Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(5): 25-30

Received: 06-07-2018
Accepted: 07-08-2018

Sushmita Sastya
College of Veterinary and Animal Sciences G.B. pant University of Agriculture & Technology Pantnagar, Uttarakhand, India

Rajeev Ranjan Kumar
College of Veterinary and Animal Sciences G.B. pant University of Agriculture & Technology Pantnagar, Uttarakhand, India

Stuti Vatsya
College of Veterinary and Animal Sciences G.B. pant University of Agriculture & Technology Pantnagar, Uttarakhand, India

Correspondence
Sushmita Sastya
College of Veterinary and Animal Sciences G.B. pant University of Agriculture & Technology Pantnagar, Uttarakhand, India

In vitro and *in-vivo* efficacy of *Eucalyptus citriodora* Leaf in gastrointestinal nematodes of goats

Sushmita Sastya, Rajeev Ranjan Kumar and Stuti Vatsya

Abstract
The study was undertaken to evaluate the anthelmintic activity of crude powder (CP), crude aqueous (CAE) and crude methanolic (CME) extracts of *Eucalypyus citriodora* leaves. *In vitro* anthelmintic activity was evaluated against freshly collected adult *Haemonchus contortus* using Adult Motility Test (AMT). However, against eggs and larvae of gastrointestinal nematodes of goats using Egg Hatch Assay(EHA) and Larval Paralysis Test(LPT) at 0.156%, 0.312%, 0.625%, 1.25%, 2.5%, 5% and 10% concentration. *In-vivo* trial was conducted in goats naturally infected with GI nematodosis by using Faecal Egg Count Reduction Test (FECRT%). In AMT an average highest corrected motility of 100% was observed from 1.25–10% followed by 66.66%, 46.66%, 46.66% at 0.625%, 0.312 and 0.156% concentration, respectively. In EHA, CAE showed better ED_{50}=0.997mg/ml and ED_{99}=105.033 mg/ml values than CME (ED_{50}= 1.820 and ED_{99}= 52.182 mg/ml). Both CAE and CME showed highest (100%) inhibition of egg hatching at 50 and 100 mg/ml and minimum (60% and 54%) at 1.56 mg/ml concentration, respectively. In LPT, CAE (ED_{50}= 5.305 and ED_{99}= 157.326mg/ml) and CME (ED_{50}= 5.503 and E_{99}= 165.447 mg/ml) showed highest 100% and 100% and lowest 25% and 22% paralysis of third stage larvae of gastrointestinal nematodes at 100mg/ml and 1.56 mg/ml concentration, respectively. In *in-vivo* anthelmintic activity, CME showed highest (35%) reduction in faecal egg count followed by CAE (21.05%) and then CP (10.34%) @ 4gm/kg b.wt. on 7, 14 and 14 day post-treatment, respectively.

Keywords: *Eucalypyus citriodora,* anthelmintic activity, GI nematodosis, Goats

Introduction
Gastrointestinal (GI) nematodes are major problem and primary cause for affecting small ruminant production throughout the worldwide including India. They produce various pathogenic effect like anaemia, villous atrophy, pimply gut, enteritis and is a major cause of morbidity (Tyasi *et al*., 2015) [38] leading to retarted growth, weight loss, reduce food consumption, lower milk production, impaired fertility and animal becomes more prone to secondary infections due to relaxation in immunity. Among these GI nematodes, *H. contortus* is most common and most pathogenic parasite because of its voracious blood feeding ability (Jabbar *et al*., 2006) [13]. Currently GI parasite control programs in small ruminants mainly depend through the use of anthelmintics. However, these are very expensive and often not easily available to the farmers in rural or remote areas and also the frequent use of the anthelmintics results in emergence of resistance (Jaiswal *et al*., 2013; Rialch *et al.,* 2013) [14, 32]. Because of increase in severity of anthelmintic resistance, increase public awareness towards the drug residues and toxicity problem, Therefore there is urgent need, not only to develop alternative or complementary control method but also extends the life and preserve efficacy of current anthelmintics available in the market (Hoste *et al*., 2002) [12]. This has given a new area of research to find out alternative therapeutic agents for the control of GI nematodosis. Several plants have been identified having anthelmintic properties are available in India (Kumar *et al*., 2014 and 2016) [19, 20]. These herbal plants are cheaper or affordable, easily available, easy to prepare and administer (Tariq *et al*., 2009) [37], are also less toxic, biodegradable, and ecofriendly (Melo *et al*., 2003) [26] so it is a good option for ordinary or small scale animal farmer. Use of traditional plants not only reduces the conventional anthelmintic treatment it also extends the life of anthelmintics (Chagas *et al.,* 2008) [5].
Eucalyptus spp. commonly called Safeda in Hindi and Blue Gum in English is basically a native plant of Australia. It is mainly cultivated for paper production, cosmetic and pharmaceutical purposes (Hasegawa *et al*., 2008) [10].

