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Original Article

Comparative anthelmintic efficacy of *Arundo donax, Areca catechu,* and *Ferula assa-foetida* against *Haemonchus contortus*

Eficácia anthelmíntica comparativa de *Arundo donax*, *Areca catechu* e *Ferula assa-foetida* contra *Haemonchus contortus*

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Abstract

In the present study, anthelmintic activities of *Arundo (A.) donax* L., *Areca (Ar.) catechu* L., and *Ferula (F.) assa-foetida* L. were determined. Leaves of *A. donax* L., latex of *F. assa-foetida* L. and seeds of *Ar. catechu* L. in different solvent fractions were subjected to *in vitro* (egg hatch assay; EHA, and adult motility assay; AMA) and *in vivo* (faecal egg count reduction test; FECRT) tests of anthelmintic activity using *Haemonchus contortus* model. In the AMA, crude aqueous methanol extracts (CAME) and ethyl acetate fractions of *F. assa-foetida* at 10 hr post-treatment showed maximum mortality of *H. contortus* at 12.5-50 mg mL^{-1}. In the EHA, CAME of *F. assa-foetida* was identified as a potent ovicide based on its low LC_{50} (16.9 µg mL^{-1}), followed in order by *Ar. catechu* and *A. donax*. Results from the FECRT also showed the extract of *F. assa-foetida* L. to be more effective than those of *Ar. catechu* L. and *A. donax* L., against the gastrointestinal parasitic nematodes. Chloroform and ethyl acetate fractions showed better anthelmintic activities against the adult worms *in vitro*, while CAME of these plants were better than their crude powders *in vivo*. It is recommended to document and investigate indigenous knowledge of possible medicinal plants to plan scientific trials that may justify their endorsement.

Keywords: *Arundo donax* L., *Areca catechu* L., *Ferula assa-foetida* L., *Haemonchus contortus*, *in vivo*, *in vitro.*

Resumo

No presente estudo, as atividades anti-helmínticas de *Arundo (A.) donax* L., *Areca (Ar.) Catechu* L. e *Ferula (F.) assa-foetida* L. foram determinadas. Folhas de *A. donax* L., látex de *F. assa-foetida* L. e sementes de *Ar. catechu* L. em diferentes frações de solvente foram submetidos a testes *in vitro* (teste de eclosão de ovos, EHA e ensaio de motilidade em adultos, AMA); e *in vivo* (teste de redução da contagem de ovos fecais, FECRT) de atividade anti-helmíntica, usando-se *Haemonchus contortus*. Na AMA, extratos aquosos brutos de metanol (CAME) e frações de acetato de etila de *F. assa-foetida*. Dez horas pós-tratamento, apresentaram mortalidade máxima de *H. contortus* em 12,5-50 mg mL^{-1}. No EHA, CAME de *F. assa-foetida* foi identificado como um ovicida potente baseado em seu baixo LC50 (16,9 µg mL^{-1}), seguido em ordem por *Ar. catechu* e *A. donax*. Os resultados do FECRT também mostraram que o extrato de *F. assa-foetida* L. é mais eficaz do que o de *Ar. catechu* L. e *A. donax* L., contra nematoides parasitas gastrointestinais. As frações clorofórmio e acetato de etila mostraram melhores atividades anti-helmínticas contra vermes adultos *in vitro*, enquanto o CAME dessas plantas foi melhor do que o pó bruto *in vivo*. Recomenda-se documentar e investigar o conhecimento indígena de possíveis plantas medicinais para planejar ensaios científicos que possam justificar seu endosso.

Palavras-chave: *Arundo donax* L., *Areca catechu* L., *Ferula assa-foetida* L., *Haemonchus contortus, in vivo, in vitro.*

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Introduction

Production of the livestock depends upon regular supplies of food, healthcare and proper husbandry practices. Changes in the climate and other factors affect the prevalence of parasitic infections and consequently the productivity, longevity, fertility, and survivorship of the livestock (Thornton et al., 2009). In small-holder dairy farming systems, gastrointestinal (GI) parasites are among the major threats to animal health and welfare and have high economic significance, especially in the resource-poor countries like Pakistan (Iqbal et al., 2007, Rizwan et al., 2017, 2019; Ahmad et al., 2020). Lack of proper awareness, underfeeding, abundant vector populations, and availability of a wide variety of hosts provide the best opportunity for the transmission of parasitic helminths (Zvinorova et al., 2016).

Although most veterinarians and farmers use anthelmintic drugs for the control of helminths as a cornerstone in the small-holder dairy farming systems in the resource-poor countries like Pakistan (Akhter et al., 2014, Khan et al., 2017), the development of widespread anthelmintic resistance (Jabbar et al., 2006), and concerns about toxicity and residues in products (Gasbarre et al., 2001) have led to the revival of awareness about the potential importance of traditional veterinary practices and the need to validate their utility (Ketzis et al., 2002, Rizwan et al., 2019). Ethnoveterinary medicine (EVM) can contribute to sustainable practices in the modern world, especially in developing countries (Confessor et al., 2009). Clinically useful efficacy, greater accessibility, and lower costs are the major benefits associated with EVM (Mwale et al., 2005; Badar et al., 2017; Qudoos et al., 2017). Consequently, surveys about documentation and scientific studies on the validation of the use of plants against parasitic infections have been conducted around the world (Bizimenyera et al., 2006; McGaw et al., 2007; Kareparamban et al., 2012; Faruque et al., 2018).

