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In vitro anthelmintic efficacy of *Citrullus colocynthis (L.) Schrad* on *Haemonchus contortus*

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ABSTRACT

Ethno-veterinary medicinal studies associated with traditional uses of the flora of the Cholistan desert have shown that fruits of *Citrullus colocynthis* are used for the treatment of helminth infections. The present research was designed to evaluate the anthelmintic efficacy of *C. colocynthis* against *H. contortus*. The *in vitro* anthelmintic effects of aqueous-methanol and ethyl acetate fruit extracts of *C. colocynthis* against *H. contortus* were determined through egg hatch and adult motility assays. The effect of four serial dilutions of 25 mg/mL of each extract compared to levamisol (0.55 mg/mL) and oxfendazole (three serial dilutions of 25 µg/mL) were studied. Both ethyl acetate and aqueous-methanol extracts paralyzed all adult worms 4h and 8h post-exposure at a dose of 25 mg/mL each. In the egg hatch assay, about 83.67% and 80.67% of *H. contortus* eggs failed to hatch with the same dose (*i.e.* 25 mg/mL) of ethyl acetate and CAME extracts, respectively. The results of the present study strongly support fruit extracts of *C. colocynthis* as a promising alternative to synthetic drugs against *H. contortus*. These findings will lead to further *in vivo* studies to investigate the bio-availability of the active ingredients of the plant and the minimum non-lethal concentration required for treatment of haemonchosis in livestock. The anthelmintic effects of *C. colocynthis* might be attributed to the presence of phenolic acids.

Key words: anthelmintic efficacy; *C. colocynthis*; *H. contortus*; egg hatch assay; adult motility assay

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Introduction

Livestock production is a foremost earning source for the agricultural sustainability of poor farmers in rural areas, especially when crop production is not a profitable source of income (KHAJURIA et al., 2013; AHMED et al., 2020). However, parasitism has always caused major problems in achieving maximum output from livestock production systems (MEHMOOD et al., 2017; IJAZ et al., 2018; NASIR et al., 2018; ZAFAR et al., 2019; AHMAD et al., 2019; BATOOL et al., 2019; LI et al., 2019a, 2019b; KHATER et al., 2020; LI et al., 2020). *Haemonchus (H.) contortus,* the causative agent of haemonchosis, is one of the major hindrances to small ruminant production (BIBI et al., 2017), and causes an estimated loss of about ten billion dollars annually to the veterinary market (ROEBER et al., 2013). A single parasite sucks approximately 0.05 mLblood daily (ALIM et al., 2016) and consequently severely damages the gastrointestinal mucosa (SINGH et al., 2015). Continuous blood loss due to *H. contortus* causes anemia, anorexia, edema, diarrhea, hypo-proteinemia, and emaciation, that ultimately lead to the death of the animal (GITHIGIA et al., 2001). Severe infection inflicts a substantial impact on milk, meat and wool production, reduces weight gain by 23-63%, and in 25% cases death occurs before weaning (SINGH et al., 2015).

Anthelmintic treatment is a leading prophylactic application used against parasitic infection (CARVALHO et al., 2012). However, frequent and prolonged use with undiscriminating administration and improper formulations of synthetic drugs has regrettably led to an upsurge in resistance in the parasites against these salts (DEVI et al., 2014). As a result, *H. contortus* is resistant to all broad range anthelmintic families such as benzimidazole, ivermectin and imidazothiazole and is hence becoming an irrefutable problem (DEVI et al., 2014). Resistant parasites become more pathogenic, prolific and acquire increased adaptability and survivability in all their free-living phases in the host (SANYAL et al., 2003). Apart from resistance against anthelmintics, toxic residues, the high cost of production and the unavailability and inaccessibility of these synthetic drugs, especially in remote rural areas, have spurred investigations to explore alternative methods (QADIR et al., 2010). Among the alternatives, botanicals have been very effective against a wide range of parasites (ABBAS et al., 2017a, 2017b, 2018, 2019; HUSSAIN et al., 2017; IDRIS et al., 2017; ZAMAN et al., 2017; KHATER et al., 2018; FAYAZ et al., 2019). Plants have many bioactive compounds that can easily kill parasites through multiple mechanisms, and ultimately reduce the chances of the development of anthelmintic resistance. Plants are not only eco-friendly and easily biodegradable, but also their bioactive compounds have less chance of bio-accumulation in animal tissues and the surrounding environment (DELFIN et al., 2017).

