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Research Article

ANTHELMINTIC ACTIVITY OF CLEOME HASSLERIANA SEED EXTRACTS

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ABSTRACT

Objective: *Cloeme hassleriana* (Spider Flower) seed extracts was investigated for anthelmintic activity. As there is no indication of publications regarding vermicidal activity of the plant, the present study was designed to investigate the anthelmintic potential of ethanolic, aqueous and total aqueous extracts of *Cloeme hassleriana.*

Methods: The Seed extracts of *Cloeme hassleriana* was investigated for anthelmintic activity using earthworms (Pheretima posthuma). Various concentrations (10-50 mg/ml) of plant extracts were tested in the bioassay. Piperazine citrate (10 mg/ml) was used as reference standard drug whereas normal saline as control. Determination of paralysis time and death time of the worms were recorded.

Results: The anthelmintic activities of the ethanol and water extracts suspended in water and fractionated successively with petroleum ether (40-60°C), diethyl ether, ethyl acetate and n-butanol. Extracts exhibited significant anthelmintic activity at highest concentration of 50 mg/ml. Fractions from the seeds of *Cloeme hassleriana* were evaluated in this study.

Conclusion: The result shows that aqueous extract possesses vermicidal activity and found to be effective as an anthelmintic.

Keywords: *Cloeme hassleriana seeds*; anthelmintic activity; Pheretima posthuma*;* Piperazine citrate and Normal Saline.

INTRODUCTION

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of under nourishment, anemia, eosinophilia and pneumonia [**1]**. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas [**2]**. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases [**3]**. Hence there is an increasing demand towards natural anthelmintic [4-10].

The genus cleome belongs to Cleomaceae family which occur in tropical and sub-tropical warm temperate regions, is related to the Capparaceae (in which it formerly included) and to the Cruciferae. There are approximately 577 species, subspecies, varieties, forms, and cultivars in this genus. Most are annual herb, some are shrub, erect or climbing, rarely trees, which are widely distribute from in India from Bihar, Orissa, Gujarat, Decan region, Konkan region; in field and places. In *Cleome* genus leaves are simple or compound containing 3 to 9 foliate, entire or serrulate. Flowers are white, yellow, pink, or purple in colour; found in branches or singly. There are four spreading sepals, four subequal petals. Sometimes sub unilateral, four to eight stamens inserted on the torn. Ovary is sessile or with many ovules on two parietal placentas. The fruit is capsular and usually elongated, sometimes inflated, sessile or stalked, split into two when ripe. They are one gelled, two valved. Valves are membranous, separated from persistent placentas. Seeds are reniform. Some species bear prickles. The seeds of the many species have been reported to contain glucocapparin and other thioglucosides from which methyl isothiocyanate and other mustard oils are released when the seeds are crushed [11-12**]**. Many of the plants of cleome species posses a good amount of medicinal properties. According to traditional use in the plants of this genus, the leaves are stimulants; the roots are stimulants, antiscorbutic and anthelmintic; the seeds are rubifacient, vesicant, anthelmintic and carminative; bark is bitter and laxative [13**]**.

The plant was identified by the taxonomists of Botanical Survey of India, Shibpur, Howrah **[14-15]**. After authentication, Dried Capsules were collected in bulk from young matured plants and collect the seeds then milled in to coarse powder by a mechanical grinder. This plant has a wide reputation among natives of being curative for intestinal-worm infections. This plant is being used by the tribals as an anthelmintic in the form of extract, prepared by soaking powdered material in water for 10-12 hours. This extract is taken orally once a day for three days to treat intestinal-worm infections. Based on this, an attempt has been made to evaluate the anthelmintic potential of *Cloeme hassleriana.*

MATERIALS AND METHODS

The powdered material was extracted with 90% ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure, which gave a light yellowish colored sticky residue (yield-12.1% w/w on dried material basis). A portion of dried ethanolic extract was suspended in water and fractionated successively with petroleum ether (40-60°C), diethyl ether, ethyl acetate and n-butanol. The yields of the fractions were found to be 26.64 %, 8.95 %, 6.39 %, and 16.33 % w/w respectively of the ethanolic extract. All the fractions were dried by distillation under reduced pressure and kept in desiccators for future use.

Qualitative Chemical Evaluation

Results of phytochemical screening of *Cloeme hassleriana* Seed Extracts are presented in Table 1 and Table 2.

Pharmacognostical Studies of *Cleome hassleriana*

Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. In this regard despite the modern techniques, identification of plant drugs by Pharmacognostical studies is more reliable. Since the plant, *Cloeme hassleriana,* is useful in traditional medicine for the treatment of some ailments, it is important to standardize it for use as a drug. As no work has been reported on the diagnostic features of *Cloeme hassleriana*, the present study reports the macro and microscopic and some other Pharmacognostical characters of the *Cloeme hassleriana* the Plant which could be used to prepare a monograph for the proper identification of the plant.