Leaves have febrifuge, carminative, stimulant, expectorant, antifungal, antibacterial, anti-inflammatory, antiseptic, antimalarial and mosquito repellent properties. It increases the flow of saliva, gastric and intestinal juices and increases the digestion and appetite and also it has anthelmintic properties (Kuamr *et al*., 2015) [18]. To concentrate on anthelmintic property of *E. citriodora*, the present was aimed to evaluate the anthelmintic activity against GI nematodes of Goats.

Materials and Methods

Plant material

Eucalyptus citriodora was selected, on the basis of their documented anthelmintic properties or literature survey. Leaves of *E. citriodora* were collected (July 2015 to April 2016) from the plants situated in G.B. Pant University of Agriculture & Technology, Pantnagar. Plant were identified and authenticated by Department of Biological Sciences, College of Basic Science & Humanities, G.B. Pant University of Agriculture and & Technology, Pantnagar.

Preparation of extract

Leaves of *E. citriodora* were cleaned manually, dried in a well aerated room protected from sun and then they were dried in the oven at 50 ^{0}C. Powder was prepared using electric mixer grinder and stored in airtight container. About 50 gm of powdered sample was soaked separately in 400 ml of distilled water and 400 ml methanol in 1 lit conical flasks, covered with aluminium foil and stirred every one hour interval initially for 2-3 times and left undisturbed for overnight at room temperature and then filtered through Whatman paper No.1 and separating funnels. Then after, filtrate was transferred into separate glass tray which then placed in an oven at 40^{0}C to dry the extract into powder. The extract residues were kept in airtight glass petridishes at 4 ^{0}C (refrigerator) for further use (Ombasa *et al.*, 2012) [30].

Seven different dilutions of each extract were prepared *viz*. 0.156% (1.56mg/ml), 0.312% (3.125mg/ml), 0.625% (6.25mg/ml), 1.25% (12.50mg/ml), 2.5% (25mg/ml), 5% (50mg/ml) and 10% (100mg/ml). Thiabendazole dissolved in Dimethyl Sulfoxide (DMSO) at same concentrations as above and used as positive control and distilled water was used as negative control.

In vitro evaluation of anthelmintic activity of *E. citriodora*

Adult Motility Test

Collection of *Haemonchus contortus* worm

Adult *H. contortus* worms were collected from the abomasii of freshly slaughtered goats at Lalkuan, Nainital. The worms were washed and cleared off debris with distilled water. Then after, they were kept in lukewarm normal saline and brought to the laboratory, Department of Parasitology, College of Veterinary & Animal Sciences, Pantnagar. The cleaned worms were transferred with the help of needle in beaker containing Lock's solution and incubated at 37 ^{0}C to acclimatize before beginning test (Bhatnagar *et al.*, 1961) [2].

Adult motility test for anthelmintic activity of CAE, CME and CP of plant leaves were conducted on adult *H. contortus* of goats. Ten worms were exposed to 15 ml of seven different dilutions in separate Petri dishes and then incubated at 37 ^{0}C±1 ^{0}C for 24 hrs. Lock's solution was used as positive and distilled water as negative control. The motility was observed after 1, 2, 4, 6, 12, 18 and 24 hrs intervals. The dead worms were easily recognized by their straight flat appearance without movement of head and tail region but for conformation the viability of the worms was determined by pinch technique (the absence of motility for an observation period of 5-6 second) as described by Neogi *et al.* (1964) [29]. The number of dead worm was recorded for each treatment.