More reliable and inexpensive herbal medicines are being developed for rapid detection of their active ingredients against the different phases of *H. contortus*. Amongst these, *Citrullus (C.) colocynthis,* from the Cucurbitaceae family, is highly xerophytic with large perennial roots, triangular leaves, ovoid brown seeds, and globular green fruit containing white pulp and monoecious flowers (PRESTON et al., 2015). The anthelmintic activity of the fruit of *C. colocynthis* (Voucher # Ch-03) has been previously documented by conducting a survey (July 2010-January 2011) among local respondents in the Cholistan desert, using closed and open ended questionnaires. About 86 plant-based remedies were recorded, of which the helminthiasis treatment was found to be most frequent. Local shepherds use dry fruit powder from *C. colocynthis* to treat helminthiasis (RAZA et al., 2014). In the current study, the anthelmintic efficacy of fruit extracts of *C. colocynthis* was investigated against the most pathogenic and prevalent *H. contortus*.

Materials and methods

Collection of plant fruit. Fruit from *C. colocynthis* was collected during the month of February, 2017. The plants were identified by a botanist from the Cholistan Institute of Desert Studies, and voucher specimen was deposited in the herbarium of the Cholistan Institute of Desert Studies (CIDSHB54). The collected fruit was washed, dried in shade, pulverized into a coarse powder using a mechanical grinder, and later stored at 4°C in air-tight bottles.

Reagents. All analytical grade chemicals were purchased from Sigma (Sigma-Aldrich, St-Quentin Fallavier, France).

Preparation of extracts in aqueous-methanol and ethyl-acetate. The preparation of fruit extracts were performed according to the methods previously adopted by IQBAL et al. (2012). For extract preparation in aqueous-methanol solution, fruit powder (50g) was mixed into sufficient amount of 70% aqueous-methanol solution for three days, with stirring three times per day for five minutes, and later filtered using porous cloth. The residual plant material was mixed again in aqueous-methanol, and the whole procedure was repeated twice. The three filtrates were then combined and the solvent was evaporated using a rotary evaporator at 40 °C under reduced pressure. Later evaporation of solvent was completed in a water bath at 65 °C. Finally, crude aqueous methanolic extract (CAME) was stored at 4 °C in the form of a paste.

The procedure was repeated similarly to prepare the extract in ethyl acetate. Thus, fruit powder (50g) was soaked in a sufficient amount of ethyl acetate and the further procedure was repeated three times as described above and finally, the paste was stored at 4 °C.

High performance liquid chromatography (HPLC) analysis for flavonoids and phenolic compounds. The hydrolysis of CAME was performed as described previously by DEK et al. (2011). Briefly, CAME (50 mg) was weighed and dissolved in 24 mL of methanol. After homogenization, 16 mL of distilled water and 10 mL of 6M HCl were added to the mixture in order. Then mixture was incubated for 2h at 95 °C. It was filtered through a 0.45 µm nylon membrane filter (Biotech, Germany) prior to HPLC analysis.

The separation of CAME by gradient HPLC (LC-10A, SHIMADZU, JAPAN) was performed using a shim-pack CLC-ODS (C118), 25 cm × 4.6 mm, 5 µm column. The chromatographic separation was carried out as mobile phase gradient: A (H2O: Acetic acid-94:6, pH = 2.27), B (acetonitrile 100%). The gradient used was 15% solvent B (0-15 min), 45% solvent B (15-30 min) and 100% solvent B (35-45 min) with 1 mL/min flow rate. A UV- visible detector (λ max 280 nm) was used for separation of flavonoids and phenolic acids.