The *in vitro* and *in vivo* anthelmintic efficacy of crude aqueous-methanol extracts (CAME) of *Arundo (A.) donax* and its various fractions (petroleum spirit, chloroform, and ethyle acetate) were reported against *Haemonchus (H.) contortus* (Sharatkumar et al., 2004; Al-Snafi, 2015). Miles et al. (1993) reported anthelmintic efficacy of *A. donax* extracts against *Oesophagostomum* sp. *Ascaris* sp., and *Paramphistomum* sp. of cattle. The anthelmintic efficacy of an ethanolic extract of *Ferula (F.) assa-foetida* against *Fasciola gigantica* was reported by Kumar & Singh (2014). An ethanolic extract of *Areca (Ar.) catechu* significantly inhibited fumarate reductase and succinate dehydrogenase activities in *Cotylophoron cotylophorum* (Dhanraj & Veerakumari, 2016). The data regarding scientific evaluation of *A. donax* against *H. contortus* models is available; however, we did not find *in vitro* and *in vivo* scientific evaluation of *F. assa-foetida* L. and *Ar. catechu* extracts against *H. contortus* models.

In Pakistan, information related to medicinal plants used for the treatment of parasitic infections and other illnesses transfers from one generation to another. However, this indigenous knowledge varies greatly in different ethnic groups and from region to region due to local variation in the availability of plants (Hussain et al., 2008). Due to geographic and climatic diversity in Pakistan, a wide variety of medicinal plants has been reported from different parts of the country (Iqbal et al., 2004; Muhammad et al., 2005; Hussain et al., 2008; Goraya et al., 2013; Badar et al., 2017). Most of these plants are used by native healers without specific knowledge about the active ingredients. It is important to document and validate plants used by local communities for medicinal purposes before their disappearance due to environmental and technological changes (Goraya et al., 2013; Badar et al., 2017). In connection with earlier studies, the objective of this work was to determine the anthelmintic activities of the leaves of *A. donax* L., latex of *F. assa-foetida* L. and seeds of *Ar. catechu* L through *in vitro* and *in vivo* assays against *H. contortus* models.

Materials and Methods

Collection of plant materials

The plants *A. donax* L., *Ar. catechu* L. and *F. assa-foetida* L were selected during a documentation survey of the herbs used for the treatment of helminthiasis in animals (Badar et al., 2017). Briefly, participatory epidemiological procedures were adopted to involve the local healers as respondents for the identification of the most commonly used plants for the treatment of worm infection in animals of the area (Hussain et al., 2008). The leaves of *A. donax* L. (voucher No. Jg8/2006), latex of *F. assa-foetida* L. (voucher No. Jg24/2006), and seeds of *Ar. catechu* L. (voucher No. Jg7/2006) were collected from the market and field of district Jhang, Punjab, Pakistan. Identification

of the plants was done by Prof. Dr. Abdul Wahid from the Department of Botany, University of Agriculture, Faisalabad, Pakistan.

Extraction and fractionation

Extraction

The leaves of *A. donax* L., latex of *F. assa-foetida* L. and seeds of *Ar. catechu* L. were dried at the room temperature. The dried specimens were ground to powder with an electric grinder. The powder was soaked for 3 days in an aqueous-methanol 30:70 suspension for the preparation of crude aqueous-methanol extracts (CAME) and filtered through muslin cloth and filter paper. The whole procedure was repeated thrice and then by the use of rotary evaporator at 40°C and low pressure, the combined filtrate was evaporated to get CAME (Tabassam et al., 2008).

Fractionation

Organic solvents, including ethyl acetate, chloroform, and petroleum spirit, were used to fractionate the crude extracts as described (Williamson et al., 1998). Briefly, after dissolving crude extract (20 g) in the distilled water (20 mL), and petroleum spirit (60 mL) in a funnel, was shaken vigorously. To separate the layers of petroleum spirit and the distilled water, the funnel was kept undisturbed for about 30 min. Another 60 mL petroleum spirit was added and the procedure was repeated until a clear petroleum spirit layer was obtained. All separated petroleum spirit layers were pooled and evaporated in a rotary evaporator. Fractions of chloroform and ethyl acetate were obtained by adopting the same procedure.

Parasitological procedures

Procurement of adult *Haemonchus contortus*

Following the methods of Alawa et al. (2003), adult *Haemonchus (H.) contortus* worms were obtained from the abomasal contents of slaughtered sheep. Each abomasum was separated from the small intestine and shifted to the Ethnoveterinary Research Center at the Department of Parasitology, University of Agriculture, Faisalabad, Pakistan and examined within 3 h of slaughter. The abomasum was split open and their contents washed gently with water in separate buckets and collected. The abomasum was divided longitudinally and the whole mucosa was examined carefully. The worms were collected gently from the infected abomasum with the help of forceps. Some of the worms were separated to use in adult motility assay; whereas, from the remaining worms, females were separated and crushed using a pestle and mortar to liberate the eggs to be used in egg hatch assays.

