspended in saturated solution of NaCl to form a convex meniscus above the test tube. After putting a coverslip above the test tube, samples were centrifuged again. Coverslip was carefully removed and eggs were washed into another test tube. This solution was then centrifuged and eggs were collected from sediment. Eggs were washed thrice with distilled water and adjusted to a concentration of 100–200 eggs/mL, using the McMaster technique [18].

EHIA was performed following the technique of Coles et al. [19]. Approximately, 100 eggs in 200 µL of water were pipetted into each well of a 48-well microtiter plate. To each of the test wells, 200 µL of each plant extract was added to a final volume of 400 µL per well. The plant extracts were tested at concentrations of 0.1, 0.25, 0.5, and 1 mg/mL. Similarly, 200 µL of albendazole (standard drug) at 0.25 mg/mL concentration and distilled water were used as a positive control and nontreated control, respectively.

Each test was done in three replicates. The plate was incubated in a humidified incubator at 37°C for 48 hrs. Thereafter, a drop of Lugol’s solution was added to stop further hatching. All unhatched eggs and L1 larvae in each well were counted. The percent inhibition of egg hatching was calculated by using the formula below [19].

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

where *P* is the number of eggs that hatched in EHIA.

##### 2.4.2. Adult Motility Assay (AMA)

AMA was conducted on mature *H. contortus* worms following the technique of Sharma et al. [20]. The test was performed in 5 cm diameter glass Petri dish. A total of about 368 adult parasites were used in the study. Four concentrations were employed for each plant extract. Ten worms were exposed in triplicate to each of the following treatments in separate Petri dishes at room temperature (25–30°C).

There were 4 groups as follows: Group I: crude methanol extract at 1.25, 2.5, 5, and 10 mg/mL of *C. quadrangularis* (four different concentrations prepared in PBS); Group II: crude methanol extract at 1.25, 2.5, 5, and 10 mg/mL of *S. molle*; Group III: albendazole at 0.25 mg/mL (positive control); and Group IV: PBS (negative control).

The inhibitions of motility of worms were used as indication of worm mortality or paralysis. Motility of worms was observed and motile worms were counted at different time intervals till 7 hrs posttreatment. Worms not showing any motility were picked out and kept in lukewarm PBS for 10 minutes and, in case of revival in motility, the observed worms were counted as alive; otherwise, they were counted as dead.

#### 2.5. Data Analysis

Data were organized, edited, and analyzed using SPSS Version 20. The data obtained from both assays were analyzed with one-way ANOVA using Tukey HSD multiple comparison test. Results were deemed statistically significant if *p* < 0.05 at 95% confidence intervals.

### 3. Results

#### 3.1. Phytochemical Screening

The phytochemical screening showed the presence of alkaloid and tannin in the both extracts whereas flavonoids and phenols were additionally present in the methanolic extract of *C. quadrangularis*.

#### 3.2. Anthelmintic Activity

Both in vitro assays showed that crude extracts of both plants have promising adulticidal and...
The adulticidal efficacy profile of the extracts, as measured by the percentage of the adult parasites killed at the end of observation period, is as follows: 100 and 95% at concentration of 10 mg/mL, 97.5 and 92.6% at concentration of 5 mg/mL, 95.08 and 91.4% at concentration of 2.5 mg/mL, and 91.4 and 89.4% at concentration of 1.25 mg/mL for the C. quadrangularis and S. molle, respectively.

3.2.2. Egg Hatching Inhibition Assay. The result of EHIA at graded concentration of crude methanolic extracts of C. quadrangularis and S. molle is shown in Table 2. The result indicated that both extracts produced a relatively comparable egg hatching inhibitory effect with albendazole. The methanolic extract of leaves of S. molle required a maximum concentration of 1 mg/mL to induce 96% egg hatch inhibition while C. quadrangularis induced 88% egg hatching inhibitory effect at the same concentration. The egg inhibitory efficacy profile of the extracts, as measured by the percentage of eggs unhatched at the end of observation period, is as follows: 40.67 and 39.33% at concentration of 0.1 mg/mL, 52 and 57% at concentration of 0.25 mg/mL, 70.33 and 74.33% at concentration of 0.5 mg/mL, and 88 and 96% at concentration of 1 mg/ml for the C. quadrangularis and S. molle, respectively.