Corrected mortality was calculated as per the formula given by Sangwan and Sangwan (1998) [33].

$$\% \text{ Corrected mortality} = \frac{\text{Total mortality - Control mortality}}{\text{Total mortality}} \times 100$$

Egg Hatch Assay

Collection of GI nematode eggs and Egg hatch assay were conducted according to the standard protocol described by World Association for the Advancement of Veterinary Parasitology guidelines (Coles *et al*., 1992) [6]. Faecal samples were collected from goats naturally infected with GI nematodes and obtain a pooled sample by mixing several samples. Sample was stored anaerobically in screw top plastic bottle filled with water upto brim. Faecal sample was broken in mortar-pestle with water to make homogenised suspension. It was filter through tea strainer of 0.15 mm in size and filtrate was transferred into 15 ml conical centrifuge tubes. Then after it was centrifuge for 2 min. at 300×g and then supernatant was discarded. The sediment was resuspended in saturated salt solution and after gentle mixing, the suspension was re-centrifuge for 3 min at 130×g. Then tubes were filled with saturated salt solution until a meniscus was formed above the tube. A cover slip was placed gently above the meniscus for few minutes. The cover slip was plucked off with the help of artery forceps and the eggs were washed twice with deionised water into a conical centrifuge tube. Isolated egg suspension was counted in 100 µl and numbers of eggs were estimated per ml and diluted to 100-150/100µl. The assay was performed in 24 well plates as per Le Jambre (1976) [21]. 100µl of fresh egg suspension were exposed to 1ml of each prepared dilution of extract. The plate was incubated at 27 °C for 48 hours. Thereafter, two drops of Lugol's iodine were added to each well to stop further hatching. A total of hundred eggs and hatched larvae (L1) were counted under compound microscope and percent inhibition of egg hatching was calculated.

Larval Paralysis Test

Faecal sample collected from each group was pooled and faecal sample was broken in mortar with help of pestle. The faeces were transfer into petridish and covered it. Then petridish was incubated at 27 °C in incubator upto 7 days. The infective Larvae (L3) were procured according to MAFF (1986) [24] using Baerman's apparatus. Test was performed in 24 well plate in which 100µl of larvae suspension containing 100-150 larvae/100µl were exposed to different dilutions of extract residues. Thiabendazoal and distilled water were used as positive and negative control, respectively. The plates were kept at room temperature (27 °C) for 24 hours. A total of hundred dead and live larvae were counted under compound microscope and percent larval paralysis was calculated.

In-vivo Evaluation of Anthelmintic Activity of *E. citriodora*

Location of Study Area

Evaluation of anthelmintic efficacy of CAE, CME and CP was carried out in goats from Department of Livestock Production & Management, College of Veterinary and Animal Sciences, Pantnagar and also in local areas, Sanjay colony and Jha colony of Pantnagar.

Grouping and dosing of animals

Total 42 adult goats age of 1-3 years of either sex naturally infected with mixed infections of gastrointestinal nematodes having >200 faecal egg count and history of not using any kind of anthelmintic treatment since last three months were selected, numbered and weighed during each trial. These animals were randomly divided into seven group *viz.* GI, GII, GIII, GIV, GV, GVI and GVII with 6 animals in each. The GI, GII and GIII groups were treated with CP, CAE and CME of *E. citriodora* leaves @ 2gm/kg b.wt. orally, while GIV, GV and GVI were treated @ 4gm/kg b.wt. orally. However, animals of GVII kept as untreated infected control.

Faecal Egg Count Reduction Test (FECRT)

Faecal samples were collected directly from rectum from individual animals of each treated and control group on 0 day (before) of treatment, 7 and 14 day post-treatment (7 and 14 DPT) and placed separately in plastic bag, marked them and transfer to laboratory. Intensity of egg per gram of faeces (EPG) of each faecal sample was determined by modified Mc Master Technique (MAFF, 1971) [25] briefly 1g of fresh faeces was accurately weighed and suspended in 14ml saturated sodium chloride NaCl. The suspension was strained through a fine sieve. The larger particles of faeces were removed and the residue was left to pass through. The suspension was stirred well in order to obtain a completely homogenous distribution of eggs in the liquid. Using a Pasteur pipette, compartment of the McMaster slide was filled, avoid air bubbles. After a few minutes eggs from the concentration solution floats up and sticks to the cover glass. Number of eggs was counted in the compartment under low magnification. The egg per gram of faeces was calculated as follows:

Egg per gram (EPG) = Number of egg in the chamber x 50

Determination of Anthelmintic Efficacy

The anthelmintic efficacy of the extracts residue was calculated using the following formula given by Dash *et al*. (1988) [7].

$$\%\ \text{efficacy} = \frac{\text{Pre - treatment EPG - Post treatment EPG}}{\text{Pre - treatment EPG}} \times 100$$

Coproculture

Larvae were cultured according to MAFF (1986) [24] using Baermann's apparatus.

