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RESEARCH ARTICLE

Antiparasitic Activity of Methanolic and Ethyl acetate Extracts of *Azadirachta indica* against *Haemonchus contortus*

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A B S T R A C T

Azadirachta indica (Neem) is an important medicinal plant containing diverse antimicrobial and antiparasitic activities. In the current scenario of emerging drug resistance, there is need of plant derived compounds having antiparasitic activity to counter infectious problem including helminths. Present research was conducted to evaluate the phytochemical composition, ovicidal and adulticidal activity of methanolic and ethyl acetate extracts of *Azadirachta indica* (Neem). The leaves of Neem were used for preparation of extracts and their composition was determined through high performance liquid chromatography (HPLC). Results showed that the methanolic extract had higher quercetin compounds than the ethyl acetate extract. The effect of these extracts on egg hatch and larval motility were tested in *in vitro* experiment. The results indicated that these were found effective to reduce the hatchability of eggs. Hatching percentage was 1.12% ± 1.01 in case of methanolic extract and 3.57% ± 0.97 in case of ethyl acetate extract. Ethyl acetate extract of *A. indica* killed all the worms at the concentration of 25 mg/mL at 6 hrs post exposure while methanolic extract showed 100 percent mortality at 10 hrs post exposure of same dose. Results were in dose-dependent manner. Ethyl acetate extract killed adult worms faster and at lower dose than methanolic extract. However, the methanolic extract of Neem had higher effectiveness than the ethyl acetate extract in preventing hatching of eggs. This research concludes that extraction solvent greatly affects the phytochemical composition and anthelmintic activities of Neem and this is effective to control the helminths.

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INTRODUCTION

Plants and plant compounds had been widely used for the control of many diseases since ancient times (Chandran *et al*., 2020; Hussain *et al*., 2021). Phytochemicals present in plants enable them to control various diseases (Ghildiyal *et al*., 2020). Parasites, especially helminths, are among the major constraints in profitable livestock farming (Zaman *et al*., 2014). They cause serious illness which may lead to death (Rehman *et al*., 2016; Akram *et al*., 2019; Shaukat *et al*., 2019; Strbac *et al*., 2020). Haemonchus is among harmful parasites which are aggressive blood feeders of ruminants (Cottingham *et al*., 2022). It causes anemia, gastric problems and may even lead to death in ruminants (Gareh *et al*., 2021). Development of resistance against chemical drugs is compelling the researchers to find out alternatives for the control of these parasites (Jimenez Castro *et al*., 2019). Vaccines are being practiced but they have limited prevention and unsure protection against the helminths including Haemonchus (Ehsan *et al*., 2020). The researchers are trying to find alternatives of these drugs. Plants and plant derived compounds are attracting the scientists due to high proportion of bioactive compounds, safe mode of actions, and economic concern (Yasmin *et al*., 2020; Jamil *et al*., 2022). Most of ancient and modern medicinal compounds are still derived from the plants of medical importance.

Azadirachta indica (Neem) is a well-known plant having great medicinal and therapeutic effects (Mahmood *et al*., 2018). Biologically active metabolites of Neem have been proven to be effective in controlling multiple infectious agents including bacteria, fungi, helminths, and insects in various experiments (Tembe-Fokunang *et al*., 2019). Multiple preparations of A. indica have been used to control various diseases but the effective preparation is still being researched (Srivastava *et al*., 2020). Various extracts are being implemented in the research to investigate a proper formulation but, still effective one needs to be researched (Latif *et al*., 2020; Reddy and Neelima, 2022).

Extracts are derived from the plants to obtain the fractions which have high concentrations of bioactive compounds (Lefebvre *et al*., 2021). Various solvents are used for the extraction of phytochemicals from the plants, so that the diversity of compounds may be achieved. Research experiments suggested that the extraction solvents have great effect on phytochemical composition of the plants. Variation in solvents resulted in variety of composition of the plants constituents extracted (Nobossé *et al*., 2018; Nawaz *et al*., 2020).

This research trial was conducted to assess the difference of phytochemical composition and in vitro anthelmintic activity of the methanolic and ethyl acetate extracts of the A. indica. In this experiment various concentrations of the extracts were used to analyze the effects of the A. indica on reduction in egg hatching and percent worm motility of Haemonchus.

