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RESEARCH ARTICLE

In vitro Anthelmintic Efficacy of Three Plant Extracts against Various Developmental Stages of *Haemonchus contortus*

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A B S T R A C T

Gastrointestinal nematodes, including *Haemonchus (H.) contortus*, have a notable impact in terms of impaired production, high fatalities, and substantial economic losses, affecting small ruminants in tropical countries, including Pakistan. The irrational use of synthetic chemicals has fueled anthelmintic resistance, particularly in *H. contortus*, as a formidable challenge, prompting a search for alternative treatment strategies. This study evaluated the anthelmintic potential of leaf extracts of three forage plants, including *Leucaena leucocephala*, *Moringa oleifera*, and *Acacia ampliceps*, against various developmental stages of *H. contortus*. Phytochemical screening revealed the presence of secondary metabolites in the extracts. *In vitro* assays were performed to evaluate the anthelmintic efficacy of the extracts on the inhibition of egg hatching, larval paralysis, and adult worm motility following the guidelines of the World Association for the Advancement of Veterinary Parasitology, while Albendazole was used as a positive control and PBS as a negative control. The highest concentrations of tannins and flavonoids in *Acacia ampliceps* and phenolic compounds in *Leucaena leucocephala* were detected in the extracts as major secondary metabolites. All the tested extracts showed significant time- and dose-dependent responses to various stages of *H. contortus*. The ED_{50} values showed that the lowest dose of *Leucaena leucocephala* (0.285), *Moringa oliefera* (0.81), and *Acacia ampliceps* (0.245) inhibited egg hatching and larval and adult motility, respectively. Extracts of *Leucaena leucocephala* exhibited significant inhibition of egg hatching (70.3%), whereas *Moringa oliefera* showed maximum percent inhibition (66.6) of larval motility. These findings suggest that these plants, traditionally used by local healers in Pakistan, may possess antiparasitic properties, encouraging further studies on the relationship between phytochemicals, extraction methods, and *in vivo* trials to explore their anthelmintic efficacy.

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INTRODUCTION

Gastrointestinal nematodes (GINs) are considered as one of the major production constraints in ruminants. Among the GINs, *Haemonchus (H.) contortus* is the most pathogenic and prevalent species in tropical countries affecting small ruminants farming, threatening food security issues through impaired production and high fatalities (Besier *et al*., 2016). In Pakistan, the impact of GI parasites including *H. contortus* is notably very high in terms of impaired production, and high fatalities, leading to substantial economic losses in the livestock industry (Kuiseu *et al.*, 2021).

Synthetic anthelmintics have historically been the mainstay of treatment for GINs, including *H. contortus* in livestock. However, a considerable rise in anthelmintic-resistant parasite populations has been unintentionally caused by the irrational use of conventional dewormers (Lamb *et al.*, 2017). *H. contortus* is a parasite that is unique among them since it has shown resistance to

certain anthelmintics, particularly affecting livestock species (Waller and Chandrawathani, 2005). A significant problem has also been posed by the environmental contamination and potential negative effects of these medications on non-target populations. The increasing apprehension over synthetic chemicals has raised questions about the efficacy of traditional anthelmintics in managing parasite diseases, leading to a search for substitute therapeutic approaches (Sawleha *et al.,* 2010).

The existing global trend for developing alternative strategies in prevention and therapeutics to combat anthelmintic resistance has reinforced the demand for research on medical plants with active bio-compounds. These bioactive compounds are secondary plant metabolites, tannins, alkaloids, saponins, flavonoids, and triterpenoids produced by the plant as a natural defense (Liu *et al.,* 2020). Although, the exact mechanism of action has not yet been explored, it is speculated that these compounds impair energy metabolism in worms, resulting in paralysis and death of parasites (Veerakumari and Munuswamy, 2000). Additionally, the utilization of plant extracts aligns with the growing interest in natural products and herbal remedies for livestock management, emphasizing the need for sustainable and environmentally conscious practice (Sandoval-Castro *et al.,* 2012).

Leucaena leucocephala (Family, Fabaceae) also known as Kubabhal locally, has shown considerable anthelmintic activity because of one of its secondary metabolites, mimosine, which is believed to contribute to its potent anthelmintic effect (Widaad *et al.,* 2022). Similarly, *Moringa oleifera*, locally known as Suhanjna, (Family, Moringaceae), has shown considerable anthelmintic action against GINs. When utilized as a natural substitute for parasite management, this plant's leaves which are well-known for their many applications and advantages have been shown to enhance goat development performance (Elghandour *et al.,* 2023). Another Fabaceae family member, *Acacia ampliceps*, locally known as Keekar, has been claimed to possess anthelmintic qualities, although detailed studies on its activity are limited. Furthermore, these plants are commonly used in ethno-veterinary practice in the country and have forage significance, providing essential nutrients and benefits for ruminant health and production.

