A Comparative Study of the Anthelmintic Potential of *Cleome Viscosa* L. and *Cleome Burmanni* W. and A.

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Pillai and Nair: Anthelmintic Potential of *Cleome viscosa* and *Cleome burmanni*

Methanol, aqueous and chloroform extracts of *Cleome viscosa* and *Cleome burmanni* were tested for anthelmintic potential against the Indian earthworm *Pheritima posthuma*. Different concentrations of the extracts ranging from 50-2000 µg/ml were tested and results expressed as time required for paralysis and death of the worms. Piperazine citrate was used as a reference standard and DMSO (1%) as the negative control. The methanol extracts of *Cleome viscosa* and *Cleome burmanni* exhibited significant anthelmintic activity. Methanol extract of *Cleome viscosa* at a concentration of 2000 µg/ml was detected to be the most effective treatment dose. Thin layer chromatography of methanol extracts of both plants revealed the presence of terpenoids.

Key words: Anthelmintic activity, *Cleome viscosa*, *Cleome burmanni*, DMSO, *Pheritima posthuma*, piperazine citrate, terpenoids

Helminthiasis is a disease caused by infestation with one or more intestinal parasitic worms. The worms usually reside in the gastrointestinal tract but may pose a threat to other organs by burrowing into them. Helminth infections cause many acute and chronic diseases among human beings as well as cattle. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia and pneumonia[1]. Anthelmintics are drugs that expel parasitic worms from the body by either stunning or killing them and are therefore also called vermifuges or vermicides. The most commonly used anthelmintic drug, piperazine, relaxes the large intestinal round worms and pinworms of man and domesticated animals so that they are eliminated with the faeces.

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A number of medicinal plants have been used to treat parasitic infections in man and animals[2,3]. The leaves and seeds of *Cleome viscosa* are being used as rubefacient and vesicant by traditional medicinal practioners in Africa and Asia. They are also used to treat infections, fever, rheumatism and headache. A perusal of the literature showed that the common weed, *Cleome viscosa* of the family Cleomaceae, has anthelmintic properities[4]. Anthelmintic activity has been reported in *Cleome viscosa* but not yet in *Cleome burmanni*. The present work intends to prove scientifically the anthelmintic potential of two species of *Cleome*, *Cleome viscosa* and *Cleome burmanni.*

The plants *Cleome viscosa* and *Cleome burmanni* collected were from Kariavattom, Thiruvananthapuram, Kerala. Fresh plants collected, were washed to remove adhered dirt, rinsed with distilled water, blotted and dried in shade. The shade-dried specimens were powdered in a mixer. This powder was used for

solvent extraction. About 15 g of the powdered plant material from each sample was subjected to Soxhlet extraction using 100 ml each of the three solvents such as methanol, chloroform and water. The extracts were concentrated under reduced pressure and preserved in refrigerator until further use.

The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al.*[5]. Anthelmintic activity was evaluated on the adult Indian earthworm *Pheritima posthuma* collected from Kerala Agricultural College, Vellayani, Thiruvananthapuram. The earthworms were raised and cultured using the commonly practiced method[6]. The earthworms were divided into 17 groups of four each. The experiment was conducted with the methanol, aqueous and chloroform extracts of both, *Cleome viscosa* and *Cleome burmanni*. Four earthworms of approximately equal size (similar type) were placed in the glass containers along with the 50 ml of the test samples. The formulations were prepared by dissolving the test extracts and reference standard in dimethylsulphoxide (DMSO, 1%). Three replicates were used for each concentration. DMSO (1%) was chosen as the common solvent for the standard drug as well as the samples of the plant extracts tested and was considered as negative control. Piperazine citrate was chosen as the reference standard at a constant dose of 200 µg/ml and served as the positive control. The rest of the treatment groups included the methanol, aqueous and chloroform extracts in five different concentrations such as 50, 100, 200, 1000 and 2000 µg/ml. Anthelmintic activity was judged at two different stages as paralysis and death. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for the death of the worms was recorded when the worms lost their motility completely followed with fading away of their body colours. Death was ascertained when the worms neither moved when shaken vigorously or when dipped in warm water (50°). Data was analyzed with one-way ANOVA using Duncan's test (SPSS 7.5 for Windows). The results are provided as mean±SEM (N=4). The value of $P<0.05$ was considered as significant.

The methanol, aqueous, and chloroform extracts of *Cleome viscosa* and *Cleome burmanni* were tested for the presence of constituents such as tannins, saponins, flavonoids, alkaloids, terpenoids and steroids using standard biochemical procedures[7]. Preliminary phytochemical screening detected terpenoids in all the three extracts (Table 1). The concentrated methanol-extract residue was chromatographed using benzene:ethylacetate (2:l) as the solvent system since anthelmintic activity was significantly high in methanol extracts. The plates were sprayed with Liebermann-Burchard's reagent after proper drying. The R_f values were noted for the separated components.