MATERIALS AND METHODS

Plants and Preparation of extracts: Fresh leaves of A. indica were collected and dried in shade. Leaves were then ground into powder. This powder was mixed in sufficient quantity of methanol and ethyl acetate. stirring was after three days of suspension, solutions were filtered through muslin cloth. This activity was repeated twice. Three filtrates were then mixed and put into the rotary evaporator for the preparation of methanolic and ethyl acetate extracts of the leaves. Mondal *et al*. (2019) method was followed for the preparation of extracts. The prepared extracts were dried and stored at 4°C for experimental use.

Phytochemical analyses: Ethanolic and ethyl acetate extracts were subjected to high performance liquid chromatography (HPLC) for estimation of their phytochemical constituents. CSW32-Chromatography station was used, and the graphs were developed using the Data Apex ® 2001 software. Shim-Pak CLC-ODS (C-18), 250mm x 46cm, 5um columns were used for the chromatography. Flow rates were adjusted @ 1 mL/minute in an ultraviolet-visible detector at a wavelength of 280nm.

Egg hatch assay: Fresh eggs were collected from female worms, washed and strained through sieve. The filtered eggs were counted and divided into 8 groups each having 3 replicates with 100 eggs in each replicate. The first 4 groups were administered the methanolic extracts of Neem @ 25, 12.5, 6.25 and 3.125 mg/mL respectively while groups 5-8 were subjected to ethyl acetate extract of Neem @ 25, 12.5, 6.25 and 3.125 mg/mL respectively. Positive control group contains Oxfendazole. The negative control was given phosphate buffer saline. All the procedures were done according to Coles *et al*. (1992).

Adult motility assay: Adult, living and motile female worms of *H. contortus* were collected from abomasum of the slaughtered sheep. They were washed and divided into 8 groups each having 3 replicates with 10 worms in each replicate. The experimental design was the same as egg hatch test. Motility of worms was observed and recorded after 2hrs interval. The procedure was followed as Rehman *et al*. (2021).

Statistical analyses: All the values were recorded, and percent eggs hatched and percent reduction in worm motility were calculated using Microsoft Excel 365®. Means were compared through Tuckey test via Minitab® 26. The significance level was adjusted to 5% ($P<0.05$).

RESULTS

Phytochemical analysis: High-Performance Liquid Chromatography (HPLC) method of the methanolic and ethyl acetate extracts was performed in the similar conditions. The results showed that the methanolic extract had the higher Quercetins than the Ethyl acetate extract (Fig. 1, 2; Table 1, 2).

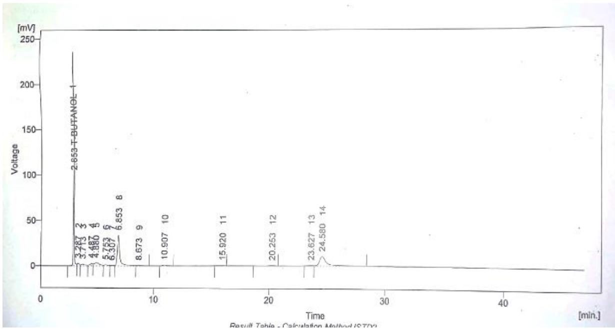

Fig. 1: HPLC diagram of methanolic extract of Neem.

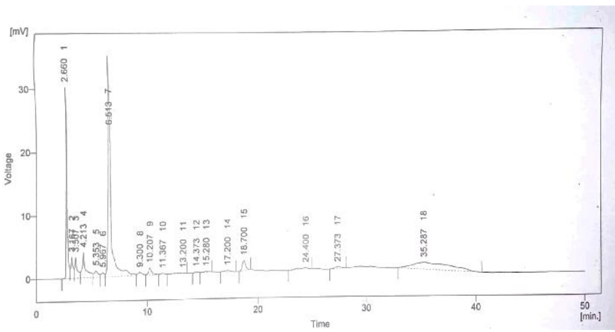

Fig. 2: HPLC diagram of ethyl extract of Neem.

Egg hatch assay: The effect of the various concentrations of Neem was determined against the hatchability of eggs of *H. contortus*. The results showed that both the extracts were effective in dose dependent manner (Table 3). Highest dose (25mg/mL) of methanolic and ethyl acetate extract allowed hatching of only 1.12 and 3.57% of eggs. Methanolic extract is more effective in inhibiting egg hatching as compared to ethyl acetate extract.