Adult Motility Assay (AMA)

CAME and fractions of chloroform, ethyl acetate and petroleum spirit at concentrations of 50, 25, 12.5, 6.25, 3.12 and 1.56 mg mL^{-1} in phosphate-buffered saline (PBS) along with levamisole (0.55 mg mL^{-1}; positive control) and PBS (negative control) were prepared. Adult live *Haemonchus contortus* were collected from abomasa of sheep procured from the local slaughter house. Minimum 10 worms were separated into individual Petri dishes and exposed to the above treatments. Each treatment was repeated thrice at the room temperature and the number of surviving and dead worms was observed at 0, 2, 4, 6, 8, 10, 12, and 24 hours post-treatment as described elsewhere (Lateef et al., 2003; Zaman et al., 2012).

Egg Hatch Assay (EHA)

Eggs were separated from female *H. contortus* by triturating them in a pestle and mortar. About 250 eggs in 1.5 mL water were placed in each well of a 24-well plate. The CAME and fractions of chloroform, ethyl acetate and petroleum spirit along with albendazole (positive control) and PBS (negative control) were used at 1.2, 12, 120, 1200 and 12000 µg mL^{-1}. The plate was incubated at 28°C for 36 hr. After incubation, eggs and larvae (dead or alive) were counted using an inverted microscope (Coles et al., 1992; Zaman et al., 2012). Each treatment was repeated in triplicate.

Faecal Egg Count Reduction Test (FECRT)

Naturally *H. contortus*- infected sheep (4-8 months-old) were selected for FECRT. The study was conducted at a local farm of Faisalabad district, Punjab, Pakistan. Animals were screened through the "Modified McMaster test". This study was approved by Research Ethics Committee, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan. Standard guidelines for the institutional animal care and use (IACU), University of Agriculture, Faisalabad, Pakistan were followed. Sheep were divided into 20 groups of 5 animals each through a complete randomized design. Groups 1 and 2 were treated with PBS (negative control) and levamisole (positive control) at 7.5 mg kg^{-1} body weight, respectively. Groups 3-5 were treated with the crude powder (CP) of *A. donax* at 1, 4, and 7 g kg^{-1} body weight and groups 6-8 were treated with the CAME of *A. donax* at 1 g, 4 g, 7 g kg^{-1} body weight, respectively. Groups 9-11 were treated with the CP of *F. assa-foetida* at 0.33, 0.66, and 1 g kg^{-1} body weight, respectively and groups 12-14 were treated with the CAME of *F. assa-foetida* at 0.33, 0.66, and 1 g kg^{-1} body weight, respectively. Similarly, groups 15-17 were treated with the CP of *Ar. catechu* at 0.33, 0.66, and 1 g kg^{-1} body weight, respectively, and groups 18-20 were treated with the CAME of *Ar. catechu* at 0.33, 0.66, and 1 g kg^{-1} body weight, respectively. Each treatment was given once *per os* and faecal egg counts were done on days 0, 4, 8, and 12 post-treatment.

Statistical analyses

A characteristic sigmoid dose-response curve was transformed into a linear function (to get a constant or fixed rate of hatching with the increase concentration of different fractions) via the probit transformation of the egg hatch test (EHT) data. Probit analysis was used to calculate the lethal concentration 50 (LC_{50}) of each extract for the EHT (Hubert & Kerboeuf, 1992). The Regression analysis was used to determine the effect of independent variables(concentration level) on the dependent variable (hatching) as described by Zaman et al. (2012). The adult motility assay (AMA) data were analyzed by the Duncan's multiple range (DMR) test to compare means of the number of dead worms. For FECRT, the results were expressed as eggs per gram (Mean±SEM) of faeces and means were compared by using DMR Test. All statistical analyses were carried out using SAS software (SAS, 1998).

Results

In vitro anthelmintic activity

In the AMA, 100% mortality (at 10^{th} hr post-exposure) of *H. contortus* was noted with the CAME and ethyl acetate fractions of *F. assa-foetida* at 12.5-50 mg mL^{-1}. The ethyl acetate fraction of *A. donax* showed maximum efficacy, followed in order by the fractions of chloroform, CAME and petroleum spirit. Among different fractions of *Ar. Catechu*, CAME showed the maximum mortality, followed in order by the fractions of ethyl acetate, chloroform and petroleum spirit. Levamisole caused 100% worm mortality within 2 hr post-exposure. The results of the AMA for the various treatments were observed as given in Table 1.

In the EHA, the CAME of *F. assa-foetida* showed the maximum ovicidal activity (LC_{50} =16.9 µg mL^{-1}), followed in order by those of *Ar. catechu* and *A. donax*. The chloroform fractions of *F. assa-foetida* and *A. donax* showed more ovicidal activity than that of *Ar. catechu*. Both the regression values and their correlations regarding the effects of the treatments on egg hatching indicated that a better concentration-dependent effect was found with the CAME of *F. assa-foetida* compared to those of *Ar. catechu* and *A. donax*. The concentration-dependent effects of petroleum spirit fractions were observed as given in Figure 1.

In vivo anthelmintic activity

Both the crude powder (CP) and crude aqueous methanol extracts (CAME) of the tested plants showed dose-dependent, variable levels of anthelmintic activity (Table 2). The anthelmintic efficacy of the CP of the plants ranged from 5.4% to 50.5%. The highest reduction (50.5%) in faecal egg count was recorded for *A. donax* at 7 g kg^{-1} than for *F. assa-foetida* at 1 g kg^{-1}. The anthelmintic efficacies of the CAME preparations were not consistent with those recorded for the CP. The effects of all plant treatments (12 days post-treatment) were statistically different from untreated controls ($P<0.05$). Differences in eggs per gram (EPG) values of the levamisole-treated group from those of plant extract-treated groups were statistically significant ($P<0.05$). However, differences in EPG values of the groups treated with different doses of the plant extracts were not statistically significant ($P>0.05$) on days 8 and 12 post-treatment, treated with CP of *F. assa-foetida* and *Ar. catechu*.