Gastrointestinal nematode larvae were identified on the basis of their morphological characters as described by Soulsby (1965) [36].

Phytochemical analysis of extracts

Extracts residue obtained from CAE and CME were tested for the presence of their phytoconstituents such as alkaloid, anthraquinones, tannins, flavonoids, saponins, glycoside, resins, triterpencs, reucing sugars, proteins and coumarins by standard procedures (Sofowara, 1982) [35].

Statistical Analysis

The data of *In vitro* trials *viz.* Egg Hatch Assay and Larval Paralysis Test were analyzed statistically using probit analysis for calculation of ED_{50} and ED_{99} by SPSS version 16. However, for *in-vivo* trial multivariat two ways ANOVA was used to analyzed the data for calculation of F-value and difference between the mean were considered significant at $p>0.05$.

Results

In-vitro* anthelmintic activity of *E. citriodora

Adult Motility Test

Crude powder showed 40, 40, 100, 100, 100, 100 and 100% mortality at 0.156%, 0.312%, 0.625%, 1.25%, 2.5%, 5% and 10% concentration respectively. CAE showed 100% mortality at all tested concentrations whereas CME and TBZ showed 0, 0, 0, 100, 100, 100 and 100% mortality at 0.156%, 0.312%, 0.625%, 1.25%, 2.5%, 5% and 10% concentration respectively. Overall, CAE showed maximum (100%) adulticidal activity against *H. contortus* followed by CP (82.86%) and minimum (57.14%) with CME and TBZ (Table-1). *E. citriodora* showed overall 79.99% corrected mortality. An average highest corrected motility of 100% was observed from 1.25–10% followed by 66.66%, 46.66% and 46.66% at 0.625%, 0.312 and 0.156% concentration, respectively (Table-1).

Egg Hatch Assay

CAE and CME of *E. citriodora* caused significant dose dependent inhibition of egg hatching (Table-2). CAE (ED_{50}= 0.997 and ED_{99}= 105.003 mg/ml) showed 100% inhibition @50 and 100mg/ml concentration. However, at 25, 12.5, 6.25, 3.125 and 1.56 mg/ml concentration, inhibition of egg hatching was 92, 86, 79, 76 and 60%, respectively. However, CME (ED_{50}= 1.820 and ED_{99}= 52.182 mg/ml) extract showed 100% inhibition of egg hatching @50 and 100mg/ml while at 25, 12.5, 6.25, 3.125 and 1.56 mg/ml concentration it was 98, 91, 73, 60 and 54%, respectively (Table-2 and 3). In EHA, TBZ showed 100% inhibition in egg hatching at all concentrations (Table-2).

Larval Paralysis Test

In LPT, both CAE and CME of *E. citriodora* also showed significant dose dependent anthelmintic activity (Table-2). CAE (ED_{50}= 5.305 and ED_{99}= 157.326mg/ml) at 100 mg/ml concentration showed 100% paralysis of L_3 larvae, however, at 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/ml concentration, paralysis of L_3 were 98, 78, 71, 50, 36 and 25%, respectively. However, CME (ED_{50}= 5.503 and E_{99}= 165.447 mg/ml) also showed 100% paralysis of L_3 larvae at 100mg/ml concentration. However, it was 89, 83, 79, 52, 30 and 22% at 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/ml concentration, respectively (Table-2 and 3). TBZ caused 100% paralysis of third stage larvae (L_3) of gastrointestinal nematodes in all tested concentrations (Table-2).

In-vivo* anthelmintic Efficacy of *E. citriodora

Coproculture examination revealed the presence of *Haemonchus contortus* (68-85%) as predominant GI nematode followed by *Oesophagostomum columbianum* (8-21%), *Trichostrongylus colubriformis* (5-8%) and *Strongyloides* spp. (2-4%) in animals. CME showed highest (34.11%) reduction in faecal egg count followed by CAE (30.55%) and lowest (7.5%) against CP @ 2gm/kg b.wt. on 7 DPT, however, it was 12.56, -27.08 and -12.57%, respectively on 14 DPT.