Adult motility assay: Adult motility assays were performed against the adult female *H. contortus,* and the results are given in the Table 4 and Table 5. Ethyl acetate extract with dose of 25 mg/mL killed/ inhibited motility of all adult worms at 6hrs after administration of dose while methanolic extract caused 100 percent mortality of adult worms after 10hrs of administration of same dose. Ehtyl acetate extract at dose of 3.125mg/mL killed 100 percent worms 8hrs post-treatment. Ehtyl acetate extract proved to be better in killing adult worms in comparison to methanolic extract.

Table 1: Phytochemical composition of methanolic extract of Neem

Sr. No	Retention time	Area (mV. s)	Compounds	Concentration (ppm)
1.	2.853	994.969	Quercetin	52.73
2.	4.880	118.196	Gallic Acid	4.255
3.	15.920	2.791	Chlorogenic acid	0.217
4.	20.253	4.656	M-Coumaric Acid	0.05
5.	24.580	411/707	Cinnamic Acid	45.19

Table 2: Phytochemical composition of ethyl acetate extract of Neem

Sr. No	Retention time	Area (mV. s)	Compounds	Concentration (ppm)
1.	2.660	196.754	Quercetin	10.42
2.	4.213	90.158	Gallic Acid	3.24
3.	13.200	5.961	Vanillic Acid	0.36
4.	14.373	3.181	Benzoic Acid	0.33
5.	15.280	78.562	Chlorogenic acid	0.30
6.	17.200	109..434	P-Coumaric Acid	0.12

Table 3: Effect of various concentrations of methanolic and ethyl acetate extract of Neem on percent egg hatchability of *H. contortus*

Concentrations	Percent (%) Eggs Hatched	
	Methanolic extract	Ethyl Acetate extract
25 mg/mL	1.12 ± 1.01^{d}	3.57 ± 0.97^{de}
12.5 mg/mL	8.25 ± 3.8^{cd}	12.11 ± 4.73^{d}
6.25mg/mL	22.09 ± 5.47^{c}	31.02 ± 5.03^{c}
3.125mg/mL	39.47 ± 10.12^{b}	55.06 ± 4.39^{b}
Positive Control	0.49 ± 0.49^{d}	0.49 ± 0.49^{e}
Negative control	96.17 ± 2.47^{a}	96.17 ± 2.47^{a}

Difference among means with different superscript is statistically significant (P<0.05).

DISCUSSION

In this study, the effect of extraction solution on phytochemical properties and *in vitro* anthelmintic activity is evaluated. The result of this study showed that there is great variation in the composition of methanolic and ethyl acetate extracts of Neem. The methanolic extract of Neem had a high proportion of active constituents and higher Quercetin components. Ethyl acetate extract had the less amount of active phenolics and flavonoids in it. The methanolic extract of Neem had higher efficiencies in both *in vitro* trials. Both the extracts were significantly effective to control the egg hatchability and worm motility at 25 mg/mL concentration. The results were in dose dependent manner. Multiple researchers have conducted similar studies using plant extracts for *in vitro* efficacy against *H. contortus* (Elandalousi *et al.,* 2013; Politi *et al.,* 2018; Orengo *et al.*, 2022).

The results of our study are in line with the results of Alowanou *et al.*, (2019) who mentioned that the herbal extract of *Bridelia ferruginea, Mitragyna inermis* and *Combretum glutinosum* plants were effective to reduce worm motility and egg hatchability of the *H. contortus* in *in vitro* environment. Similar results have been obtained by Rehman *et al.* (2021), who conducted a research experiment to control the worm motility and egg hatchability of the *H. contortus* in *in vitro* environment using an extract of *Citrullus colocynthis.* Kuiseu *et al.* (2022) conducted similar research using the herbal extract of *Adansonia digitata* and *Anogiessus leiocarpus*. The results of their study suggested that the herbal extracts of *Adansonia digitata* and *Anogiessus leiocarpus* were effective against helminths. Many other studies are also in line with the results of current study (Sisay *et al*., 2021; Sebai *et al.,* 2021).