Determination of anthelmintic activity

Adult motility assay. Adult motility assay was performed following the methodology of IQBAL et al. (2012) with modifications. About ten mature adult worms of the same size were collected from the abomasum of freshly slaughtered sheep, washed in PBS and transferred to separate petri dishes containing different concentrations of one of the plant extracts. Two-fold serial dilutions of both extracts were prepared from a 25 mg/mL concentration of stock solutions by mixing in PBS. Three replications of each concentration and of each control group were carried out as follows:

Aqueous-methanol extract: 25, 12.5, 6.25, 3.12 mg/mL
Ethyl acetate extract: 25, 12.5, 6.25, 3.12 mg/mL
Levamisol: 0.55 mg/mL
PBS: 20 mL/petri plate

Motility was observed under an inverted microscope at intervals of 0, 2, 4, 6, 8, 10, 12 hours. The worms that did not show any motility in either the head or tail region were picked out and kept in lukewarm PBS for five minutes, and these worms were only counted as alive if their motility revived.

Egg hatch assay. Egg hatch assay was performed following the guidelines of the "World Association for the Advancement of Veterinary Parasitology" with modifications (ALAWA et al., 2003). To release the eggs, female worms were triturated in a mortar containing PBS. The mixture was filtered using a mesh sieve with 80 µm pores, and then the sieve was washed with PBS. The collected fluid was diluted to a concentration of 200 eggs/mL. Four doses of each plant extract and three concentrations of oxfendazole were used in triplicates in 24 multi-well plates as follows:

Aqueous-methanol extract: 25, 12.5, 6.25, 3.125 mg/mL
Ethyl acetate extract: 25, 12.5, 6.25, 3.125 mg/mL
Oxfendazole: 25, 12.5, 6.125 µg/mL
PBS: 1 mL/well

After incubation at 28 °C/48h unhatched eggs were counted under an inverted microscope. The percentage ratios of unhatched eggs were calculated by dividing the final number of unhatched eggs by the initial number of unhatched eggs.

Statistical analysis. The collected data on unhatched eggs was subjected to probit analysis, and data on the adult motility assay were analyzed using SPSS. $P<0.05$ was considered as the statistically significant level.

Results

Adulticidal effects. The adulticidal effect of ethyl acetate and aqueous methanol fruit extract of *C. colocynthis* is presented in Table 1. Ethyl acetate extract of *C. colocynthis,* even at lower concentrations (6.25 mg/mL and 3.125 mg/mL), revealed its maximum effect at the end of the time period, *i.e.* 12h. The dose of 25 mg/mL paralyzed all the worms in only 4h after the start of the experiment. The highest tested concentration (25 mg/mL) of aqueous-methanolic extract paralyzed all the worms 8h post-exposure, while the lower doses showed their extreme activity in about 10h after the start of the experiment. Therefore, the inhibitory potential of ethyl acetate extract was found to be greater than the aqueous-methanol extract. However, both extracts demonstrated a dose dependent effectiveness against the motility and mortality of the worms, ratifying their anthelmintic activity. Moreover, ethyl acetate extract exhibited comparable efficiency to Levamisole at higher dose, of 25 mg/mL. No death was recorded up until the end of the experiment (12h) by PBS, that was used as a negative control.

Ovicidal effects. Table 2 shows the ovicidal effect of *C. colocynthis* ethyl acetate and aqueous methanol fruit extract. Ethyl acetate extract of *C. colocynthis* exhibited maximum ovicidal efficacy (83.67%) at the highest tested dose of 25 mg/mL ($P<0.05$). Serially diluted concentrations of ethyl acetate extract inhibited egg hatching in a dose dependent manner. The highest dose of aqueous methanolic extract (25 mg/mL) inhibited the development of 80.67% eggs into larvae, while lower doses displayed a similar pattern of inhibition of egg hatching to the ethyl acetate extract - in a dose dependent manner. Ethyl acetate extract demonstrated slightly higher effectiveness than the aqueous-methanol extract.

