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# *In vitro* efficacy of *Tribulus terretris* L. & *Ziziphus mauritiana* Lam. as potential anthelmintics against *Haemonchosis contortus*

**ABRAR HUSSAIN[1], AQSA KHALIL[1], SAIRA ZAHEER[1], MUHAMMAD LATEEF[2] & MUHAMMAD MANSHA[1]**

[1]*Division of Science and Technology, University of Education, Lahore, Pakistan*
[2]*Department of parasitology, University of Veterinary and Animal Sciences Lahore, Pakistan*

## ABSTRACT

Ruminants are common victims of helminthic infections which curb the efficient production of livestock. Chemical anthelmintic are used to control parasitic infections but their persistent use develop resistance in parasites. In this connection local plants/ their parts are being used to explore their anthelmintic potential. The aim of the present study was to determine the anthelmintic activity of *Tribulus terrestris* L. and *Ziziphus mauritiana* Lam. Whole plant of *Tribulus terrestris* L. and leaves of *Ziziphus mauritiana* Lam. were used. The plant materials were dried, ground firmly, macerated in ethanol and then the crude ethanolic extract (CEE) was prepared. Adult motility assay (AMA) was carried out on adult *Haemonchus contortus* to analyze the anthelmintic efficacy of CEE of the whole plant and the leaves of *Tribulus terrestris* L and *Ziziphus mauritiana* Lam. The motility of insects was assessed at concentration of 2, 4, 6 & 8 mg/ml after 2, 4, 6 & 8 hours of post treatment and dead worms were noted. The 8mg/ml concentration of CEE of *Tribulus terrestris* L. and *Ziziphus mauritiana* Lam. exhibited 90% and 93% mortality rate, respectively, after 8 hours of post treatment. The positive control, levamisole showed 100% mortality at 0.5mg/ml concentration. It is concluded that *Tribulus terrestris* L. and *Ziziphus mauritiana* Lam. possess wormicidal potential and their extracts have anthelmintic activity against *Haemonchosis contortus*.

**Key Words:** Anthelmintic, *Tribulus terrestris* L., *Ziziphus mauritiana* Lam., *Haemonchus contortus, a*dult motility test (AMA)

## INTRODUCTION

Helminthic infections are documented as frequent cause of many diseases among humans and cattle's (Chaturvedi *et al*., 2009; Charlier *et al*., 2014). Among helminthic worms, *Haemonchus contortus* is considered the most predominant and economically devastating worm species thriving in warm and humid areas of the world (Rahman *et al*., 2011). Pharmaceutically derived anthelmintic drugs are being used on wide scale to control *H. contortus* in domestic animals (Verma *et al*., 2014). The widely used anthelmintic drugs to control helminthic worms are, Piperazine, Benzimidazoles, Morantel, Levamisole, and Niclosamide (Holden-Dye, 2007). The widespread and inappropriate use of these anthelmintic drugs may occasionally become source of resistance development in parasitic strains. The use of these drugs against helminthic infections is in question these days (Alemu *et al*., 2014). Different species of gastrointestinal nematodes show different level of resistance to major groups of anthelmintic drugs (Shalaby, 2013). The resistance in parasites has reduced productivity alongwith the enhancement of risk of contamination and has increased the overall budget for managing the infections ( Dewanjee *et al*., 2007; Saddiqi *et al*., 2010). To overcome the issue of parasitic resistance and helminthic infections herbal anthelmintics are being explored (Hussain *et al*., 2011).

Local medicinal plants are being used as alternative of antiparasitic drugs in developing countries (Satrija *et al*., 2001; Jagatheesan & Senthilkumar, 2011). The extracts of medicinal plants are employed as single or in combination with other drugs against various diseases and disorders. These natural anthelmintics are considered a unique and useful source for control

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***Corresponding author**: ranaabrar299@gmail.com

of helminthic infections (Bahmani *et al*., 2014). The bio analysis of local plants can reveal regimen of effective compounds which may be environment friendly with wide killing potential (Eguale *et al*., 2007; Humeera *et al*., 2013; Yadav *et al*., 2014). Moreover, the extracts of these plants have been evaluated *in vitro* and *in vivo* and found successful against variety of infections (Iqbal *et al*., 2005 & Nawaz *et al*., 2014). The active ingredients of these herbal drugs showing anti parasitic activity may include cysteine proteinases, or secondary metabolites, such as glycosides, alkaloids and tannins (Githori *et al*., 2006).