The anthelmintic efficacy of herbals has been proven and it is linked to phenolics, and flavonoids present in the plants (Lima *et al.,* 2021). The phenolics like Quercetin, Gallic acid, and Benzoic acids etc. have known biomedical activities (Salem *et al.,* 2021). They are capable of inducing apoptosis and cell death in the helminths (Mahmoudvand *et al.,* 2022). They can cause cell-cycle arrest (Liu *et al.,* 2022) i.e., lead to disturbing growth and differentiation of the worms. They are also supposed to be involved in the energy uptake mechanisms of the worm which leads to their death (Adak and Kumar, 2022). Although exact mechanisms of action are under investigation, methanolic and ethyl acetate extracts of Neem contained high fractions of Quercetin, and phenolic acids, these may be responsible for the anthelmintic activities of Neem. Although, methanolic extract had the higher proportion of compounds but the ethyl acetate had a rich fraction of these compounds. It can be Justified that these compounds were the reason of anthelmintic properties of Neem (Rahaman *et al.*, 2022).

Differences in the activity of methanolic and ethyl acetate extracts of Neem can be attributed to differences in the amount and type of phenolics in the phytochemical composition of both extracts. Multiple scientists have suggested that the variation in the composition is a primary factor in bioactivities (Ma *et al.,* 2021). Due to these variations, they have differences in their effect on egg hatch and worm motility of *H. contortus*.

Table 4: Effect of various concentrations of methanolic extract of Neem on the percent reduction in motility of female worms

Treatment groups	Times (hours)						
	0	2	4	6	8	10	12
25 mg/mL	0.0 ± 0.0^{l}	6.66 ± 5.77^{jk}	26.66 ± 5.77^{hi}	50 ± 17.32^{e}	96.66 ± 5.77^{b}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
12.5 mg/mL	0.0 ± 0.0^{l}	10.0 ± 10.0^{j}	30.0 ± 10.0^{h}	46.66 ± 15.27^{f}	73.33 ± 11.54^{d}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
6.25 mg/mL	0.0 ± 0.0^{l}	3.33 ± 5.77^{k}	23.33 ± 5.77^{i}	36.66 ± 5.77^{g}	73.33 ± 5.77^{d}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
3.125 mg/mL	0.0 ± 0.0^{l}	0.0 ± 0.0^{l}	6.66 ± 5.77^{jk}	20.0 ± 10.0^{i}	46.66 ± 15.27^{f}	80.0 ± 10.0^{c}	100.0 ± 0.0^{a}
Positive Control	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
Negative control	0.0 ± 0.0^{l}	0.0 ± 0.0^{l}	0.0 ± 0.0^{l}	0.0 ± 0.0^{l}	0.0 ± 0.0^{l}	0.0 ± 0.0^{l}	0.0 ± 0.0^{l}

Values carrying different superscript letters have statistically significant difference (P<0.05).

Table 5: Effect of various concentrations of ethyl acetate extract of Neem on the percent reduction in motility of female worms

Treatment groups	Times (hours)						
	0	2	4	6	8	10	12
25 mg/mL	0.0 ± 0.0^{i}	33.33 ± 5.77^{f}	70.0 ± 5.77^{c}	100.0 ± 5.77^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
12.5 mg/mL	0.0 ± 0.0^{i}	23.33 ± 5.77^{g}	60.0 ± 7.32^{d}	90.0 ± 10.0^{b}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
6.25 mg/mL	0.0 ± 0.0^{i}	23.33 ± 5.77^{g}	43.33 ± 15.27^{e}	90.0 ± 7.32^{b}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
3.125 mg/mL	0.0 ± 0.0^{i}	13.33 ± 5.77^{h}	56.66 ± 5.77^{d}	90.0 ± 10.0^{b}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
Positive Control	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
Negative control	0.0 ± 0.0^{i}	0.0 ± 0.0^{i}	0.0 ± 0.0^{i}	0.0 ± 0.0^{i}	0.0 ± 0.0^{i}	0.0 ± 0.0^{i}	0.0 ± 0.0^{i}

Values carrying different superscript letters have statistically significant difference ($P<0.05$).

Conclusions: Present research concludes that Neem has a variety of multiple bioactive compounds. The method of extraction has a great impact on the type, amount, and diversity of these compounds. Depending upon these variations, the biomedical activity like anthelmintic activity is varied. This research shows that the Neem has *in vitro* anthelmintic activities and recommends that further trials should be conducted to evaluate *in vivo* biomedical activities.

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Authors contribution: TR, MA, AAEM, SKA, LAAS, designed study plan; TR, ZS, MR performed *in vitro* tests; ZAB, AAEM, SKA, LAAS, MA, MAZ performed statistical analyses.