In the countries with agricultural foundation like Pakistan, investigating the anthelmintic potential of forage plants as alternative treatment options is vital for the nation's livestock industry. This research direction holds significant promise for addressing the challenges posed by anthelmintic resistance and ensuring sustainable livestock production. By evaluating the *in-vitro* anthelmintic efficacy of *L. leucocephala, M. olifera,* and *A. ampliceps* plant extracts against various developmental stages of *H. contortus*, this study contributes to the exploration of potential alternative treatment options for managing parasitic infections in small ruminants in Pakistan.

MATERIALS AND METHODS

Plant identification and extraction: Plants, *Leucaena leucocephala, Accacia ampliceps and Moringa oliefera* were collected from Bahadurnagar farm, Okara, and identified at the Department of Botany, Government College University, and Lahore. The leaves of each plant were rinsed, shade dried, and ground into powder. Approximately 75g of each plant's powder was extracted using a methanol: water (70:30) solvent in a Soxhlet apparatus. The extraction yield was determined as $(w_1 \times 100)/w_2$), where w_1 is the extracts dried weight and w_2 is the plant powder weight. The extracts were then standardized to 100mg/ml in Dimethyl Sulfoxide (DMSO) and stored at 4ºC for future use (Zangueu *et al.,* 2018).

Phytochemical screening: It was carried out by using various protocols, such as titrimetric method for Tannins, Folin-Ciocalteu's for phenolic and aluminum chloride colorimetric method for flavonoids estimation (Sharma *et al.,* 2021). The crude protein percentage was estimated by Kjeldahl method (Kaska and Mammadov, 2019).

In vitro bioassays: The plant extracts were tested against *H. contortus* with Albendazole as a positive and PBS as a negative control. The standardized extracts and Albendazole were two fold serially diluted in Phosphate Buffer Solution (PBS) to create desired concentrations (Table 1) (Eguale *et al.,* 2011). The evaluation criteria for the anthelmintic activity of the extracts included inhibition of egg hatching, larval paralysis and inhibition of adult worm motility. The assays followed World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines with minor modifications (Coles *et al.,* 1992). All assays were conducted in triplicate and replicated thrice.

Egg hatch assay: In this assay, per rectum feces from donor goat were collected and eggs were isolated through flotation method to form egg suspension. Subsequently, in each well of 24-well micro titration plate, 100µL aliquots containing approximately 100 eggs, formulated extracts and Albendazole concentrations, and PBS were added. After incubation at 27°C for 48 hours, one drop of Lugols iodine was added to halt further hatching. The numbers of hatched and un-hatched eggs were counted under microscope. Egg hatch inhibition was calculated using the following formula (Chan-Pérez *et al.,* 2016).

$$\%EHI = 100\ (1-X_1 / X_2)$$

Where X_1 is the hatched eggs in extracts and X_2 is hatched eggs in the negative control.

Table 1: Different doses of plant extracts

Group 1: *Leucaena leucocephala*	Group 2: *Accacia ampliceps*	Group 3: *Moringa oliefera*	Group 4: Albendazole	Group 5: PBS
5mg/ml	5mg/ml	5mg/ml	1mg/ml	PBS solution , pH 7.2
2.5mg/ml	2.5mg/ml	2.5mg/ml	0.5mg/ml	
1.2mg/ml	1.2mg/ml	1.2mg/ml	0.1mg/ml	
0.6mg/ml	0.6mg/ml	0.6mg/ml		
0.3mg/ml	0.3mg/ml	0.3mg/ml		

Larval paralysis assay: The retrieved eggs were cultured at 27°C for 7 days in a sawdust and charcoal filled jar. Following the culture, L3 larvae were collected using Baermann's technique (Várady and Čorba, 1999). The assay was conducted within 3 hours of larvae harvesting, with the larval suspension adjusted to 100 larvae/ 200ul, formulated concentrations of extracts and Albendazole and PBS distributed in 24–well micro titration plates. Following incubation at 27°C for 24 hours, the larvae were examined under microscope and categorized as motile and non-motile observing the sinusoidal movement. The larval motility inhibition index was calculated as (Váradyova *et al.,* 2018):

$$\%LMI = (T - M / T \times 100)$$

Where T is larvae in PBS and M are larvae in treatment groups.