*Tribulus terrestris* L. (Bakhra) is a local plant of Pakistan and is abundantly found in warm areas of Asia, Africa, Europe, America and Australia (Parimala *et al*., 2011). Its various parts contain pharmaceutically significant compounds like flavonoids, glycosides, saponins and alkaloids. The medicinal compounds derived from this plant possess diuretic, aphrodisiac, immunomodulatory, antidiabetic, anti-inflammatory, analgesic, antibacterial, anthelmintic, anticancer and larvicidal potential (Chhatre *et al*., 2014).

*Ziziphus mauritiana* Lam. (common name "Bairy"), is another widely found plant in Punjab, Pakistan. The different parts of the plant have proved their therapeutic significance against pulmonary ailments, ulcers, liver diseases, asthma, fever and epilepsy. Its anthelmintic role has largely been accepted (Hussain *et al*., 2011). The crude methanolic leave extract of plant reflect antibacterial potential against variety of bacteria like *Bacillus subtilis, Escherichia coli*, *Staphylococcus aureus* and antifungal activity against different fungi (Mahesh & Satish, 2008).

The current study is carried out to evaluate the anthelmintic activity of *Tribulus terrestris* L. and *Ziziphus mauritiana* Lam against adult *Haemonchus contortus* worms (common parasites of ruminants). The study will improve the existing regimen of wormicides and will have direct impact on ruminant health and thus help boosting the economy of the country.

## MATERIALS AND METHODS

### Plant Material Used

The plants used for analysis of anthelmintic activity have been listed in Table No-1. The plants were selected because of their use in ethno-veterinary system of Pakistan. The plant materials (leaves, roots and stem) were selected from the fields of Arifwala, Punjab. Both plants were identified and validated by botany department of University of Education, Lahore. The selected plant parts were dried, ground firmly in powdered form and stored at 4°C.

**Table-I. Plants and their parts used for Anthelmintic evaluation**

| Plant Name | Part Used | Family | Local Name |
|---|---|---|---|
| *Tribulus terrestris* L. | Whole plant | Zygophyllaceae | Bakhra/Kharkhisk |
| *Ziziphus mauritiana* Lam. | Leaves | Rhamnaceae | Bairy |

### Crude Ethanolic Extract (CEE)

The method used for formation of crude extract of plant material was adopted from Jabbar *et al*. (2007). Selected plant material (20gm) was placed in 200ml of ethanol for 3 days at room temperature for maceration. The material was filtered and the filtrate was concentrated at 46°C to produce a thick and dark colored crude extract. The yield was estimated by the following formula and was stored at -4°C.

Extract yield = weight of dry matter before maceration) – weight (dry matter after maceration)

Percentage yield = (extract yield/ weight of dry matter before maceration) × 100.

The percentage yield of *Tribulus terrestris* L. and *Ziziphus mauritina* Lam was 93% and 96% respectively.

Formulations containing four concentrations of the two extracts like 2mg/ml,

4mg/ml, 6mg/ml and 8mg/ml were prepared in phosphate buffer saline (PBS). The negative control (PBS) and the positive control (levamisole, 0.5mg/ml) were freshly prepared.

**Test for Anthelmintic Activity**

Adult Motility Assay (AMA) was carried out for the determination of *in vitro* anthelmintic activity, as described by Singh *et al*. (1985). *Haemonchus contortus* worms (adult) were recovered from abomasi of freshly slaughtered goats. Various concentrations (of CEE such as 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml were applied to 10 worms in petri dishes. The experiment was replicated in triplicate. Levamisole (0.5mg/ml) was taken as positive control and PBS as negative control.

The movement of worms was noted for each treatment, when no movement of any sort could be observed except when the worms were shaken vigorously. The reading was taken after every two hours in experimental and control petri dishes for eight hours. The worms' resistance to motility in different concentrations of extract was assessed as a benchmark for anthelmintic activity. The dead worms were counted after 2, 4, 6, and 8 hours intervals.

**Statistical Analysis**

The data was analyzed by the statistical program, COSTAT VERSION 6.303. Statistical significance of the data ($p<0.05$) was calculated by three-way ANOVA. When ANOVA indicated significant effects ($p<0.05$), LSD (Latin Square Design) test was used to compare the means.