CME showed highest (35%) reduction in faecal egg count followed by CAE (13.15%) and lowest (6.89%) against CP @ 4gm/kg b.wt. on 7 DPT, however, it was 33.80, 21.05 and 10.34%, respectively on 14 DPT (Table-4). Non-significant difference was found at different doses between CP, CAE and CME.

Phytochemical Analysis of Extracts

On phytochemical analysis of *E. citriodora*, CAE revealed the presence of alkaloids, flavonoid, reducing sugar, saponins, tannins and triterpenes. However, CME contained alkaloids, coumarin, reducing sugar, saponin, tannins and triterpenes.

Table 1: Average% corrected mortality of CP, CAE and CME of *E. citriodora* and TBZ against adult *H. contortus*

Extracts	Average% mortality at different% concentration							Average% Corrected mortality
	0.156	0.312	0.625	1.25	2.5	5	10	
CP	40	40	100	100	100	100	100	82.86
CAE	100	100	100	100	100	100	100	100
CME	0	0	0	100	100	100	100	57.14
Average% Corrected mortality	46.66	46.66	66.66	100	100	100	100	
TBZ	0	0	0	100	100	100	100	57.14

CP- Crude Powder, CAE- Crude aqueous extract, CME- Crude methanol extract

Table 2: Percent efficacy of *Eucalyptus citriodora* extracts and TBZ on eggs hatching inhibition and larvae paralysis of GI nematodes of Goats

Concntration (mg/ml)	% inhibition of egg hatching			% larvae paralysis		
	CAE	CME	TBZ	CAE	CME	TBZ
1.56	60	54	100	25	22	100
3.125	76	60	100	36	30	100
6.25	79	73	100	50	52	100
12.5	86	91	100	71	79	100
25	92	98	100	78	83	100
50	100	100	100	98	89	100
100	100	100	100	100	100	100

Table 3: ED_{50} and ED_{99} (mg/ml) of crude aqueous and methanolic extracts of *E. citriodora* in Egg Hatch Assay and Larval Paralysis Test

E. citriodora	Tests	ED_{50} mg/ml			ED_{99} mg/ml		
		ED_{50}	Lower limit	Upper limit	ED_{99}	Lower limit	Upper limit
CAE	EHA	0.997	0.315	1.812	105.003	44.295	624.484
	LPT	5.305	3.537	7.416	157.326	75.054	575.437
CME	EHA	1.820	0.984	2.659	52.182	26.892	183.281
	LPT	5.503	3.818	7.490	165.447	83.543	510.828

Table 4: *In-vivo* anthelmintic efficacy of *Eucalyptus citriodora* against mixed infection of gastrointestinal nematodes in goats

Groups		GI	GII	GIII	GIV	GV	GVI
Treatment & dose rate (per kg b.wt)		CP @ 2gm	CAE @ 2gm	CME @ 2gm	CP @ 4gm	CAE @ 4gm	CME @ 4gm
% FECRT	(7DPT)	7.5	30.55	34.11	6.89	13.15	35
	(14DPT)	-12.57	-27.08	12.56	10.34	21.05	33.80

Discussion

In the present study of *In vitro* trial of *Eucalyptus citriodora*, crude aqueous extract showed maximum (100%) adulticidal activity against *H. contortus* followed by crude powder (82.86) and minimum (57.14%) with crude methanolic extract (Table-6). Kuamr *et al.* (2015) [18] also reported adulticidal activity of *Eucalyptus globulus* against *H. contortus*. They also observed that methanolic extract had maximum efficacy.

In EHA both extracts showed 100% inhibition of egg hatching @50 and 100mg/ml, similar observation has also been reported by Kanojiya *et al.* (2015) [16]. However, Kuamr *et al.* (2015) [18] also observed dose-dependent inhibition of egg hatching with maximum (100%) against methanolic extract @50mg/ml. In contrary to the present findings, Macedo *et al.* (2010) [23] and Macedo *et al.* (2011) [22] reported 99.27% and 98.80% inhibition of egg hatching against *Eucalyptus staigeriana* and *Eucalyptus citriodora* essential oil @ 1.35mg/ml and 5.3mg/ml, respectively. The variations in inhibition of egg hatching at lower concentration might be due to the differences in method, type of extraction, collection time, maturity of leaves, soil type and geographical areas in which plant is grown (Peach and Tracey, 1955) [31]. It might also be due variation in species of plants which contain different phytochemical constituents responsible for anthelmintic activity.