Adult Worm Motility Assay (AWMA): Adult worms were obtained from the abomasum of naturally infected goats slaughtered at a local slaughterhouse. After transport to the laboratory, the abomasa were opened and rinsed with saline for worm recovery. Following the protocol of (Hounzangbe-Adote *et al.,* 2005), ten vigorously motile worms were exposed to the formulated concentrations of the plant extracts, Albendazole and PBS in 5cm plastic petri dishes at room temperature. The anthelmintic activity was gauged by worm mortality inhibition. Worms were monitored every 3-hours for 24 hours. Immotile worms were placed in lukewarm water for 10sec to revive motility. The count of motile and dead worms was recorded. Worm motility index was calculated using the following formula (Zangueu *et al.,* 2018):

AWMI =Number of immotile worms / total number of worms for each concentration × 100

Statistical analysis: The ED_{50} was estimated by using Probit analysis. In-vitro test data underwent one-way and two ANOVA for analysis. Comparisons of time interval with mean percentage were assessed through Repeated Measure Design using R Statistical Language version 4.2.2. The Least Square difference was performed as a post hoc test. The difference between means was taken significant at $P<0.05$.

RESULTS

Phytochemical screening: The yield percentage of plant extracts were 13.8 for *L. leucocephala*, 24.9 for *A. ampliceps* and for *M. oliefera* 9.3. The phytochemical analysis revealed major secondary metabolites like tannins, flavonoids, and phenolic compounds. *A. ampliceps* showed higher levels of flavonoids and tannins, whereas *L. leucocephala* exhibited greater phenolic content and a higher percentage of crude protein (Table 2).

Egg hatch assay: In Egg Hatch Assay, all three extracts exhibited significant inhibition of egg hatching among various dose levels compared to negative control ($p<0.05$). Likewise various concentrations of albendazole also showed significant inhibitory effect ($P < 0.05$). The inhibitory effect was dose dependent for all three extracts (Table 4). Among all extracts, *L.leucocephala* showed highest inhibition percentage at higher tested concentration along with lowest ED_{50} value for inhibition of egg hatch (Table 3).

Table 2: Concentration of various secondary metabolites of three plants

	Phytoanalysis			
Plant	Total Flavonoids (Rutin equilents ug/g F.wt)	Total Phenols (TPC uM/g. F.wt)	Total Tannins (uuM/g.f.wt.)	CP%
L. leucocephala	2603.522	80100	7300	27.9
A. ampliceps	2921.582	79300	9700	10.2
M. oliefera	2455.138	70700	9400	23.6

Table 3: ED_{50} of Plant Extracts against various stages of *H. contortus*

Extract	Assay	LD50	95% confidence limit		Regression equation	R2
			Upper limit	Lower limit	Y=bx +a	
L. leucocephala	AWMA	0.299	0.376	0.192	Y=3.47x+1.82	0.792
	EHIA	0.285	0.6021	0.0225	Y=0.41x+0.22	0.921
	LMIA	2.1	4.0757	1.3697	Y=0.60+-0.19	0.885
A. ampliceps	AWMA	0.245	0.616	0.0004	Y=0.61x+0.37	0.95
	EHIA	1.704	5.5587	0.8531	Y=0.39+-0.09	0.883
	LMIA	3.527	11.5331	2.0563	Y=0.53+-0.29	0.842
M. oliefera	AWMA	0.431	1.763	10.2041	Y=0.90x+0.32	0.81
	EHIA	5.085	71.8607	2.3958	Y=0.40+-0.28	0.942
	LMIA	0.81	1.463	0.298	Y=0.43+0.04	0.692

Table 4: Mean percent inhibition ±SEM of plant extracts on hatching of *H. contortus* eggs

Conc. mg /ml	*L. leucocepahala*	*A. ampliceps*	*M. oliefera*
5**	70.3 ± 0.33^{ia}	58.6 ± 0.33^{iia}	51.6 ± 0.33^{iiia}
2.5**	64 ± 0.33^{ib}	51.6 ± 0.33^{iib}	42.6 ± 0.33^{iiib}
1.2 **	58.3 ± 0.33^{ic}	47.6 ± 0.33^{iic}	40.0 ± 0.0^{iiic}
0.6**	54.0 ± 0.33^{id}	41.0 ± 0.0^{iid}	34.6 ± 0.33^{iiid}
0.3**	50.6 ± 0.33^{ie}	39.0 ± 0.0^{iie}	31.6 ± 0.33^{iiie}
Control Albendazole		Mean % Inhibition	
0.1		86.33 ± 0.33^{b}	
0.25		89.66 ± 0.33^{a}	
0.5		90.33 ± 0.33^{a}	
PBS		2.666 ± 0.33^{p}	

Means with different superscript (alphabets) differ significantly within a row and column ($P<0.05$).