## RESULTS

The findings of the present study revealed that both plants, *Tribulus terrestris* L. and *Ziziphus mauritiana* Lam had remarkable anthelmintic potential against adult *Haemonchus contortus* worms. *Tribulus terrestris* L. showed increase in mortality rate with increase in concentration and time. The highest mortality rate (90%) was recorded at 8mg/ml of concentration after 8 hour of treatment. *Ziziphus mauritiana* Lam. exhibited a slightly different behavior than *Tribulus terrestris* L. with an equal mortality rate at 2mg/ml, lower at 4mg/ml and then rapid increase at 6mg/ml (73%) and 8mg/ml (93%) after 6 and 8 hours of treatment as shown (Figure 1). Table III shows the mean effect of CEE (*Tribulus terrestris* L) at different concentrations for 2, 4, 6, 8hours of treatment. The mean effect of *Tribulus terrestris* L. at a concentration of 2mg/ml after 2 and 4 hours of post treatment was insignificant while at rests of the concentration it was significant ($p<0.05$). Taking a concentration of 4mg/ml after 4 and 6 hours of post treatment, the mean effect was insignificant while at rests of the concentration it was significant ($p<0.05$). Similarly, the concentration of 6 and 8mg/ml after 2 and 8 hours of post treatment were insignificantly different while rests of the combinations were significantly different ($p<0.05$) (table III). On the other hand, taking time period of post treatment as fixed variable and the concentration as varying factor, it was observed that mean effect was insignificant for 2 hours of post treatment. The mean effect for 4 hours of post treatment at 4 and 6 mg/ml concentration was insignificant while a rest of the concentration was significant ($p<0.05$). Similarly, mean effect after 6 hours of treatment at 2mg/ml and 4 mg/ml concentration was significant and at 6mg/ml and 8mg/ml concentration was also significant ($p<0.05$). Further, mean effect after 8 hours of treatment was significant at 2mg/ml and 4mg/ml concentration while with rest of the concentration nit was insignificant. Table IV represents similar results of post treatment of *Ziziphus mauritiana* Lam on mortality of *Haemonchus contortus* at different time intervals.

**Table III: *In vitro* effect of crude extract of *Tribulus terrestris* L. at different concentrations on mortality of adult *Haemonchus contortus* after 2, 4, 6 and 8 hours of post treatment**

| Concentration | 2 hours | 4 hours | 6 hours | 8 hours |
|---|---|---|---|---|
| 2 mg/ml | b1.33±0.33a | b0.33±0.33b | a3.00±0.00a | ab1.67±0.33a |
| 4 mg/ml | b0.67±0.33a | ab1.67±0.33b | ab2.00±0.58a | a2.33±0.33a |
| 6 mg/ml | b0.67±0.33a | ab1.67±0.67b | a3.00±0.58a | b1.33±0.33a |
| 8 mg/ml | b1.33±0.67a | a3.33±0.33a | ab2.67±0.67a | b1.67±.88a |

Means followed by the same letter do not differ significantly in a row ($p<0.05$)
Means preceded by same letter do not differ significantly in a column ($p<0.05$)

**Table IV: *In vitro* effect of crude extract of *Ziziphus mauritiana* Lam. at different concentrations on mortality of adult *Haemonchus contortus* after 2, 4, 6 and 8 hours of post treatment.**

| Concentration | 2 hours | 4 hours | 6 hours | 8 hours |
|---|---|---|---|---|
| 2 mg/ml | ab1.67±0.33a | b0.67±0.33b | ab1.33±0.67a | a2.67±0.33a |
| 4 mg/ml | b0.67±0.33a | b0.33±0.33b | ab1.67±0.67a | a2.33±0.33a |
| 6 mg/ml | a1.33± 0.67a | a1.67±0.33b | a2.00±0.58a | a2.33±0.33a |
| 8 mg/ml | b1.67±0.67a | a3.33±0.33a | ab2.00±0.58a | ab2.33±0.33a |

Means followed by the same letter do not differ significantly in a row($p<0.05$)
Means preceded by same letter do not differ significantly in a column($p<0.05$)