REFERENCES

Adak M and Kumar P, 2022. Herbal anthelmintic agents: a narrative review. J Trad Chines Med 42(4):641.

Akram MZ, Zaman MA, Jalal H, et al., 2019. Prevalence of gastrointestinal parasites of captive birds in Punjab, Pakistan. Pak Vet J 39(1):132-134.

Alowanou GG, Olounladé PA, Akouèdegni GC, et al., 2019. In vitro anthelmintic effects of Bridelia ferruginea, Combretum glutinosum, and Mitragyna inermis leaf extracts on Haemonchus contortus, an abomasal nematode of small ruminants. Parasitol Res 118(4):1215-1223.

Chandran H, Meena M, Barupal T, et al.,. 2020. Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. Biotech Rep 26:e00450.

Coles GC, Bauer C, Borgsteede FHM, et al., 1992. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol 44(1-2):35-44.

Cottingham SL, Walden HDS, Wisely SM, et al., 2022. Gastrointestinal Parasites of Farmed White-tailed Deer (Odocoileus virginianus) in Florida: WEC453/UW498, 10/2022. EDIS 2022 (5).

Ehsan M, Hu RS, Liang QL, et al., 2020. Advances in the development of anti-Haemonchus contortus vaccines: challenges, opportunities, and perspectives. Vaccin 8(3):555.

Elandalousi, Boubaker R, Akkari H, et al., 2013. Thymus capitatus from Tunisian arid zone: chemical composition and in vitro anthelmintic effects on Haemonchus contortus. Vet Parasitol 197(1-2):374-378.

Gareh A, Elhawary NM, Tahoun A, et al., 2021. Epidemiological, morphological, and morphometric study on Haemonchus spp. Recovered from goats in Egypt. Front Vet Sci 8:705619.

Ghildiyal R, Prakash V, Chaudhary VK, et al., 2020. Phytochemicals as antiviral agents: recent updates. In Plant-derived Bioact, 279-295. Springer.

Hussain K, Abbas RZ, Abbas A, et al., 2021. Anticoccidial and Biochemical Effects of Artemisia Brevifolia Extract in Broiler Chickens. Braz J Poultry Sci 23(4):01-06. http://dx.doi.org/10.1590/1806-9061-2020-1377.

Jamil M, Aleem MT, Shaukat A, et al., 2022. Medicinal Plants as an Alternative to Control Poultry Parasitic Diseases. Life 12:449. https://doi.org/10.3390/life12030449.

Jimenez Castro PD, Howell SB, Schaefer JJ, et al., 2019. Multiple drug resistance in the canine hookworm Ancylostoma caninum: an emerging threat? Parasite Vect 12(1):1-15.

Kuiseu J, Olounlade PA, Houssoukpe CG, et al., 2022. In vitro Effects of Anogeissus leiocarpus and Adansonia digitata on Two Life-cycle Stages of Haemonchus contortus, a Gastrointestinal Parasite of Small Ruminants. Anim Vet Sci 10(1):1-7.

Latif MJ, Hassan SM, Mughal SS, et al., 2020. Therapeutic potential of Azadirachta indica (neem) and their active phytoconstituents against diseases prevention. J Chem Cheml Sci 10(3):98-110.

Lefebvre T, Destandau E and Lesellier E, 2021. Selective extraction of bioactive compounds from plants using recent extraction techniques: A review. J Chromat A1635:461770.

Lima CS, Matheus HP, Yousmel AG, et al., 2021. Anthelmintic effect of Pterogyne nitens (Fabaceae) on eggs and larvae of Haemonchus contortus: Analyses of structure-activity relationships based on phenolic compounds. Indust Crops Prod 164: 113348.

Liu Y, Meng Y, Bian J, et al., 2022. 2-Methoxy-5 ((3, 4, 5-trimethosyphenyl) seleninyl) phenol causes G2/M cell cycle arrest and apoptosis in NSCLC cells through mitochondrial apoptotic pathway and MDM2 inhibition. J Biochem Mol Toxic: e23066.

Ma D, Wang C, Feng J, et al., 2021. Wheat grain phenolics: a review on composition, bioactivity, and influencing factors. J Sci Food Agric 101(15):6167-6185.

Mahmood MS, Amir HW, Abbas RZ, et al., 2018. Evaluation of antiviral activity of Azadirachta indica (Neem) bark extract against Newcastle disease virus. Pak Vet J 38(1):25-28.