Table 5: Mean percent paralysis ±SEM of plant extracts on motility of *H. contortus* infective larvae

Conc. mg /ml	*L. leucocepahala*	*A. ampliceps*	*M. oliefera*
5**	*61±0*^{bc}	*56±0*^{ef}	*66.6±0.67*^{a}
2.5**	*59.6±0.33*^{cd}	*53±0.33*^{fg}	*62.3±0.33*^{b}
1.2 **	*59±0*^{cd}	*53±3*^{g}	*57.6±0.33*^{de}
0.6**	53.3±0.33^{g}	*51.6±0.33*^{g}	*56.3±0.33*^{e}
0.3**	*51.6±0.33*^{g}	*48±0*^{h}	*46.3±0.67*^{h}
Control Albendazole		Mean % Inhibition	
0.1		*78.66±0.33*^{b}	
0.25		*84.66±0.33a*	
0.5		*86.66±0.33*^{a}	
PBS		*2.67±0.33*^{k}	

Means with different superscript (alphabets) differ significantly within a row and column (P<0.05).

Table 6: Mean percent inhibition ± SEM of plant extracts on *H. contortus* adult worms' motility

Plant	Conc.	3h	6h	9h	12h	15h	18h	21h	24h
L.	5	15.5±0.33^{opq}	24.4±0.33^{mno}	38.8±0.88^{jklm}	55.5±0.88^{fghij}	72.2±2.19^{cdef}	86.6±1.53^{abcd}	93.3± 1.15^{ab}	100±0.0^{a}
leucocepahala	2.5	2.2±0.33^{pq}	6.6±0.58^{pq}	13.3±0.58^{opq}	36.4±0.58^{klm}	54.4±0.88^{ghij}	67.7±0.88^{efgh}	76.6±1.53^{bcde}	90±0.58^{ab}
	1.2	0.0±0.0^{q}	.11±0.33^{pq}	17.7±0.33^{nop}	34.4±1.45^{lmn}	44.4±1.2^{ijkl}	61.11±0.88^{efghi}	70±0.88^{defg}	86.6±0.58^{abcd}
	0.6	0.0±0.0^{qm}	2.2±0.33^{pqm}	16.6±0.58^{opq}	40±0.58^{jklm}	52.2±0.88^{hijk}	57.7±0.67^{fghi}	72.2±1.15^{cdef}	88.8±0.33^{abc}
	0.3	0.0±0.0^{q}	1.1±0.33^{pq}	16.6±0.58^{opq}	36.6±0.58^{klm}	47.7±0.58^{ijkl}	55.5±0.88^{fghij}	68.8±0.88^{efgh}	77.7±1.2^{bcde}
A. ampliceps	5	11.1±0.33^{opq}	23.3±0.58^{no}	35.5±0.88 ^{klmn}	42.2±1.76^{ijklm}	67.7±1.2^{efg}	80±0.58^{bcde}	92.2± 0.88^{ab}	98.8±0.33^{a}
	2.5	1.1±0.33^{q}	5.5±0.33^{pq}	10±0.58^{pq}	33.3±0.58^{klmn}	51.1±0.88^{hi}	64.4±0.88	72.2±1.53^{def}	86.6±0.58^{abc}
	1.2	0.0±0.0^{q}	1.1±0.33 ^{q}	14.4±0.33^{op}	30±1.53^{mn}	37.7±1.2^{jklm}	56.6±1.53^{gh}	65.5±0.88 ^{fg}	83.3±0.58^{bcd}
	0.6	0.0±0.0^{q}	1.1±0.33 ^{q}	14.4±0.33^{opq}	30±0.0 ^{klmn}	37.7±0.58^{ijkl}	56.6±0.33^{hij}	65.5±0.67^{fg}	83.3±0.58^{bcd}
	0.3	0.0±0.0^{q}	1.1±0.33 ^{q}	11.1±0.33^{opq}	31.1±0.38^{lmn}	41.1±1.45	45.5±0.88^{hijk}	64.4±0.88^{fg}	74.4±1.2^{cdef}
M. oliefera	5	6.6±0.0^{lm}	16.6±0.58^{jkl}	25.5±1.45^{ij}	35.5±2.19 ^{ghi}	57.7±1.76 ^{def}	71.1±0.33^{bcd}	85.5± 0.88^{ab}	94.4±0.88^{a}
	2.5	0.0±0.0 ^{m}	2.2±0.33^{lm}	5.5±0.33^{lm}	28.8±0.33^{ij}	44.4±1.33 ^{fgh}	58.8±1.76 ^{def}	62.2±1.2^{cde}	78.8±0.33^{b}
	1.2	0.0±0.0 ^{m}	0.0±0.0 ^{m}	10±0.58^{klm}	23.3±1.53^{ijk}	31.1±1.2^{hij}	48.8±1.76^{efg}	58.8±1.45 ^{def}	74.4±0.88^{bc}
	0.6	0.0±0.0 ^{m}	0.0±0.0 ^{m}	7±0.33^{lm}	26.6±0.58^{ij}	34.4±0.88^{ghi}	37.7±0.33 ^{ghi}	57.7±1.45^{def}	74.4±1.2^{bc}
	0.3	0.0±0.0 ^{m}	0.0±0.0 ^{m}	7.7±0.33^{lm}	23.3±1 ^{ijk}	33.3±0.58^{hi}	37.7±0.33 ^{ghi}	58.8±1.76 ^{def}	71.1±1.2^{bcd}
Albendazole	0.1	40±1.15^{e}	100.66±0.88^{b}	100±0.0^{a}	___	___	___	___	___
	0.25	100±0.58^{d}	100±0.67^{a}	100±0.0^{a}	_____	___	___	___	___
	0.5	100±0.58^{c}	100±0.0^{a}	___	___	___	___	___	___
PBS	--	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	20.0±0.0	20.0±0.0