Flavonoids and phenolic compounds. The data from the HPLC analysis of *C. Colocynthis* aqueous methanolic extract in the current study showed that the amount of phenolic acids (*m*-coumaric acid, gallic acid, vanillic acid) varied from 2.23 ± 0.01 to 10.48 ± 0.03 ppm, while the amount of flavonoids (quercetin) was 2.49 ± 0.02 ppm.

Table 1. *In vitro* anthelmintic efficacy of various *C. colocynthis* ethyl acetate extract concentrations against *H. contortus,* in comparison with Levamisol and PBS

	0 hour	2 hour	4 hour	6 hour	8 hour	10 hour	12 hour
Lev 0.55 mg/mL	0.00 ± 0.00^{i}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}
PBS	0.00 ± 0.00^{i}	0.00 ± 0.00^{i}	0.00 ± 0.00^{i}	0.00 ± 0.00^{i}	0.00 ± 0.00^{i}	0.00 ± 0.00^{i}	0.00 ± 0.00^{i}
3.125 mg/mL	0.00 ± 0.00^{i}	0.00 ± 0.00^{i}	1.67 ± 0.68^{g}	4.33 ± 0.33^{e}	7.00 ± 0.00^{c}	9.00 ± 0.00^{b}	10.00 ± 0.00^{a}
6.25 mg/mL	0.00 ± 0.00^{i}	0.00 ± 0.00^{i}	0.67 ± 0.33^{h}	3.33 ± 0.67^{ef}	6.00 ± 1.00^{cd}	9.00 ± 0.58^{b}	10.00 ± 0.00^{a}
12.5 mg/mL	0.00 ± 0.00^{i}	1.00 ± 0.58^{g}	4.00 ± 1.53^{e}	8.00 ± 1.00^{bc}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}
25 mg/mL	0.00 ± 0.00^{i}	9.67 ± 0.33^{ab}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}

Table 2. *In vitro* anthelmintic efficacy of various *C. colocynthis* aqueous-methanol extract concentrations against *H. contortus* in comparison with Levamisol and PBS

	0 hour	2 hour	4 hour	6 hour	8 hour	10 hour	12 hour
Lev 0.55 mg/mL	0.00 ± 0.00^{k}	5.67 ± 0.33^{f}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}
PBS	0.00 ± 0.00^{k}	0.00 ± 0.00^{k}	0.00 ± 0.00^{k}	0.00 ± 0.00^{k}	0.00 ± 0.00^{k}	0.00 ± 0.00^{k}	0.00 ± 0.00^{k}
3.125 mg/mL	0.00 ± 0.00^{k}	0.00 ± 0.00^{k}	3.00 ± 0.00^{h}	5.00 ± 0.00^{f}	8.33 ± 0.67^{bc}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}
6.25 mg/mL	0.00 ± 0.00^{k}	1.67 ± 0.33^{j}	3.67 ± 0.33^{g}	6.67 ± 0.33^{e}	8.33 ± 0.33^{bc}	9.67 ± 0.33^{ab}	10.00 ± 0.00^{a}
12.5 mg/mL	0.00 ± 0.00^{k}	2.00 ± 0.58^{j}	4.00 ± 0.58^{g}	7.67 ± 0.33^{cd}	9.00 ± 0.00^{b}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}
25 mg/mL	0.00 ± 0.00^{k}	2.67 ± 0.67^{hi}	5.67 ± 0.67^{fe}	8.33 ± 0.33^{bc}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}	10.00 ± 0.00^{a}

Table 3. The percentage of egg hatch inhibition by *C. colocynthis* ethyl acetate extract on *H. contortus* eggs