4. Discussion

The problem of anthelmintic resistance, toxicity, and the increasing concern over the presence of drug residues in animal products has led to a renewal of interest in the use of plant based drugs. Plant materials evaluated in the current study had been identified from various sources to serve as anthelmintic agents by traditional healers of Ethiopia. The in vitro tests using free living stages of parasitic nematodes offer a means of evaluating the anthelmintic activity of new plant compounds [21]. In vitro techniques are preferred to in vivo methods due to their low cost, simplicity, and rapid turnover [22]. In the current study, a statistically significant association was noted between graded concentrations of the extracts, the exposure test-time interval, and adult parasite mortality.

The whole plant of C. quadrangularis is documented to possess medicinal properties in ethnomedicinal surveys conducted by ethnobotanists in traditional system of medicine [23]. Moreover, Luseba et al. [24] reported that the methanol extract and dichloromethane extract of stems of C. quadrangularis possess antimicrobial activity. The present study showed 100% efficacy of the plant extract against the parasite at the concentration of 10 mg/ml which was the highest efficacy value and was comparable with the standard anthelmintic, albendazole. The egg hatching inhibitory effect of this plant extract was 88% at the concentration of 1 mg/ml.

In the folk medicine, S. molle is an extensively studied medicinal plant throughout the world and has been reported to be used against wide ranges of human and livestock ailments [25–27]. In Somali Regional State of Ethiopia, S. molle is well used against endoparasites by pastoralists and agropastoralists. The leaves of S. molle are also reported to be used for the treatment of infections caused by different parasites [26]. This is evident from the current study, which showed 95% mortality of adult parasites of H. contortus at a concentration of 10 mg/ml in methanolic extracts of S. molle. The egg hatching inhibitory effect of S. molle was 96% at a concentration of 1 mg/ml. Increment on the concentration of the plant extracts resulted in increased inhibition of egg hatching indicating dose-dependent activity.

As screened in the phytochemical test of S. molle, the secondary metabolites, alkaloid and tannin, are responsible for their anthelmintic activity. In phytochemical screening of C. quadrangularis, it is revealed that the plant has secondary metabolites like alkaloids, flavonoids, tannins, and phenols. These classes of plant secondary metabolites are considered the sources of chemical components responsible for wide therapeutic activities of several medicinal plants [28]. Some studies are available for anthelmintic activity of tannins, alkaloids, and flavonoids [29, 30]. The presence of these phytochemicals may be responsible for the observed anthelmintic activity of plant extracts in the present study. Furthermore, tannins have been shown to interfere with coupled oxidative phosphorylation, thus blocking ATP synthesis in these parasites [31]. Finally, the in vitro methods provide a
### Table 1: In vitro adulticidal effect of crude extracts of *C. quadrangularis* and *S. molle* against *H. contortus*.