Table 1. Comparative efficacies of the crude aqueous methanolic extract and different fractions of *Arundo donax* (leaves), *Ferula assa-foetida* (latex) and *Areca catechu* (seeds) on the survival of *Haemonchus contortus* worms.

Treatments (in mg mL^{-1})	*Arundo donax* (leaves)				*Ferula assa-foetida* (latex)				*Areca catechu* (seeds)			
	Hours post-exposure				Hours post-exposure				Hours post-exposure			
	0	2	6	10	0	2	6	10	0	2	6	10
Levamisole 0.5	0.00^{r}	10.00±0.00^{a}	10.00±0.00^{a}	10.00±0.00^{a}	0.00^{k}	10.00±0.00^{a}	10.00±0.00^{a}	10.00±0.00^{a}	0.00^{k}	10.00±0.00^{a}	10.00±0.00^{a}	10.00±0.00^{a}
PBS	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{r}	0.00^{k}	0.00^{k}	0.00^{k}	0.00^{k}	0.00^{k}	0.00^{k}	0.00^{k}	0.00^{k}
Crude Aqueous Methanol Extract												
1.56	0.00^{l}	0.00^{l}	0.00^{l}	0.33±0.33^{kl}	0.00^{n}	0.00^{n}	1.00±0.00^{mn}	7.33±0.33^{de}	0.00^{m}	0.00^{m}	0.00^{m}	1.33±0.33^{jkl}
3.12	0.00^{l}	0.00^{l}	0.00^{l}	0.67±0.33^{ikl}	0.00^{n}	0.00^{n}	1.67±0.33^{klm}	7.00±0.58^{de}	0.00^{m}	0.00^{m}	0.33±0.33^{lm}	1.67±0.33^{jk}
6.25	0.00^{l}	0.00^{l}	0.33±0.33^{kl}	1.33±0.33^{ij}	0.00^{n}	0.00^{n}	2.33±0.33^{jkl}	8.67±0.33^{abc}	0.00^{m}	0.00^{m}	1.67±0.33^{jk}	3.00±0.58^{hi}
12.5	0.00^{l}	0.00^{l}	1.00±0.00^{ijk}	2.67±0.33^{fg}	0.00^{n}	0.67±0.33^{mn}	4.33±0.88^{gh}	10.00±0.00^{a}	0.00^{m}	0.67±0.33^{klm}	2.33±0.33^{ij}	5.67±0.67^{ef}
25	0.00^{l}	0.00^{l}	2.33±0.33^{gh}	4.33±0.33^{e}	0.00^{n}	3.33±0.33h^{ij}	6.33±0.88^{ef}	10.00±0.00^{a}	0.00^{m}	2.00±0.58^{ij}	5.00±1.16^{fg}	7.67±0.88^{cd}
50	0.00^{l}	0.67±0.33 ^{jkl}	3.33±0.33^{f}	5.67±0.33^{c}	0.00^{n}	5.67±0.33^{f}	8.00±0.58^{bcd}	10.00±0.00^{a}	0.00^{m}	4.00±0.58^{gh}	7.33±0.33^{cd}	9.00±0.58^{ab}
Chloroform fractions												
1.56	0.00^{o}	0.00^{o}	0.00^{o}	1.00±0.00^{i-o}	0.00^{p}	0.00^{p}	1.00±0.58^{nop}	2.67±0.33^{jm}	0.00^{m}	0.00^{m}	0.00^{m}	0.67±0.33^{klm}
3.12	0.00^{o}	0.00^{o}	0.67±0.33^{mno}	1.33±0.33^{kn}	0.00^{p}	0.00^{p}	2.00±0.58^{lmn}	3.67±0.33^{hij}	0.00^{m}	0.00^{m}	0.67±0.67^{klm}	1.67±0.33^{hk}
6.25	0.00^{o}	0.33±0.33^{no}	1.33±0.33^{kn}	2.33±0.33^{hk}	0.00^{p}	0.67±0.33^{op}	2.33±0.33^{klm}	5.00±0.58^{efg}	0.00^{m}	0.00^{m}	1.00±0.00^{jm}	2.33±0.33^{fi}
12.5	0.00^{o}	0.67±0.67^{mno}	2.33±0.33^{hk}	3.67±0.33^{dg}	0.00^{p}	1.67±0.33^{mno}	4.00±0.58^{ghi}	7.00±0.58^{c}	0.00^{m}	0.00^{m}	2.33±0.33^{fi}	3.00±0.00^{efg}