Products	Concentrations	Percentage inhibition of hatching
Oxfendazole (µg/mL)	25 µg/mL	77.67 ± 2.96
	12.5 µg/mL	59.33 ± 6.94
	6.25 µg/mL	46.67 ± 5.70
PBS	1 mL/well	37.00 ± 2.08
Ethyl acetate	25 mg/mL	83.67 ± 1.20
fruit extract (mg/mL)	12.5 mg/mL	77.30 ± 13.1
	6.25 mg/mL	73.00 ± 4.51
	3.125 mg/mL	58.00 ± 1.00

Table 4. The percentage of egg hatch inhibition by *C. colocynthis* aqueous-methanol extract on *H. contortus* eggs

Products	Concentrations	Percentage inhibition of hatching
Oxfendazole (µg/mL)	25 µg/mL	82.33 ± 2.73
	12.5 µg/mL	60.67 ± 3.53
	6.25 µg/mL	61.33 ± 8.37
PBS	1 mL/well	40.00 ± 2.65
Aqueous-methanol	25 mg/mL	80.67 ± 3.33
fruit extract (mg/mL)	12.5 mg/mL	75.00 ± 1.15
	6.25 mg/mL	69.00 ± 4.51
	3.125 mg/mL	57.00 ± 4.16

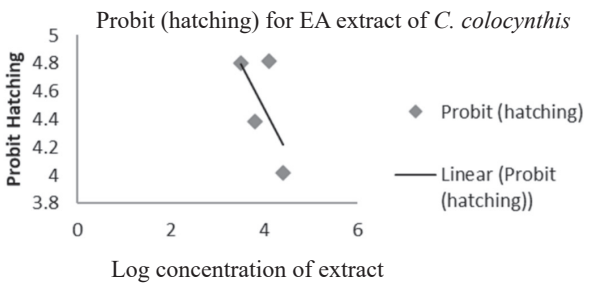

Fig. 1. Linear response of different *C. colocynthis* ethyl acetate extract concentrations on the hatching of *H. contortus* eggs.

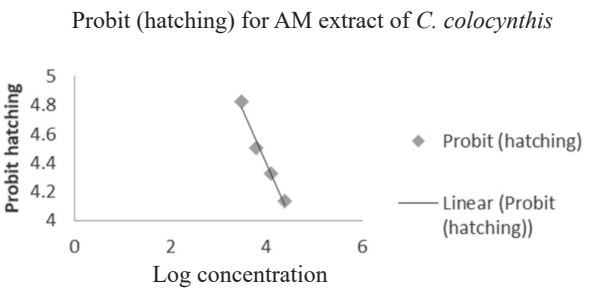

Fig. 2. Linear response of different *C. colocynthis* aqueous methanol extract concentrations on the hatching of *H. contortus* eggs

Discussion

Haemonchosis causes an estimated loss to veterinary market of about ten billion dollars annually, and sometimes exceeds the livestock income (ROEBER et al., 2013). Therefore, research and the potential use of novel anthelmintic plant medicines as a substitute for synthetic therapeutics, such as *C. colocynthis* fruit extracts, offers the possibility of cheaper, ecofriendly and easily accessible phyto-therapeutic products.

In vitro experimentation plays an imperative role in this context as it shows the direct interactive effect of plant materials on different stages of the life of parasites (FERRIERA et al., 2013). Inhibition of egg hatch assay (COLES et al., 1992), and adult (HOUNZANGBE-ADOTE et al., 2005) and larval (KOTZE et al., 2006) motility assays are usually used for testing the anthelmintic activity of natural drugs.