Mahmoudvand H, Al-Abodi HR, Zolfagharkhani P, et al., 2022. Anti-helminthic effects and cellular mechanisms of Astragalus ecbatanus extract against Echinococcus granulosus protoscoleces. J Para Dis 46(4):1047-1054.

Mondal M, Hossain M, Das N, et al., 2019. Investigation of bioactivities of methanolic and ethyl acetate extracts of Dioscorea pentaphylla leaf along with its phenolic composition. J Food Measure Charac 13(1):622-633.

Nawaz Haq, Shad MA, Rehman N, et al., 2020. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds. Braz J Pharm Sci 56.

Nobossé P, Fombang EN and CMF Mbofung, 2018. Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh moringa oleifera L. leaves. Food Sci Nutri 6:2188-98.

Orengo KO, Mbaria JM, Ndichu M, et al., 2022. Preliminary Phytochemical Composition and In Vitro Anthelmintic Activity of Aqueous and Ethanol Extracts of Olea africana against Mixed Gastrointestinal Worms in Dogs. Evi Complement Alter Med .

Politi FAS, Junior AAS, Fantatto RR, et al., 2018. Chemical Composition and In vitro Anthelmintic Activity of Extracts of Tagetes patula Against a Multidrug-Resistant Isolate of Haemonchus contortus. Chem Biodiv 15(2):e1700507.

Rahaman A, Azmal SA, Matin SMA, et al., 2022. Effect of Neem and Tamarind Leaves as Anthelmintic in Cattle. J Dis Med Plants 8:13-5.

Reddy IV and Neelima P, 2022. Neem (Azadirachta indica): A review on medicinal Kalpavriksha. Intern J Econ Plants 9(1):59-63.

Rehman T, Khan MN, Abbas RZ, et al., 2016. Serological and coprological analyses for the diagnosis of Fasciola gigantica infections in bovine hosts from Sargodha, Pakistan. J Helminth 90:494–502.

Rehman T, Iqbal KJ, Anwer A, et al., 2021. In vitro anthelmintic efficacy of Citrullus colocynthis (L.) Schrad on Haemonchus contortus. Veterinarski Arhiv 91(3):309-318.

Salem MZM, Mohamed AA, Ali HM, et al., 2021. Characterization of phytoconstituents from alcoholic extracts of four woody species

and their potential uses for management of six Fusarium oxysporum isolates identified from some plant hosts. Plants 10(7):1325.
Sebai E, Abidi A, Serairi R, et al., 2021. Essential oil of Mentha pulegium induces anthelmintic effects and reduces parasite-associated oxidative stress in rodent model. Exp Parasitol 225:108105.
Shaukat A, Medmood K, Shaukat I, et al., 2019. Prevalence, Haematological Alterations and Chemotherapy of Bovine Anaplasmosis in Sahiwal and Crossbred Cattle of District Faisalabad, Punjab, Pakistan. Pak J Zool 51(6):2023-2032.
Sisay A, Negesse T and Nurfeta A, 2021. Anthelminthic effects of extracts of indigenous browses from mid rift valley of Ethiopia. Ethio Vet J 25(2):132-143.
Srivastava SK, Agrawal B, Kumar A, et al., 2020. Phytochemicals of Azadirachta indica source of active medicinal constituent used for cure of various diseases: A Review. J Sci Res 64(1):385-90.
Strbac F Bosco A, Amadesi A, et al., 2020. Ovicidal potential of five different essential oils to control gastrointestinal nematodes of sheep. Pak Vet J 41 (3):353-358.
Tembe-Fokunang EA, Charles F, Kaba N, et al., 2019. The potential pharmacological and medicinal properties of neem (Azadirachta indica A. Juss) in the drug development of phytomedicine. J Compl Altern Med Res 7(1):1-18.
Yasmin S, Nawaz M, Anjum AA, et al., 2020. Phytochemical analysis and in vitro activity of essential oils of selected plants against Salmonella enteritidis and Salmonella gallinarum of poultry origin. Pak Vet J 40:139-144.
Zaman MA, Sajid M, Sikandar A, et al., 2014. Point prevalence of gastrointestinal helminths and their association with sex and age of the buffaloes in lower Punjab, Pakistan. Int J Agric Biol 16:1229–1231.