Means with different superscript (alphabets) differ significantly within a row and column (P<0.05).

Larval paralysis assay: The inhibitory effect on the larval motility post treatment with extracts and albendazole is presented in Table 5. Notably, *M. oliefera* exhibited significant anthelmintic activity across all concentrations against larvae (L_3), inducing highest motility inhibition in contrast to the negative control (P<0.05). ED_{50} value for larval paralysis was also found to be lower for the same plant (Table 4). Larval paralysis percentage was dose dependent for all extracts (p<0.05).

Adult worm motility assay: A significant time and concentration dependent effect was observed against all plant extracts and albendazole as compared to negative control (p<0.05). While there were no significant differences in adult worm motility inhibition among the plant extracts, *L. leucocephala* showed complete mortality within 24 hours of exposure (Table 6). *A. ampliceps* exhibited the lowest ED_{50} value to inhibit adult worm motility (Table 3). In the negative control, 80% of worms remained motile after 24 hours.

DISCUSSION

H. contortus is the most pathogenic hematophagous parasite that is a major threat to the small ruminant industry due to the emergence of resistance against commercially available anthelmintic drug residues, which accelerates substantial production and economic losses globally. Goats (*Capra hircus*), which are natural browsers, are more susceptible to the adverse effects of *H. contortus* owing to digestion impairment, which affects the digestion process and leads to decreased production (Kearney *et al.,* 2016). The search for plant active compounds to discover new dewormers and better parasitic treatments is a continuous process (Kuiseu *et al.,* 2021).

The three plants evaluated in the current study were selected on the basis of their ready accessibility in Pakistan, palatability, chemical profile, and the presence of biologically active compounds with pharmacological properties, making them attractive alternatives to synthetic chemicals and feed for small ruminants. The variation in extraction yields might be attributed to the unique nature of each plant species, their distinct chemical profiles, and varying environmental conditions. Moreover, the choice of extraction solvent and testing procedure can significantly influence the concentration and classification of secondary plant bioactive compounds (Marie-Magdeleine *et al.,* 2009). Understanding these factors is crucial for selecting the most effective plant species and optimizing the extraction conditions for maximum anthelmintic potent (Kamaraj and Rahuman, 2011).