C. colocynthis has been extensively used by farmers as a traditional treatment against helminthiasis in Cholistan (RAZA et al., 2014). Its anthelmintic efficiency has also been previously explored against *Pheretima postumaas* (TALOLE et al., 2013), *H. contortus* (ULLAH et al., 2013) and *Orthocoelium scoliocoelium* (SWARANAKAR and KUMAWAT, 2014). Petroleum ether, ethanol and aqueous extract of *C. colocynthis* at 40 mg/mL inhibited the motility of *Pheretima posthumaas* after an average time of 10.14, 2.36 and 4.33 min respectively (TALOLE et al., 2013). A combination of aqueous-methanolic extract of *C. colocynthis* (fruit), *Curcuma longa* (rhizome) and *Peganum harmala* (seeds) at 100 mg/mL leads to the death of all *H. contortus* worms 4h post exposure (ULLAH et al., 2013). Furthermore, an alcoholic extract of fruit pulp of *C. colocynthis* at 40 mg/mL for 5h rendered complete inhibition of motility of *Orthocoelium scoliocoelium* parasites (SWARNAKAR and KUMAWAT, 2014). The combined synergistic action of aqueous-methanolic extract of three plants including *C. colocynthis* previously reported by ULLAH et al. (2013) against *H. contortus* was only against one of its life cycle stages (adult worms) at 100 mg/mL. However, in the current study, aqueous methanolic and ethyl acetate extracts of the plant inhibited worm motility at 25 mg/mL at 8h and 4h post-exposure, respectively. AHMED et al. (2019) investigated the effect of *C. colocynthis in vivo* against albendazole resistant *Haemonchus* in lambs. An extract of *C. colocynthis* at a dose of 200 mg/Kg body weight caused a 95.57% reduction in fecal egg count, while a dose of 50 mg/Kg caused a 55.07% reduction in fecal egg count. This difference may be due to the fact that AHMED et al. (2019) performed an *in vivo* experiment on albendazole resistant worms. AHMED et al. (2019) observed no untoward response to admiinistration of 200 mg/mL of *C. colosynthis* to lambs. However, they did not perform any proper study involving histological and biochemical tests to reveal any lethal effect at this dose. From these studies, it is conceivable that the ingredient absorption of plant extracts vary between different worms. The difference in anthelmintic activity of plants may be attributed to difference in solubility of solid materials of plants. Solubility is affected by dissimilarities in polarity of plant components and solvent (MALU et al., 2009)

Egg hatch assay was first designed for analysis of benzimidazole resistance in helminthes, and is now used for screening the anthelmintic efficiency of plants (IQBAL et al., 2012). In egg hatch assays, the active compounds of the plant extract pierce the egg shell, paralyze the first stage larvae, and thus inhibit egg hatching (PONE et al., 2011). Various investigations have explored the varying efficacy of different plants against the eggs of *H. contortus*. Aqueous and alcoholic extracts of *Foenicum vulgare* induced complete inhibition of hatching, while aqueous and alcoholic extracts of *Acokanthera schimperi* inhibited hatching of 53.6% and 87% *Haemonchus* eggs, respectively (GETACHEW et al., 2012). Similarly, an aqueous extract of *Saba senegalensis* at 15 mg/mL (BELEMLILGA et al., 2016), and ethanolic and aqueous extracts of *Moringa oleifera* at 15.6 mg/mL (DELFIN et al., 2017) inhibited hatching of 93.63%, 95.89% and 81.72% eggs of this parasite, respectively. Moreover, hatching of 46.6% eggs was inhibited with *Eucalyptus globulus* leaf methanolic extract, 6.4% with *Annona squamosa*, 51.2% with *Syzigium cumini*, 82.7% with *Catharanthus roseus* (KUAMR et al., 2015), 79.6% with methanolic and acetonic extract of *Khaya senegalensis* (CHINA et al., 2016)

and absolute inhibition with aqueous extracts of *Capparis spinosa* (AKKARI et al., 2016). PONE et al. (2011) described that the active components of plants inhibit egg hatching by paralyzing the first stage larvae inside the egg shell. Despite these numerous studies, the inhibitory effect of *C. colocynthis* on egg hatching of any helminth has not yet been investigated. Ethyl acetate and aqueous-methanol fruit extracts of *C. colocynthis* in the current study caused inhibition of hatching of more than 80% eggs and showed their effectiveness against the parasite. It is remarkable to note that most plant extracts showed a statistically significant anthelmintic effect against the different life-cycle stages of parasites and lower the chances of the development of parasitic resistance (HOUNZANGBE-ADOTE et al., 2005).