In Larval Paralysis Test, crude aqueous showed better ED_{50} (5.305 mg/ml) and ED_{99} (157.326mg/ml) values than crude methanolic extracts (ED_{50}= 5.503 and ED_{99}= 165.447 mg/ml). However, Kanojiya *et al.* (2015) [16] observed that methanolic extract revealed better ED_{50} (6.352 mg/ml) and ED_{99} (305.48mg/ml) in comparison to aqueous extract (ED_{50}=6.878 and ED_{99}= 878.027mg/ml) of *Eucalyptus globulus*. It might be due to differences in plant used in the experiment. In the present study, crude aqueous extract showed better dose-dependent larval paralysis. The dose-dependent larvicidal activity of *H. contortus* has also been reported by Macedo *et al.* (2010) [23].

In *in-vivo* trial, crude methanolic extract of *Eucalyptus citriodora* showed better anthelmintic efficacy than crude aqueous extract and crude powder. Anthelmintic activity of *Eucalyptus* spp. against gastrointestinal nematodes has also been reported by several workers (Mesquita *et al*., 2013; Macedao *et al.*, 2010 and Kanojiya *et al.*, 2015) [27, 23, 16]. In general, higher anthelmintic efficacy of herbal plants have been observed at higher doses by several workers, but in this trial, crude powder and crude aqueous extract of *Eucalyptus citriodora* sowed higher efficacy at low dose (@2gm/kg b.wt.) than higher dose (@4gm/kg b.wt.). It might be due to drug metabolism could have taken longer time with increase in doses and due to early saturation of aqueous extract of the

individual plants during preparation of extracts (Kimani *et al.*, 2014) [17].

The result of coproculture indicated that *E. citriodora* had lower anthelmintic activity against *H. contortus* than other parasites. The results are in agreement with the findings of Kanojiya *et al.* (2015) [16].

The phytochemical results are partially in agreement with the finding of Javed *et al*. (2012) [15] where they reported alkaloids, flavonoids, tannins, glycosides, saponins, triterpenoids in aqueous and in addition to steroids and phenols in methanolic extract. However, presence of tannins, flavonoids and steroids in crude aqueous and methanolic extracts have been observed by Sharma *et al*. (2009) [34] while Bhagat *et al*. (2012) [1] found the presence of anthraquinon, saponins, tannins, flavonoids and cardiac glycosides as phytoconstituents of the methanolic extract.

E. citriodora showed anthelmintic activity against mixed infection of gastrointestinal nematodes of goats might be due to presence of tannins, alkaloids, saponins, reducing sugars, coumarins, flavonoids and triterpenes. Condensed tannins can impair vital process like feeding and reproduction of the parasites or may bind and disrupt the integrity of cuticle of the parasites (Dave *et al*., 2009; Zafar *et al*., 2009) [8, 40]. Min and Hart (2003) [28] reported that condensed tannins can improve protein nutrition by binding to plant proteins in the rumen that results into preventing microbial degradation and increasing the flow of amino acids in upper part of intestine. Alkaloid plays an important role in the interaction of plants with their environment (Fester, 2010) [9]. Alkaloid inhibits essential enzymatic system in parasites and also plays an important role in inhibition of mobility of larvae. In several studies, alkaloid isolated from higher plants has been used as anthelmintic agents (Watts *et al*., 2010) [39]. Havsteen (2002) [11] reported that many plant extracts which showed a high anthelmintic activity revealed the presence of flavonoids along with other phytoconstituents. It is possible, that different developmental stages of helminths might posses different susceptibility to selected flavonoids as observed by Braguine *et al.* (2012) [4].

Conclusion

Results of present study indicate that *E. citriodora* showed low to moderate anthelmintic activity and did not reach the required therapeutic dose to controlling gastrointestinal nematodosis. But, it may be included in integrated parasite management to achieve sustainable parasite control in goats.

Acknowledgement

The authors thankfully acknowledge the Director, Experiment Station and Dean, College of Veterinary and Animal Science, G. B. Pant University of Agriculture & Technology, Pantnagar for providing financial assistance and all the necessary facilities during the entire course of study.