The methanol, aqueous and chloroform extracts of *Cleome viscosa* and *Cleome burmanni* exhibited anthelmintic activity in a dose-dependant manner. Among the extract concentrations chosen for the study, the extracts at 200, 1000 and 2000 µg/ml concentration exhibited significant anthelmintic effect. At each concentration, the methanol extract was most effective followed by the aqueous and chloroform extracts, respectively. The extracts at 2000 µg/ml concentration gave the shortest time for paralysis and death of worms. The methanol extract of *Cleome viscosa* at 2000 µg/ml concentration was detected to be the most effective. At this dose, the extract could cause paralysis of the earthworms in 10 min and death in 34 min while the methanol extracts of *Cleome burmanni* could cause paralysis in 29 min and death in 49 min. The methanol extract of C*leome viscosa* at the dose, 200 µg/ml had an effect comparable with that of the standard drug piperazine citrate at the same concentration. Almost all the

TABLE 1: PHYTOCHEMICALS TESTED IN *CLEOME VISCOSA* AND *CLEOME BURMANNI*

Phytochemicals	ME		AE		CE	
	C. v	*C. b*	*C. v*	*C. b*	*C. v*	*C. b*
Tannins	+	+	-	-	-	+
Saponins	+	+	+	+	+	+
Flavonoids	+	-	+	+	+	+
Alkaloids	-	-	-	-	+	-
Terpenoids	+	+	+	+	+	+
Steroids	+	+	-	+	+	+

ME: Methanol extract; AE: Aqueous extract and CE: Chloroform extract, **C. v - Cleome viscosa and* *C. b - Cleome burmanni*

TABLE 2: ANTHELMINTIC ACTIVITY OF EXTRACTS OF *CLEOME VISCOSA* AND *CLEOME BURMANNI*

Treatment	Conc. µg/ml	*Cleome viscosa*		*Cleome burmanni*	
		Paralysis (min) Mean±SEM	Death (min) Mean±SEM	Paralysis (min) Mean±SEM	Death (min) Mean±SEM
1% DMSO		-	-	-	-
Piperazine citrate	200	23.33±0.577^{e}	59±1^{e}	23.33±0.577^{a}	59±1^{b}
Methanol	50	-	-	-	-
	100	-	-	-	-
	200	26.66±0.577^{f}	58.66±0.577^{e}	40.33±0.577^{e}	69.33±0.577^{d}
	1000	20.33±0.577^{c}	48.33±0.577^{c}	34.33±0.577^{c}	59.0±1^{b}
	2000	10.33±0.577^{a}	34.33±0.577^{a}	29.0±0.0^{b}	49.66±0.577^{a}
Aqueous	50	-	-	-	-
	100	-	-	-	-
	200	30.33±0.577^{g}	65.33±0.577^{f}	55.33±0.577^{g}	83.66±0.577^{g}
	1000	22.00±0.0^{d}	53.33±1.155^{d}	41.33±1.155^{f}	71±1^{e}
	2000	13.33±0.577^{b}	40.66±1.155^{b}	39.00±0.0^{d}	60.33±577^{c}
Chloroform	50	-	-	-	-
	100	-	-	-	-
	200	55.33±0.577^{j}	90.33±0.577^{i}	70±0.0^{h}	99.33±0.577^{h}
	1000	48.0±0.0^{i}	80.66±0.152^{h}	54.66±0.577^{g}	84.33±0.577^{g}
	2000	39.66±0.577^{h}	73.00±1.0^{g}	42±0.0^{f}	75.33±0.577^{f}

'-'indicates absence of anthelmintic effect; Similar superscripts indicate homogenous sets. SEM- Standard error of mean.

extracts of both *Cleome viscosa* and *Cleome burmanni* could cause paralysis and death within a period less than 90 min, except the chloroform extract of *Cleome burmanni* at 200 µg/ml, which took 99 min for causing death of worms (Table 2). Statistical analysis revealed that the extracts of both *Cleome viscosa* and *Cleome burmanni* had a significant anthelmintic effect. The types of extract used as well as their different concentrations were detected to have an impact on the time taken for the paralysis and death of earthworms.

Terpenoids were earlier reported to be present in species of *Cleome*. Terpenoids and the glucosinolates such as glucocapparin and glucocleomin have been reported to be the major organoleptic compounds in *Cleome viscosa*[8]. The methanol extracts of *Cleome viscosa* and *Cleome burmanni* when subjected to thin layer chromatography (TLC) specific for terpenoids, showed the presence of three bands at R_f values of 0.78, 0.76 and 0.75 for *Cleome viscosa* and R_f values of 0.77, 0.75 and 0.74 for *Cleome burmanni*. These R_f values were found to correspond to that of certain triterpenoids[7].

The observed terpenoids might have been responsible for the anthelmintic activity exhibited by extracts of two species of *Cleome*. These results suggest that the extracts of both *Cleome viscosa* and *Cleome burmanni* could be used as effective vermicides.

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