25	0.00^{o}	1.33±0.33^{kn}	3.00±0.00^{fi}	4.33±0.67^{cde}	0.00^{p}	2.33±0.33^{klm}	5.00±0.58^{efg}	9.67±0.33^{a}	0.00^{m}	0.00^{m}	2.67±0.67^{eh}	4.33±0.33^{cd}
50	0.00^{o}	2.33±0.67^{hk}	4.67±0.33^{cd}	6.67±0.67^{b}	0.00^{p}	4.33±0.88^{fgh}	7.00±0.58^{c}	10.00±0.00^{a}	0.00^{m}	0.33±0.33^{lm}	3.33±0.88^{ef}	5.33±0.67^{bc}
Ethyle acetate fractions												
1.56	0.00^{o}	0.00^{o}	0.00^{o}	0.67±0.33^{mno}	0.00^{m}	0.00^{m}	1.67±0.33^{jk}	4.00±0.58^{fg}	0.00^{o}	1.00±0.00^{lo}	0.00^{o}	1.00±0.00^{lo}
3.12	0.00^{o}	0.00^{o}	0.00^{o}	1.33±0.33^{lo}	0.00^{m}	0.33±0.33^{lm}	2.33±0.33^{ij}	5.33±0.33^{d}	0.67±0.33^{mno}	1.67±0.33^{klm}	0.67±0.33^{mno}	1.67±0.33^{klm}
6.25	0.00^{o}	0.00^{o}	1.00±0.58^{mno}	2.67±0.33^{hk}	0.00^{m}	1.33±0.33^{jk}	3.67±0.67^{fgh}	8.00±0.58^{b}	1.33±0.33^{kn}	2.33±0.33^{ijk}	1.33±0.33^{kn}	2.33±0.33^{ijk}
12.5	0.00^{o}	0.67±0.33^{mno}	2.33±0.33^{il}	3.33±0.67^{fi}	0.00^{m}	1.67±0.33^{jk}	5.00±0.58^{de}	10.00±0.00^{a}	2.33±0.33^{ijk}	3.67±0.33^{fgh}	2.33±0.333^{ijk}	3.67±0.33^{fgh}
25	0.00^{o}	1.00±0.58^{mno}	3.33±0.88^{fi}	4.33±0.67^{ef}	0.00^{m}	2.33±0.33^{ij}	6.67±0.33^{c}	10.00±0.00^{a}	3.67±0.67^{fgh}	5.00±0.58^{de}	3.67±0.667^{fgh}	5.00±0.58^{de}
50	0.00^{o}	3.00±1.16^{gj}	5.00±0.57^{de}	7.00±0.58^{bc}	0.00^{m}	3.33±0.33^{gh}	8.00±0.58^{b}	10.00±0.00^{a}	4.67±1.20^{def}	6.67±0.88^{bc}	4.67±1.202^{def}	6.67±0.88^{bc}
Petroleum spirit fractions												
1.56	0.00^{l}	0.00^{l}	0.00^{l}	0.67±0.33^{jkl}	0.00^{m}	0.00^{m}	0.00^{m}	1.67±0.33^{hk}	0.00^{j}	0.00^{j}	0.00^{j}	0.00^{j}
3.12	0.00^{l}	0.00^{l}	0.00^{l}	1.00±0.00^{ijk}	0.00^{m}	0.00^{m}	0.67±0.33^{klm}	2.00±0.58^{gj}	0.00^{j}	0.00^{j}	0.00^{j}	0.33±0.33^{ij}
6.25	0.00^{l}	0.00^{l}	0.33±0.33^{kl}	1.33±0.33^{hij}	0.00^{m}	0.33±0.33^{lm}	1.67±0.33^{hk}	2.67±0.33^{fgh}	0.00^{j}	0.00^{j}	0.00^{j}	0.67±0.33^{hij}
12.5	0.00^{l}	0.00^{l}	0.67±0.33^{jkl}	1.67±0.33^{ghi}	0.00^{m}	1.00±0.58^{jm}	2.33±0.88^{fi}	4.33±0.33^{cd}	0.00^{j}	0.00^{j}	0.33±0.33^{ij}	1.33±0.33^{fgh}
25	0.00^{l}	0.67±0.67 ^{jkl}	1.33±0.33^{hij}	2.33±0.33^{efg}	0.00^{m}	1.33±0.33^{il}	3.00±0.58^{fg}	4.67±0.88^{cd}	0.00^{j}	0.00^{j}	0.67±0.33^{hij}	2.00±0.00^{e}
50	0.00^{l}	1.00±0.58 ^{ijk}	2.33±0.33^{efg}	4.00±0.58^{bc}	0.00^{m}	2.33±0.67^{fi}	4.67±0.33^{cd}	6.33±0.33^{b}	0.00^{j}	0.00^{j}	2.00±0.58^{ef}	3.00±0.58^{c}