References

1. Bhagat M, Sharma V, Saxena AK. Anti- proliferative effect of leaf extract of *Eucalyptus citriodora* human cancer cells *in vitro* and *in-vivo*. Ind. J Biochem and Biophysics. 2012; 49:451-457.
2. Bhatnagar SS, Santapau H, Desa JDH, Rac TN. Biological activity of Indian medicinal plants. Ind. J Med. Ressearch. 1961; 49:799-813.
3. Bhardwaj P, Varshneya C, Sharma V. Nematicidal activity of methanolic flower extract of *Tagetes patula* against *Haemonchus contortus* worms. Ind. J Anim. Sci. 2014; 85:241-243.
4. Braguine CG, Bertanha CS, Goncalves UO, Magalhaes LG, Rodrigues V, Gimenez VMM *et al*. Schistosomicidal evaluation of flavonoids from two species of Styrax against *Schistosoma mansoni* adult worms. Pharmaceut. Biology. 2012; 50:925-929.
5. Chagas ACS, Vieira LS, Freitas AR, Araújo MR, Araújo-Filho JA, Aragão WR *et al*. Anthelmintic efficacy of neem (*Azadirachta indica*) and the homeopathic product Fator Vermes in Morada Nova sheep. Vet. Parasitology. 2008; 151:68-73.
6. Coles GC, Bauer FHM, Borgsteede S, Greerts S, Klei TR, Taylor MA, Waller PJ. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitology. 1992; 44:35-44.
7. Dash K, Hall K, Barger IA. The role of arithmetic and geometric worm egg counts in faecal egg count reduction test and in monitoring strategic drenching programs in sheep. Aust. Vet. Journal. 1988; 65:66-68.
8. Dave S, Patil UK, Bhaiji A, Baghel US, Yadav SK, Sharma VK. *In vitro* anthelmintic activity of leaves of *Spinacia oleracea*. Int. J Toxicological and Pharmacological Research. 2009; 1:21-23.
9. Fester K. Plantalkaloids. Publishedonline, 2010. doi:10.1002/ 9780470015902. A 0001914
10. Hasegawa T, Takano F, Takata T, Niiyama M, Ohta T. Bioactive monoterpene glycosides conjugated with gallic acid from the leaves of *Eucalyptus globules*. Phytochemistry. 2008; 69:747-753.
11. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther. 2002; 96:67-202.
12. Hoste H, Chartier C, Lefrileux Y, Goudeau C, Broqua C, Pors I *et al*. Targeted application of anthelmintics to control trichostrongylosis in dairy goats: result from a 2-year survey in farms. Vet. Parasitology. 2002; 110:101-108.
13. Jabbar A, Iqbal Z, Kerboeuf D, Muhammad G, Khan MN, Afaq M. Anthelmintic resistance; the state of play revisited. Life Science. 2006; 79:2413-2431.
14. Jaiswal AK, Sudan V, Shanker D, Kumar P. Emergence of Ivermectine resistance in a semi-organized farm of Mathura district- India. Veterinarski Arhiv. 2013; 83:275-280.
15. Javed S, Shoaib A, Mahmood Z, Mushtaq S, Iftikhar S. Analysis of phytochemical constituents of *Eucalyptus citriodora* L. responsible for antifungal activity against post-harvest fungi. Nat. Prod. Research. 2012; 26:1732-1736
16. Kanojiya D, Shanker D, Sudan V, Jaiswal AK, Parashar R. *In vitro* and *In-vivo* efficacy of extracts of leaves of *Eucalyptus globulus* on ovine gastrointestinal nematodes. Parasitology Research. 2015; 114:141-148.
17. Kimani D, Kareru PG, Karanja JM, Njonge FK, Githira PN, Kutima HL *et al*. *In-vivo* activity of two herbal plant mixtures against gastrointestinal nematodes in ruminants. J. Applied Chemestry. 2014; 8:21-28.
18. Kuamr S, Kumar A, Kumar N, Sharma N, Singh M, Gopinath D *et al*. *In vitro* anthelmintic activity and phytochemical analysis of for tropical plants against *Haemonchus contortus*. Int. J Advanced Res. 2015; 6:541-548.
19. Kumar RR, Vatsya S, Yadav CL. *In vitro* anthelmintic