| Treatment        | Concentration | 1 hr         | 2 hr         | 3 hr         | 4 hr         | 5 hr         | 6 hr         | 7 hr         |
|------------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| **C. quadrangularis** |               |              |              |              |              |              |              |              |
|                  | 10 mg/mL      | 4.00 ± 0.58<sup>acd</sup> | 5.33 ± 0.33<sup>bcd</sup> | 7.33 ± 0.33<sup>bcd</sup> | 8.67 ± 0.33<sup>acd</sup> | 9.33 ± 0.33<sup>acd</sup> | 10.00 ± 0.00<sup>acd</sup> | 10.00 ± 0.00<sup>acd</sup> |
|                  | 5 mg/mL       | 2.33 ± 0.33<sup>bcd</sup> | 3.67 ± 0.33<sup>bcd</sup> | 4.33 ± 0.33<sup>bcd</sup> | 5.00 ± 0.58<sup>abc</sup> | 6.33 ± 0.33<sup>abcd</sup> | 7.00 ± 0.58<sup>abc</sup> | 7.33 ± 0.33<sup>abcd</sup> |
|                  | 2.5 mg/mL     | 0.67 ± 0.33<sup>b</sup> | 2.00 ± 0.00<sup>ab</sup> | 3.33 ± 0.33<sup>bcd</sup> | 4.67 ± 0.33<sup>ab</sup> | 6.33 ± 0.33<sup>abcd</sup> | 7.00 ± 0.58<sup>abc</sup> | 7.33 ± 0.33<sup>abcd</sup> |
|                  | 1.25 mg/mL    | 0.67 ± 0.33<sup>b</sup> | 1.67 ± 0.33<sup>b</sup> | 2.67 ± 0.33<sup>bc</sup> | 3.67 ± 0.33<sup>ab</sup> | 4.67 ± 0.33<sup>ab</sup> | 5.33 ± 0.33<sup>abc</sup> | 5.33 ± 0.33<sup>abcd</sup> |
|                  | 10 mg/mL      | 2.00 ± 0.58<sup>bcd</sup> | 3.00 ± 0.58<sup>bcd</sup> | 4.33 ± 0.33<sup>bcd</sup> | 6.00 ± 0.58<sup>abc</sup> | 6.67 ± 0.66<sup>abcd</sup> | 7.00 ± 0.58<sup>abc</sup> | 7.33 ± 0.33<sup>abcd</sup> |
|                  | 1 mg/mL       | 0.33 ± 0.33<sup>b</sup> | 1.33 ± 0.33<sup>b</sup> | 2.33 ± 0.33<sup>bc</sup> | 3.33 ± 0.33<sup>ab</sup> | 3.67 ± 0.33<sup>ab</sup> | 5.00 ± 0.00<sup>bc</sup> | 5.33 ± 0.33<sup>bc</sup> |
| **S. molle**     |               |              |              |              |              |              |              |              |
|                  | 5 mg/mL       | 0.33 ± 0.33<sup>b</sup> | 1.33 ± 0.33<sup>b</sup> | 2.33 ± 0.33<sup>bc</sup> | 4.67 ± 0.33<sup>ab</sup> | 4.67 ± 0.33<sup>ab</sup> | 5.00 ± 0.00<sup>bc</sup> | 5.33 ± 0.33<sup>bc</sup> |
|                  | 2.5 mg/mL     | 0.00 ± 0.00<sup>b</sup> | 0.67 ± 0.33<sup>b</sup> | 1.67 ± 0.33<sup>bc</sup> | 3.00 ± 0.58<sup>ab</sup> | 3 ± 0.58<sup>ab</sup> | 3.67 ± 0.33<sup>ab</sup> | 4.33 ± 0.33<sup>bc</sup> |
|                  | 1.25 mg/mL    | 0.00 ± 0.00<sup>b</sup> | 0.00 ± 0.00<sup>b</sup> | 0.00 ± 0.00<sup>b</sup> | 0.67 ± 0.33<sup>bcd</sup> | 0.67 ± 0.33<sup>bcd</sup> | 1.33 ± 0.33<sup>bcd</sup> | 2.67 ± 0.33<sup>bcd</sup> |
| **Albendazole**  | 0.25 mg/mL    | 5.33 ± 0.33<sup>acd</sup> | 8.33 ± 0.33<sup>acd</sup> | 9.33 ± 0.33<sup>acd</sup> | 10.00 ± 0.00<sup>acd</sup> | 10.00 ± 0.00<sup>acd</sup> | 10.00 ± 0.00<sup>acd</sup> | 10 ± 0.00<sup>acd</sup> |
| **PBS**          | 1 mL          | 0.00 ± 0.00<sup>b</sup> | 0.00 ± 0.00<sup>b</sup> | 0.00 ± 0.00<sup>b</sup> | 0.67 ± 0.33<sup>bcd</sup> | 0.67 ± 0.33<sup>bcd</sup> | 1.33 ± 0.33<sup>bcd</sup> | 2.67 ± 0.33<sup>bcd</sup> |

Values are mean ± SEM. All superscripts indicate significance at *p* < 0.05, <sup>a</sup> compared to untreated (PBS), <sup>b</sup> compared to albendazole, <sup>c</sup> compared to lowest dose of methanolic extract of *C. quadrangularis*, and <sup>d</sup> compared to lowest dose of methanolic extracts of *S. molle*. 
means to screen rapidly for potential anthelmintic activities of different plant extracts. Due to drug biotransformation, interaction with food materials, and absorption variations, the results obtained by the in vitro method could not be extrapolated to in vivo activity. Therefore, results should be ascertained by in vivo evaluation.