Number of dead worms (Mean±SE), Values having common alphabet superscripts in the columns indicate insignificant results at 95% confidence interval.

Table 2. Faecal egg count reduction (Mean±SEM) in naturally infected sheep population with gastrointestinal parasites through crude powder and crude aqueous methanol extracts of the leaves of *Arundo donax*, latex of *Ferula assa-foetida* and seeds of *Areca catechu* in comparison with Levamisole

Days PT	Crude Powder			Crude Aqueous Methanol Extract			Untreated	Levamisole
	Ferula assa-foetida						-	-
	0. 33 g/kg	**0.66 g/kg**	**1 g/kg**	**0. 33 g/kg**	**0.66 g/kg**	**1 g/kg**	**0 g/kg**	**7.5 mg/kg**
0	1570.3±73.5^{a}	1562.0±79.7^{a}	1528.7±87.5^{a}	1528.7±87.5^{a}	1495.3±83.5^{a}	1487.0±89.5^{a}	1587.0±70.4^{a}	1587.0±70.4^{a}
4	1400.0±51.6^{ab} (10.16)	1341.7±58.3^{b} (13.44)	1225.0±70.4^{b} (19.23)	1316.7±60.1^{b} (13.19)	1158.3±47.3^{b} (21.91)	950.0±51.6^{b} (35.59)	1641.7±67.6^{a} (-4.23)	58.3±15.4^{b} (96.30)
8	1258.3±52.3^{bc} (19.25)	1175.0±55.9^{bc} (24.19)	991.7±98.7^{bc} (34.61)	1125.0±52.8^{c} (25.83)	566.7±27.9^{c} (61.79)	316.7±16.7^{c} (78.53)	1758.3±74.6^{a} (-11.64)	8.3±8.3^{b} (99.47)
12	1183.3±60.1^{c} (24.06)	1100.0±50.0^{c} (29.03)	891.7±113.6^{c} (41.21)	908.3±53.9^{d} (40.11)	458.3±23.9^{c} (69.10)	183.3±16.7^{c} (87.57)	1825.0±79.3^{a} (-15.87)	0.0±0.0^{b} (100.0)
	Areca catechu							
0	2470.3±77.0^{a}	2445.3±84.5^{a}	2303.7±93.8^{a}	2395.3±92.1^{a}	2387.0±88.4^{a}	2362.0±86.9^{a}	2478.7±78.4^{a}	2479.0±86.6^{a}
4	2325.0±72.7^{ab} (5.42)	2275.0±82.4^{ab} (6.51)	2116.7±85.3^{b} (11.50)	1716.7±65.4^{b} (27.97)	1483.3±55.8^{b} (37.55)	925.0±38.2^{b} (60.64)	2516.7±95.4^{a} (-2.03)	66.7±10.5^{b} (97.31)
8	2225.0±61.6^{bc} (9.49)	2133.3±71.5^{bc} (12.33)	1891.7±82.1^{bc} (20.91)	1591.7±61.1^{b} (33.21)	1308.3±41.7^{b} (44.91)	625.0±21.4^{c} (73.40)	2616.7±118.8^{a} (-6.08)	0.0±0.0^{b} (100.0)
12	2083.3±64.1^{c} (15.25)	2016.7±71.5^{c} (17.12)	1683.3±70.3^{c} (29.62)	1350.0±57.7^{c} (43.36)	1066.7±44.1^{c} (55.09)	425.0±21.4^{d} (81.91)	2566.7±128.2^{a} (-4.05)	0.0±0.0^{b} (100.0)
	Arundo donax						-	-
	1 g/kg	**4 g/kg**	**7 g/kg**	**1 g/kg**	**4 g/kg**	**7 g/kg**	**0 g/kg**	**7.5 mg/kg**
0	1710.3±84.3^{a}	1710.3±84.3^{a}	1695.3±78.4^{a}	1695.3±78.4^{a}	1695.3±78.4^{a}	1678.7±81.5^{a}	1753.7±78.0^{a}	1778.7±77.2^{a}
4	1591.7±75.7^{ab} (6.83)	1425.0±71.6^{b} (16.58)	1308.3±67.6^{b} (22.28)	1641.7±84.1^{a} (2.47)	1591.7±83.1^{a} (5.44)	1475.0±69.2^{ab} (11.50)	1800.0±82.7^{a} (-3.35)	50.0±0.0^{b} (97.17)
8	1491.7±66.3^{ab} (12.68)	1208.3±45.5^{c} (29.27)	1091.7±45.5^{c} (35.15)	1600.0±76.4^{a} (4.95)	1508.3±83.1^{a} (10.40)	1308.3±63.8^{bc} (21.50)	1841.7±87.9^{a} (-5.74)	16.7±16.7^{b} (99.05)
12	1391.7±62.5^{b} (18.53)	1058.3±41.7^{c} (38.05)	833.3±38.0^{d} (50.50)	1533.3±79.2^{a} (8.91)	1400.0±76.4^{a} (16.83)	1141.7±67.6^{c} (31.50)	1883.3±81.3^{a} (-8.13)	16.7±16.7^{b} (99.05)

Values in the parentheses indicate the percentage reduction of faecal egg count.