activity of *Prunus persica* against *Haemonchus contortus*. Progressive Res. 2014; 9:466-468.
20. Kumar RR, Vatsya S, Yadav CL. Anthelmintic activity and Phytochemical analysis of *Chenopodium album* against *Haemonchus contortus*. J Vet. Sci. 2016; 2:53-57.
21. Le Jambre LF. Egg hatch as an *in vitro* assay of Thiabendazole resistance in nematodes. Vet. Parasitol. 1976; 2:385-391.
22. Macedo ITF, Bevilaqua CML, Oliveira LMB, Camurça-Vasconcelos ALF, Vieira LS, Amóra SSA. Evaluation of *Eucalyptus citriodora* essential oil on goat gastrointestinal nematodes. Rev. Bras. Parasitol. Vet. 2011; 20:223-227.
23. Macedo ITF, Bevilaqua CML, Oliveira LMB, Camurça-Vasconcelos ALF, Vieira L da S, Oliveira FR *et al*. Anthelmintic effect of *Eucalyptus staigeriana* essential oil against goat gastrointestinal nematodes. Vet. Parasitol. 2010; 173:93-98.
24. MAFF. Ministry of Agriculture, Fisheries and Food, Manual of veterinary parasitological laboratory techniques. HMSO, London, 1986, 1-152.
25. MAFF. Manual of Veterinary Parasitology Laborator Parasitology. HMSO, London, 1971, 5-288.
26. Melo ACFL, Reis IF, Bevilaqua CML, Vieira LS, Echevarria FM, Melo LM. Nematodeos resistentes a anti-helminticos em rebanhos de ovinos e caprinos do estado do Ceara, Brasil. Ciencia Rural. 2003; 33:339-344.
27. Mesquita MA, Júnior JBS, Panassol AM, Oliveira EF, Vasconcelos ALCF, Paula HCB *et al*. Anthelmintic activity of *Eucalyptus staigeriana* encapsulated oil on sheep gastrointestinal nematodes. Parasitol. Res. 2013; 112:3161-3165.
28. Min BR, Hart SP. Tannins for suppression of internal parasites. J Anim. Sci. 2003; 81:102-129.
29. Neogi NC, Baliga PAC, Srivastava RK. *In vitro* anthelmintic activity of some indigenous drugs. J. Ind. Med. Res. Assoc. 1964; 41:435-437.
30. Ombasa O, Karem PG, Rukunga G, Mbaria J, Keriko JM, Njonge FK. *In vitro* Anthelmintic effects of two Kenyan plant extracts against *Haemonchus contortus* Adult worm. Int. J Pharmacol. Res. 2012; 2(3):113-116.
31. Peach K, Tracey MV. Morden methods of plant Analysis. Springer- Verlag, Berlin, 1995, 197-225.
32. Rialch A, Vatsya S, Kumar RR. Detection of Benzimidazole resistance in gastrointestinal nematodes of sheep and goats of sub-Himalayan region of northern India using different tests. Vet. Parasitology. 2013; 198:312-318.
33. Sangwan N, Sangwan AK. *In vitro* effect of leaf extract of *Melia azedarach* on mortality of *H. contortus*. Ind. J Ani. Res.1998; 32:70-72.
34. Sharma A, Rathore MK, Sharma N, Sharma K. Phytochemical evaluation of *Eucalyptus citriodora* Hook, and *Lawsonia inermis* Linn. Biosciences Biotechnology Res. Asia. 2009; 6:639-645.
35. Sofowara A. Medicinal plants and traditional medicine in Africa. Chicchesfer, John Walley, 1982.
36. Soulsby EJL. Text book of veterinary clinical parasitology Helminths. Blackwell Scientific publication, Oxford, 1965; I:334-381.
37. Tariq KA, Chishti MZ, Ahmad F, Shawl AS. Anthelmintic activity of extracts of *Artemisia absinthium* against ovine nematodes. Vet. Parasitology. 2009; 160:83-88.
38. Tyasi TL, Lv ZC, Gxasheka M, Nkohla MB. Effectiveness of *Elephantorhiza elephantina* as traditional plant used as the alternative for controlling coccidian infections in goats. J Bio. Agric. Healthcare. 2015; 5:163-167.
39. Watts KR, Tenney K, Crews P. The structural diversity and promise of antiparasitic marine invertebrate-derived small molecules. Curr. Opinion Biotechnol. 2010; 21:808-818.
40. Zafar I, Bachaya HA, Nisar MK, Jabbar A, Gilani AH, Islam UD. *In vitro* and *in-vivo* anthelmintic activity of Terminalia arjuna bark. Int. J Agri. Biol. 2009; 11:273-278.