Besides their role in inhibition of egg hatching, these plant anthelmintics also affect various worm developmental stages. In this context, an aqueous extract of *Saba senegalensis* inhibited the motility of 97.77% *H. contortus* worms at 15.00 mg/mL (BELEMLILGA et al., 2016), an acetonic extract of *Khaya senegalensis* inhibited the motility of 75% *H. contortus* worms at 2400 µg/mL (CHINA et al., 2016), a herbal complex prepared from four herbs; *Cinnamomum verum*, *Capsicum annuum*, *Origanum vulgare* and *Rosmarinus officinalis* caused 100% *H. contortus* mortality at 100 mg/mL (ZAMAN et al., 2020), while a methanolic extract of the same plant caused inhibition of all the *H. contortus* worms even at a half the dose (1200 µg/mL) of the acetonic extract (CHINA et al., 2016).

The current study showed that the amount of phenolic acids (*m*-coumaric acid, gallic acid, vanillic acid) varied from 2.23 ± 0.01 to 10.48 ± 0.03 ppm, and the amount of flavonoids (quercetin) was found to be 2.49 ± 0.02 ppm. UMA and SEKAR, (2014) performed phytochemical screening of this plant, and labeled alkaloids and saponins as the active ingredients that might contribute to its anthelmintic activity.

Conclusion

It is concluded from the current project that bioactive compounds isolated from fruit extracts of *C. colocynthis* could be a strong novel substitute for commercial anthelmintic drugs for the control of this extremely prevalent nematode, *H. contortus*. For this purpose, safety and toxicity studies on *C. colocynthis* should be conducted *in vivo* to ascertain the minimum non-lethal concentration required for treatment of haemonchosis in livestock.

Statement of novelty

For the first time, the *in vitro* anthelmintic activity of aqueous-methanol fruit extracts of *C. colocynthis* against the eggs and adults of *H. contortus* has been studied; the promising alternative to synthetic drugs against this highly prolific parasite is strongly advocated.

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SAŽETAK

Istraživanja etno veterinarske medicine povezana sa tradicionalnom primjenom flore iz pustinje Cholistan pokazala su da u liječenju invazija uzrokovanih helmintima mogu biti uporabljeni plodovi *Citrullus colocynthis*. Ovo istraživanje je oblikovano s ciljem da se procjeni anthelmintička djelotvornost *C. colocynthis* u kontroli oblića *Haemonchus contortus*. Testovi izlijeganja iz jaja i pokretljivosti odraslih jedinki korišteni su za mjerenje antihelmintičkih učinaka vodene otopine metanola i etil acetate ekstrahiranih iz *C. colocynthis.* Analiziran je učinak četiri serijska razrjeđivanja od 25 mg/mL svakog ekstrakta u usporedbi s levamisolom (0,55 mg/mL) i oksfendazolom (tri serijska razrjeđivanja od 25 µg/mL). Oba ekstrakta, i etil acetata i vodene otopine metanola, paralizirali su sve odrasle crve 4h i 8h nakon izlaganja u dozi od 25 mg/mL. U testu izlijeganja jaja, pri istoj dozi (25 mg/mL), 83,67% jaja H. contortus nije se izleglo nakon uporabe ekstrakta etil acetat, odnosno 80,67% nakon uporabe otopine metanola. Rezultati ovog istraživanja snažno podupiru ekstrakte ploda *C. colocynthis* kao obećavajuće alternative sintetskim lijekovima protiv oblića *H. contortus*. Za očekivati je daljnja *in vivo* istraživanja kako bi se utvrdila biodostupnost aktivnih sastojaka biljke i minimalna nesmrtonosna koncentracija potrebna za liječenje invazija stoke sa *H. contortus*. Anthelmintički učinci *C. colocynthis* se mogu pripisati prisutnosti fenolnih kiselina.

Ključne riječi: antihelmintička djelotvornost; *C. colocynthis*; *H. contortus*; test izlijeganja jaja; test pokretljivosti odraslih parazita

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