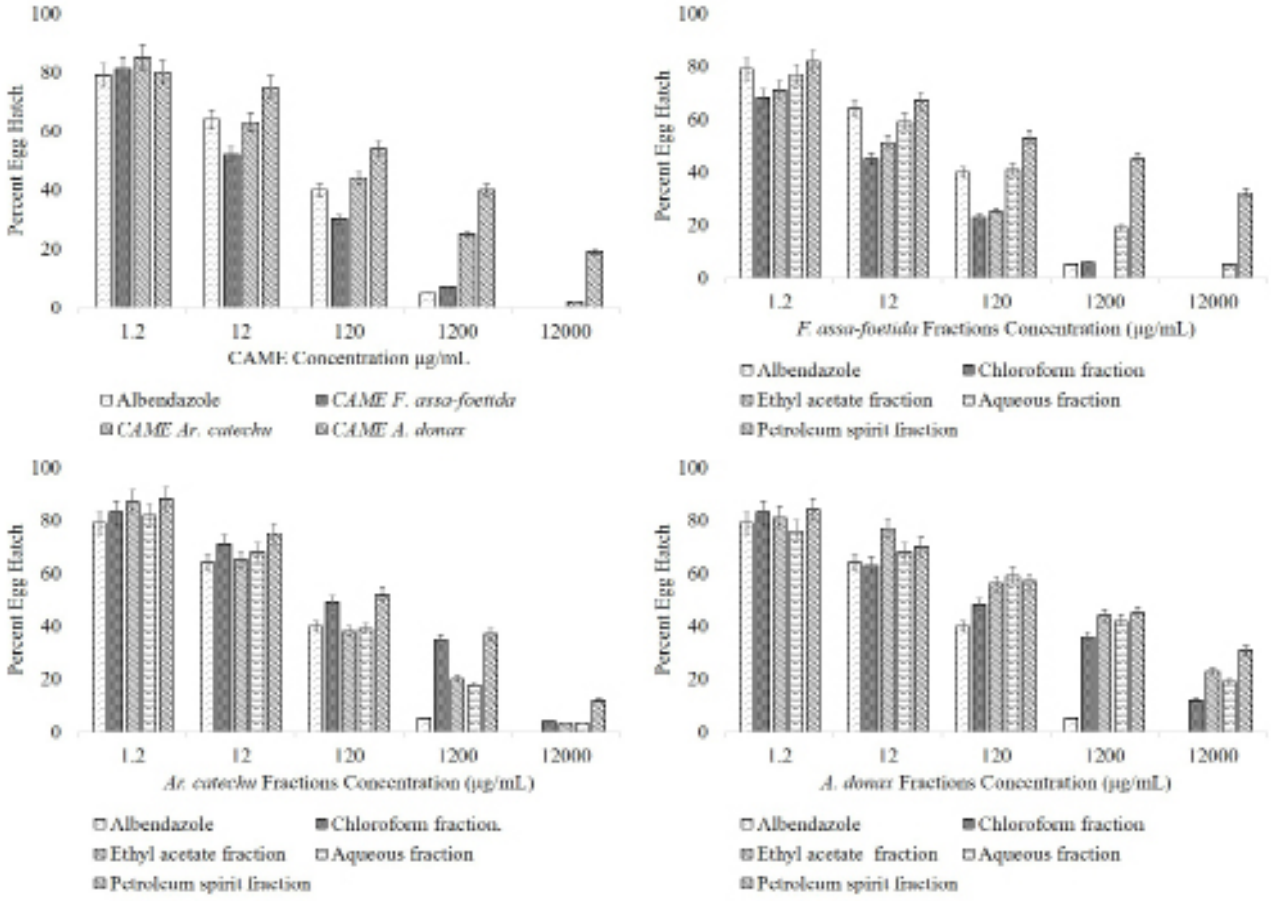

Figure 1. *In vitro* hatching (%) of *Haemonchus contortus* eggs through crude aqueous methanol extract (CAME) and fractions of *Ferula (F.) assa-foetida, Areca (Ar.) catechu* and *Arundo (A.) donax*.

Discussion

Parasitic infection is a key problem responsible for reduction in the productivity of animals, partly due to poor feed utilization (Pedreira et al., 2006). *H. contortus* is one of the principal parasitic nematodes of small ruminants, and the cause of huge economic losses (Radostits et al., 2000). The control of haemonchosis and other parasites relies mainly on the use of anthelmintics (Khan et al., 2017). The efficacy of these chemicals is threatened by the development and spread of resistance. However, success in the development of new drugs for parasitic infections is limited (Kaminsky et al., 2008; Kumarasingha et al., 2016). Searching for new, safe, and effective anthelmintic compounds from plants is a promising way to control parasitic infection (Waller et al., 2001; Kumarasingha et al., 2016). Nature has provided a variety of medicinal plants, e.g., *Trachyspermum ammi (L.) Sprague*, *Punica granatum* L., *Nicotiana tabacum* L., *Artemisia brevifolia*, *Allium sativum*, *Ferula asafoetida*, *Syzygium aromaticum* and *Withania coagulans* Dunal, to cure parasitic diseases. The chemical compounds present in these plants have different solubilities in various solvents (Iqbal et al., 2004; Kumar & Singh, 2014; Atma et al., 2017). Different solvents can dissolve different chemical compounds (solutes) that underlie biological activities (Iqbal et al., 2007; McGaw et al., 2007; Altemimi et al., 2017). Such studies will help to identify potential candidates for alternative control through plant-based, novel anthelmintics. In the present study, solvents were used to fractionate plant extracts in order to identify those with the highest activity against the *H. contortus* model.

Various fractions of *F. assa-foetida* have been isolated, including gum, resins, volatile oils, coumarin derivatives and various monoterpenes, ferulic acid, farnesiferoles, disulfides, symmetric trisulfides and tetrasulfides (Hofer et al., 1984; Kajimoto et al., 1989; Kapoor, 1990). The resin of *F. assa-foetida* has various properties, such as anticoagulant, smooth muscle relaxant, anti-diabetic, anticarcinogenic, antioxidant, antispasmodic, antihepatotoxic, antiulcerogenic, anticholesterolemic, anti-inflammatory, antifertility, antifungal, antiparasitic and anthelmintic (Kareparamban et al., 2012, Amalraj & Gopi, 2016). The gum extract of *F. assa-foetida* has been used to cure diarrhea, constipation, abdominal pain, and parasitic infections (Fernch, 1971). Inhibition of the growth of *Shigella sonnei* and