Phyto-chemicals, such as flavonoids, phenolic compounds, and tannins, are secondary metabolites that work independently or synergistically to create an overall inhibitory effect against worms; hence, they are known for

their anthelmintic activity. In this study, phytochemical analysis revealed that *A. ampliceps* had higher concentrations of flavonoids and total tannins than the other two plants, whereas *L. leucocephala* had the highest total phenolic content (Table 2). Several studies have also reported the presence of these metabolites in various plants, including shrubs (El-Nuby and Alam, 2020: Mumed *et al.,* 2022). However, the qualitative and quantitative differences in the extracts are obvious and might be related to the effects of the ecological conditions of the plants, parts of the plants used, varieties of plants, extraction methods, solvents, laboratory techniques, and type of helminths selected (Eguale *et al.,* 2011).Therefore, these plants offer a promising avenue for the development of natural anthelmintic alternatives to synthetic chemicals, thereby contributing to the sustainability of small ruminant production.

L. leucocephala, with its high crude protein (CP%) content, demonstrated promising results in inhibiting egg hatching, while *M. oleifera*, with a CP% of 23.6, showed encouraging outcomes in controlling the larval stage of the parasite. These findings suggest that the protein content of these plants may play a crucial role in their anthelmintic activity, with *L. leucocephala* targeting the early stages of the parasite's life cycle and *M. oleifera* affecting the later stages (Table 3). These results are in accordance with the findings of Widaad *et al.* (2022). As per author's knowledge, the prior published literature for estimation of anthelmintic efficacy (*in-vitro*) of plants meagerly focused this aspect of the evaluating subjects.

The usage of protein-rich forages can help in reducing the fecal egg counts in animals infected with GINs. This is attributed to the intricate interplay of various proteins that are involved in the mechanical and enzymatic processes that facilitate the hatching and release of the L_1 stage of *H. contortus*. These processes are made possible by a range of enzymes, including proteases. The high protein content in the diet may exhibit anthelmintic activity due to the presence of a cascade of proteins, including hemolysin and proteases, which disrupt the developmental process of *H. contortus*. This protein-mediated interference impairs the nematode's development and survival, providing a potential natural alternative to synthetic anthelmintics for controlling GIN infections in livestock (Salles *et al.,* 2014).

The anthelmintic activity of the tested plants against all three stages of *H. contortus* was concentration/time dependent. These findings are in accordance with findings of Cabardo and Portugaliza (2017). However, efficacy of each plant extract varies against different developmental stages (Table 4, 5, 6). It is because of integrity of cell membrane of parasites, egg cuticles, adult and larvae along with the biochemical profile of plant and various extraction methods and solvents being used can alter the mechanism of action of the plant extracts (Soares *et al.,* 2015).

The overall inhibitory effect in egg hatch assay was observed highest for *L. leucocephala* whereas *M. oleifera* inhibited larval motility significantly as of other two plants. The mean inhibition was significantly different ($p<0.05$) between plant extracts, control groups and between different plant concentrations. Several studies available previously described anthelmintic activity of various plants including *L. leucocephala* and *M. oliefera* either with different solvent extraction or plant part against larvae or egg stages (Alonso-Díaz *et al.,* 2011: Eguale *et al.,* 2011). The results explained that the highest inhibition activity of *L. leucocephala* extracts in egg hatch was due to the synergistic effect of phyto-chemical contents present. Despite the higher flavonoids and total tannin contents, the overall anthelmintic activity of *A. ampliceps* remained intermediate in all three assays compared to the other two plants. The reason might be due to composition of its bioactive compounds and their interaction with parasite (Aderibigbe *et al.,* 2022).

Although albendazole has demonstrated superior anthelmintic activity compared to plant extracts (Table 4, 5, 6) the latter still exhibits significant efficacy, as they hold significant promise for sustainable and potentially more efficacious treatment options. Further research and optimization of plant extract formulations can advance anthelmintic therapy and address parasitic infection challenges by investigating extraction methods, compound combinations, longer incubation periods, and dosage regimens (Ouattara *et al.,* 2020).

Conclusion: *H. contortus* poses a significant threat to the small ruminant industry due to emerging drug resistance. The search for plant-based dewormers continues, with three plants from Pakistan showing promise as natural alternatives to synthetic chemicals. Phytochemical analysis has revealed varying concentrations of bioactive compounds, which influence anthelmintic potency. Protein-rich forages, such as *L. leucocephala* and *M. oleifera*, have demonstrated significant anthelmintic activity against different stages of *H. contortus*. Although albendazole remains more potent, plant extracts offer sustainable and potentially effective alternatives. Further research is warranted to optimize formulations and treatment strategies for these plant-based alternatives.

Author contributions: MA, MO, MR, KA and GAC developed the project. FA and NS performed the statistical analysis on the data. All authors reviewed and approved the structure and writing of the manuscript. There is no conflict of interest among authors.

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