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SCREENING FOR ANTHELMINTIC ACTIVITY OF *CLEOME VISCOSA* PLANT EXTRACT *IN VITRO*

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Abstract: The anthelmintic effect of aqueous and alcoholic extracts of the plant *Cleome viscosa* was studied *in vitro* by egg hatch assay method against the gastrointestinal nematodes of small ruminants. The results revealed that both aqueous and alcoholic extracts of the plant showed significant anthelmintic activity at 8% level and the effect was dose dependant.
Keywords: *Cleome viscosa*, Anthelmintic, Egg hatch assay.

Introduction

Parasitic diseases remain as major constraint to livestock farming throughout the world. Gastrointestinal nematodes are of major economic importance in domesticated livestock, since intestinal parasitism ends up in production loss. Chronic use of chemical anthelmintics has led to the development of resistance, problem of tissue residues and environmental pollution (Jenina Demeler et al.,2012). This has led to the use of natural products by Indian small holder livestock farmers (Alagesaboopathy,2009).

Cleome viscosa Linn (Syn. *Cleome icosanra*) a member of the family Capparidaceae is a widely distributed, sticky herb with yellow flowers and strong penetrating odour. It is known as Naaikkadugu in Tamil and found throughtout the greater part of India. The plant is reported to be antipyretic, analgesic and anticonvulsant (Parimaladevi *et al*., 2003, Sharma *et al*., 2010, Amrita *et al*., 2010). But data on the anthelmintic activity of the plant are very limited. So this study aims at examining the *invitro* effect of crude alcoholic and aqueous extracts of *Cleome viscosa* for their anthelmintic activity against the gastrointestinal nematodes of small ruminants.

Materials and Methods

Preparation of plant extracts

Fresh samples of the plant *Cleome viscosa* were collected in and around the campus of

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Veterinary College and Research Institute, Namakkal. They were authenticated by a botanist and then shade dried thoroughly and powdered by using a laboratory mixer. Twenty grams of the powdered sample was mixed with 100 ml of distilled water and 100 ml of 95% ethanol separately to prepare its aqueous and alcoholic extracts. Both of them were kept on an electrical shaker for three hours at room temperature and then left to stand overnight. Then the mixtures were filtered into conical flasks using Whatman filter paper No.1.

The filtrate was then concentrated by air drying to yield a solid (aqueous) and a semi-solid (alcoholic) masses whose weights were then determined. From these concentrates different concentrations (1%, 2%, 4% and 8%) of the extracts were prepared by using respective solvents.

Egg hatch assay

Collection of eggs for assay

Pooled faecal samples were collected from resistance suspected flock and brought to the laboratory in an air tight container. In the laboratory, the faecal sample was transferred to a 5 lit plastic container, to which sufficient quantity of tap water was added and then thoroughly kneaded with hand till homogenous suspension was obtained. The faecal suspension was strained through series of metal strainer with different pore size to obtain suspension devoid of coarse particles. This suspension was then kept undisturbed for 30 minutes in order to allow the eggs and other minute particles get sedimented at the bottom of the jar. Thereafter, the supernatant was discarded, while the sediment was transferred to glass beaker. To this saturated salt solution was added and kept undisturbed for 5 minutes to allow the eggs get floated up to the top layer of solution. Then carefully the top layer of the solution was decanted into another glass beaker, to which triple the quantity of tap water was added and allowed to stand for 10 minutes. Then, the supernatant was discarded and sediment was equally transferred to 15 ml polyallomer tubes and saturated salt solution was added up to three fourth of the tube capacity. The tubes were then centrifuged at 1000 rpm for 1 to 2 min. After centrifugation, the polyallomer tube was clamped just below the meniscus and the contents above the clamp was transferred into 15 ml polystyrene tubes and washed twice with distilled water by centrifugation. The sediments with little quantity of water retained at the bottom of the tube were pooled in an eppendorf tube and shaken well to obtain suspension. From this suspension, 100 ul was pipetted out; eggs were counted and re-suspended in such a manner that 100 ul of the suspension contained approximately 100eggs (Ponnudurai *et al*., 2005).

Test procedure

Egg hatch assay was performed in two different ‘24 well plates’ to screen the activity of aqueous and alcoholic extracts separately as per the method described by Jackson *et al.* (2001). A 100 ul of egg suspension (with approximately 100 eggs) was added to all the 6 wells of a row in both the test plates. Then 10 ul of different concentrations of the extracts (1%, 2%, 4% and 8%) were added in first four wells. Fifth and sixth wells served as negative and positive control respectively. For negative control 10 ul of distilled water and 10 ul of 95% ethanol were added in the respective plates. Similarly for positive control 10 ul of standard anthelmintic solution containing 0.2 ug of thiabendazole was added to the well in both the test plates. Then the volume of all the wells was made up to 2 ml (2000 ul) by adding 1890 ul of distilled water. The plates were then incubated at 20’c for 48 hours. Following incubation, one drop of helminthological iodine was added to each well and the larvae and unhatched eggs were counted using an inverted tissue culture microscope.

Results and Discussion

Then mean egg hatchability percentage of 1%, 2%, 4% and 8% alcoholic extracts of *Cleome viscosa* was 92.59, 92.34, 82.09 and 23.05 respectively. The positive and negative controls showed a mean hatchability percentage of 33.48 and 84.70 respectively.

The mean egg hatchability percentage of 1%, 2%, 4% and 8% aqueous extract of *Cleome viscosa* was 92.85, 92.20, 73.11 and 24.13 respectively. The positive and negative controls showed a mean hatchability percentage of 32.20 and 97.19 respectively.

From the data it is evident that both aqueous and alcoholic extracts of *Cleome viscosa* show marked anthelmintic activity by way of inhibition of hatchability of the egg. There was dose dependent increase in the effect with both aqueous and alcoholic extracts. The effect was highly significant at 8% level of aqueous and alcoholic extracts which was better than the positive control.

Somboon and Supanee, (2006) studied the biological activity of *Cleome spp.* extracts against the rice weevil, *sitophilus oryzae* L and found that the ethanolic extract of aerial part of *C.viscosa* highly inhibited the oviposition of the insects.

Nadkarni, (2001) studied about the major chemical compounds and medicinal properties of various herbal plants including *Cleome viscosa* and revealed that cleomiscosin A, B, C and D, cleosandrin and cleomeolide were the major chemical compounds of *Cleome viscosa.* Cleomiscosins and cleosandrins are coumarinolignoids, basically belonging to phenolic group of compounds (Anil *et al.,* 1985 and Nair,1979). The anthelmintic activity of

coumarinolignoids and other phenolic compounds have been well documented (Abdul Aziz *et al*., 2014 and Liu *et al*., 2012). Disophenol is the drug of choice for treatment of hook worms in dogs and sheep (Hall *et al.,*1981).

Cleomeolide is a diterpene lactone, chemically (Paquette *et al.,*1993). Ivermectin, a macrocyclic lactone is a widely used endectocide in veterinary practice (Geary and Morino,2012). Andrographolide, another diterpene lactone is proved to have antimalarial activity (Kirti Mishra *et al*., 2011). The above facts pave way for the hypothesis that the presence of the above chemical constituents in the extract of *Cleome viscosa* could have contributed to the anthelmintic effect of the plant *in vitro*.

Summary and Conclusion

The aqueous and alcoholic extracts of *Cleome viscosa* show marked anthelmintic activity. The bioactive principles of the plant, responsible for the activity are to be elucidated by further studies which may pave way for development of a cost effective clinically efficacious anthelmintic.

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