5. Conclusion

The current study evidenced that the methanolic extracts of aerial parts of C. quadrangularis and leaves of S. molle have a promising in vitro anthelmintic activity against adult and oval stages of H. contortus. However, the anthelmintic activity of C. quadrangularis was superior to S. molle. Fractionation of the crude extracts and further anthelmintic efficacy studies involving other parasite development stages are warranted.

Abbreviations

AMA: Adult motility assay  
EHIA: Egg hatch inhibition assay  
PBS: Phosphate buffer saline  
NaCl: Sodium chloride.

Data Access

Vouchers and dried leaves used for this study are stored at the herbarium at the Addis Ababa University, College of Natural Sciences. The datasets supporting the conclusion of this study are available from the corresponding author on reasonable request.

Ethical Approval

Ethical approval was obtained from the Research Ethics Committee of the Directorate of Research, Publication and Technology Transfer, Jigjiga University, Ethiopia.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors’ Contributions

Solomon Assefa, the corresponding author, contributed to the literature reviewing as well as to the writing of the manuscript and carried out the analyses and interpretation of data. Selamawit Zenebe and Teka Feyera participated in the study design, the interpretation of the results, and the writing of the paper. Selamawit Zenebe conducted the experiment. All authors approved the final manuscript.

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Table 2: In vitro EHIA effect of crude extracts of C. Quadrangularis and S. molle against H. contortus, 48 hours posttreatment.

| Treatment      | Concentration | Number of unhatched eggs | Number of L1 larvae | Percentage of inhibition |
|----------------|---------------|--------------------------|---------------------|-------------------------|
|                | 0.1 mg/mL     | 54.3 ± 3.06<sup>a</sup>  | 45.67 ± 3.05<sup>b</sup>  | 40.67 ± 1.53<sup>ab</sup>  |
| C. quadrangularis | 0.25 mg/mL   | 66.00 ± 2.00<sup>b</sup>  | 37.00 ± 2.00<sup>b</sup>  | 52.00 ± 4.35<sup>bd</sup>  |
|                | 0.5 mg/mL     | 77.00 ± 2.64<sup>c</sup>  | 23.00 ± 2.64<sup>c</sup>  | 70.33 ± 4.04<sup>bd</sup>  |
|                | 1 mg/mL       | 89.00 ± 3.60<sup>bc</sup> | 11.00 ± 3.60<sup>bc</sup> | 88.00 ± 5.29<sup>bd</sup>  |
|                | 1 mg/mL       | 97.00 ± 2.00<sup>b</sup>  | 3.00 ± 2.00<sup>b</sup>   | 96.00 ± 2.52<sup>bc</sup>  |
| S. molle       | 0.5 mg/mL     | 43.00 ± 3.00<sup>b</sup>  | 57.00 ± 3.00<sup>b</sup>  | 74.33 ± 3.22<sup>b</sup>  |
|                | 0.25 mg/mL    | 56.00 ± 1.00<sup>b</sup>  | 44.00 ± 1.00<sup>b</sup>  | 57.00 ± 3.60<sup>b</sup>  |
|                | 0.1 mg/mL     | 69.33 ± 4.50<sup>b</sup>  | 30.67 ± 4.50<sup>b</sup>  | 39.33 ± 3.78<sup>b</sup>  |
|                | 0.05 mg/mL    | 98.33 ± 5.50<sup>b</sup>  | 1.67 ± 5.50<sup>d</sup>   | 99.67 ± 5.50<sup>bd</sup>  |
| Albenbazoide   | 0.25 mg/mL    | 94.00 ± 2.00<sup>d</sup>  | 6.00 ± 2.00<sup>d</sup>   | 7.33 ± 2.08<sup>bd</sup>  |

Values are mean ± SEM. All superscripts indicate significance at p < 0.05, <sup>a</sup> compared to untreated (distilled water), <sup>b</sup> compared to albendazole, <sup>c</sup> compared to lowest dose of methanolic extract of C. quadrangularis, and <sup>d</sup> compared to lowest dose of methanolic extracts of S. molle.
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