Staphylococcus aureus has also been reported by the gum extract of *F. assa-foetida* (Kapoor, 1990). Gundamaraju (2013) reported significant anthelmintic activity of *F. assa-foetida* at a concentration of 100 mg mL^{-1}. Paralysis, as well as lethality, of an aqueous extract of *F. assa-foetida* was comparable to piperazine citrate at a concentration of 100 mg mL^{-1}. Polyphenolic compounds and flavonoids are the main phytochemical components of crude extracts. Polyphenolic compounds such as tannins have been documented as anthelmintics (Bate-Smith, 1962; Iqbal et al., 2007). Kumar & Singh (2014) reported the LC_{50} of an ethanolic extract of the dried-root latex powder of *F. assa-foetida* (4.88 mg mL^{-1}) followed in order by the dried clove powder of *A. sativum* (3.48 mg mL^{-1}), and dried flower bud powder of *Syzygium aromaticum* (2.95 mg mL^{-1}) against *Fasciola gigantica*. The possible anthelmintic effects of *F. assa-foetida* may be due to interference with energy generation in parasites by uncoupling oxidative phosphorylation or due to presence of tannins in the extracts that can bind to glycoprotein on the cuticle of the parasite and cause death (Mali & Wadekar, 2008).

Areca catechu nuts were used as anthelmintics and appear in the British pharmacopoeia for the treatment of parasitic infections (Raghavan & Baruah, 1958). The nuts of *Ar. catechu* contain tannins, gallic acid, oil gum, volatile oils, lignins (Huang, 1992), alkaloids, phenolic compounds, beta-sitosterol, catechins, amino acids and various saline substances (Duke, 1992; Wang et al., 1997; Chu, 2001). An ethanolic extract of *Ar. catechu* significantly inhibited fumarate reductase and succinate dehydrogenase activities in *Cotylophoron cotylophorum* (Dhanraj & Veerakumari, 2016). The combined efficacy of *Ar. catechu* and *Anredera (An.) cordifolia* showed significant elimination of *Ascaridia galli* (60%) in chickens and reduced EPG of faeces. However, in a group treated with alkaloids and saponins contained in *Ar. catechu* and *An. cordifolia* showed antagonistic activity (Prastowo et al., 2017). Baby & Raphael (2014) reported dose-dependent anthelmintic activity of ethanolic extract of *Ar. catechu* root against *Pheretima posthuma*. The results of the study conducted by Yamson et al. (2019) revealed that there was no movement of *Fasciola* sp. when exposed to 40% ethanol extract *Ar. catechu*. Most of the pharmacological and biological effects, such as inhibition of acetylcholinesterase, stimulation of the relaxed bowel and cholinomimetic effects of *Ar. catechu*, are due to the condensed tannins (Kapoor, 1990; Amudhan et al., 2012). Direct and/or indirect pharmacological effects of the condensed tannins on the GI parasites have been reported (i.e., Butter et al., 2000; Molan et al., 2000; Iqbal et al., 2007; Naumann et al., 2017). Condensed tannins can interfere with physiological functioning of parasites, development of infective larval stages and hatching of parasite eggs (Molan et al., 2000).

In Ayurvedic medicine, a decoction of *A. donax* was used as haemostatic, in toothache, as a diuretic and as an emollient (Passalacqua et al., 2007). It is also used to diminish the secretion of milk and to stimulate menstrual discharge (Chopra et al., 1956). It is a potential biomass plant with an invasive potential in riparian areas (Nackley & Kim, 2015; Al-Snafi, 2015) and is also used for phytoremediation in Ni-contaminated soils (Atma et al., 2017). Five indole-3-alkylamine bases (5-methoxy-N-methyl-tryptamine, bufotenidine, bufotenine, N-dimethyltryptamine, and dehydrobufotenine) have been isolated from rhizomes of *A. donax* (Ghosal et al., 1969). Antispasmodic and hypotensive effects against serotonin, acetylcholine, and histamine-induced spasms by a defatted, ethanolic extract of *A. donax* rhizomes have been reported (Al-Snafi, 2018). In a study conducted by Sharatkumar et al. (2004), 25-50 mg mL^{-1} *A. donax* showed significant anthelmintic effects. The highest mortality of *H. contortus* after levamisole (used as a reference drug) was 56.7%, with the CAME of *A. donax* at the concentration of 50 mg mL^{-1} at 10 hr post-exposure. Among various fractions of *A. donax* against *H. contortus,* ethyl acetate was the most active, followed in order by chloroform, aqueous and petroleum spirits extracts. In a FECRT, 50.5% reduction of eggs was recorded by the CP of *A. donax* in naturally infected sheep. However, *A. donax* exhibited ovicidal activity, with LC_{50} of 200.1 µg mL^{-1}. Around 55% efficacy of *A. donax* extracts was recorded against various GI parasites of cattle (Miles et al., 1993).

In conclusions, the preparations of *F. assa-foetida* were toxic to *H. contortus in vitro*. In the AMA and EHA, CAME showed maximum mortality and ovicidal activity. However, the ethyl acetate fraction of *A. donax* showed maximum efficacy against adult worms, while chloroform fractions of *F. assa-foetida* and *A. donax* showed maximum ovicidal activity. *In vivo* efficacy of CP and CAME in this study ranged from 5.4% to 50.5%. The higher reduction (50.5%) in faecal egg count was recorded for *A. donax* at the dose rate of 7 g kg^{-1}. Differences in the values of EPG between the levamisole-treated group and those treated with plant extracts were statistically significant ($P<0.05$).

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