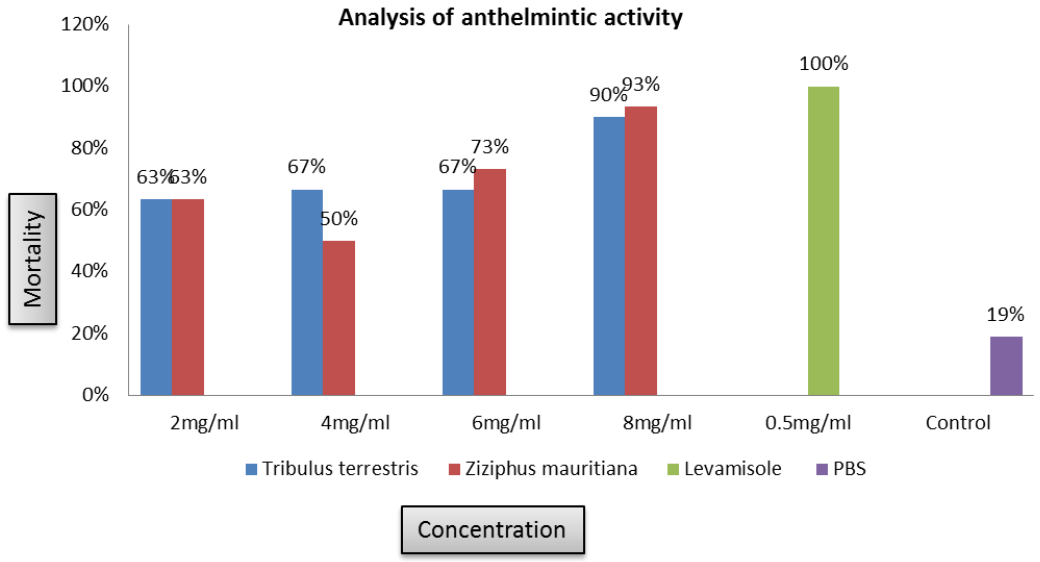


**Fig. 1:** *In vitro* evaluation of crude ethanolic extract of *Ziziphus mauritiana* Lam. and *Tribulus terrestris* L at 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml concentration against adult *Haemonchus contortus*.

## DISCUSSION

The findings of the current study showed that both plants, *Tribulus terrestris* L. and *Ziziphus mauritiana* Lam. had extensive anthelmintic potential. The wormicidal efficacy of the said plants rapidly increased with increase of treatment time. *Tribulus terrestris* L. exhibited 90% mortality and *Ziziphus mauritiana* Lam. showed 93% mortality at 8mg/mL of dose. The efficacy of the said plants was comparable to levamisole, the positive control which exhibited 100% mortality within first two hours of treatment at low dose (0.5mg/ml).

Our findings are in line with the results of Deepak *et al*. (2002) who showed that *Tribulus terrestris* L. had effective anthelmintic (Deepak *et al*., 2002) and anticancer (Bedir *et al*., 2002) potential. Similarly, *Ziziphus mauritiana* Lam. (Rhamnaceae) was known to possess good anthelmintic (Hussain 2008 & Hussain *et al*., 2011) and antioxidant (Naz *et al.,* 2013) activities.

The anthelmintic activity of these plants was further supported by phytochemical screening, a state of the art technique. It showed that extract of *Ziziphus mauritiana* Lam. contained flavonoids, tannins and saponins (Dahiru *et al*., 2005) which were attributed with good anthelmintics potential (Molan *et al*., 2000a,b; Iqbal *et al*., 2002; Goyal *et al*., 2012). Similarly, Deepak *et al.* (2002) reported that *Tribulus terrestris* L. contained tribulosin and sitosterol glucoside as active components responsible for anthelmintic activity which is in line with our work.

The results of present study are supported by Deepak *et al*. (2002) who documented that 50% methanol extract of *Tribulu sterrestris* L. on fractionation and chromatographic separation produced tribulosin, saponin and β-sitosterol-D-glucoside. These compounds revealed anthelmintic activity at wide scale. Similarly, *Ziziphus mauritiana* Lam. showed anthelmintic activity by impeding hatching of nematode eggs. Our findings are consistent with Diehl *et al*. (2004) who showed that ethanolic leaf extract of *Ziziphus mauritiana* Lam caused 90% larval mortality against *H. contortus*.

## CONCLUSION

*Tribulus terrestris* L. and *Ziziphus mauritiana* Lam. have substantial anthelmintic potential against nematode worms. The plants can be used in future as pharmaceutical agents if their anthelmintic activity is evaluated against other worms and parasites. Studies to determine the biologically active compounds of these plants will have positive impact on animals and human health.

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