Table 1: Preliminary phytochemical investigation of *Cloeme hassleriana*

Experiment	Inference						
	Powder Drug	Ethanolic extract	Ethanolic Extract fractions				Aqueous extract
			Petroleum ether fraction	Diethyl ether fraction	Ethyl acetate fraction	n-butanol fraction	
Test for Carbohydrates							
1. Molisch's Test	+	+	-	-	-	-	+
2. Fehling's Test	+	+	-	-	-	-	+
3. Benedict's Test	+	+	-	-	-	-	+
4. Barfoed's Test	+	+	-	-	-	-	+
5. Test for Gums and Mucilages	-	-	-	-	-	-	-
Test for Proteins and Amino Acid							
1. Ninhydrin Test	-	-	-	-	-	-	-
2. Biuret Test	-	-	-	-	-	-	-
3. Tannic Acid Test	-	-	-	-	-	-	-
Test for Fixed Oils and Fats							
1. Spot Test	-	+	+	-	-	-	-
2. Saponification Test	-	+	+	-	-	-	-
Test for Sterols and Triterpenoids							
1. Libermann-Burchard's Test	+	+	+	+	+	-	-
2. Salkowski's Test	+	+	+	+	+	-	-
3. Test of Triterpenoids	+	+	+	+	+	-	-
Test for Glycosides							
1. Baljet's Test	-	-	-	-	-	-	-
2. Legal's Test	-	-	-	-	-	-	-
3. Bornstrager Test	-	-	-	-	-	-	-
4. Modified Bornstrager Test	-	-	-	-	-	-	-
5. Cyanogenetic Glycoside Test	-	-	-	-	-	-	-
6. Antimony Trichloride Test	-	-	-	-	-	-	-
Test for Saponins							
1. Foam Test	+	+	-	+	+	+	+
2. Hemolytic Test	+	+	-	+	+	+	+
Test for Flavonoids							
1. Ferric Chloride Test	+	+	-	+	+	+	+
2. Shinoda Test	+	+	-	+	+	+	+
3. Lead Acetate Test	+	+	-	+	+	+	+
4. Fluorescence Test	+	+	-	+	+	+	+
5. Action of Alkali and Acid	+	+	-	+	+	+	+
Test for Tannins and Phenolic Compounds							
1. Ferric Chloride Test	+	+	-	-	+	+	+
2. Test with Heavy Metals	+	+	-	-	+	+	+
3. Nitric Acid	+	+	-	-	+	+	+

Test							
4. Phenazone Test	+	+	-	-	+	+	+
Test for Alkaloids							
1. Mayer's Test	-	-	-	-	-	-	-
2. Dragendroff's Test	-	-	-	-	-	-	-
3. Wagner's Test	-	-	-	-	-	-	-
4. Hager's Test	-	-	-	-	-	-	-

'+' and **'-'** indicates presence and absence respectively.

Table 2: Summary of preliminary phytochemical investigation of *Cleome h*assleriana

Extracts/Powder	Phytoconstituents present
Water Extract	Carbohydrates, Saponins, Flavonoids,
Ethanolic extract	Carbohydrates, Lipids, Sterols, Triterpenoids, Flavonoids, Tannins, Saponins.
Pet-ether fraction	Lipids, Sterols, Triterpenoids.
Diethyl ether fraction	Sterols, Triterpenoids, Saponins, Flavonoids
Ethyl acetate fraction	Flavonoids, Tannins, Saponins
N-Butanol fraction	Flavonoids, Tannins, Saponins.
Powder Drug	Carbohydrates, Sterols, Saponins, Flavonoids, Tannins.

Preparation of Standard Solution

Piperazine citrate is taken as standard drug and the concentration of the standard drug was dissolved in 100ml of normal saline solution to get 1, 2, and 4ml of solution. Normal saline alone was used as control.

Evaluation of anthelmintic activity

Adult earth worms (Pheretima posthuma) were collected (due to their anatomical and physiological resemblance with the intestinal round worm parasites of human beings) Earth worms was thoroughly washed with normal saline to remove the adhering material. Petri dishes of equal size were collected and 20ml of normal saline alone was poured in the first petridish, 20ml of Piperazine citrate solution of concentration 1, 2 and 4mg/ml were poured in second, third and fourth Petri dishes, respectively. Then 20ml (4mg/ml) of the test solutions that is, the aqueous, ethanolic extracts, Petroleum ether Fraction, Diethyl ether Fraction, Ethyl acetate Fraction and n-butanol Fraction of *Cloeme hassleriana* were taken in fifth, sixth, seventh, Eighth, Ninth, Tenth and Eleventh Petridishes respectively. Placed Eleven earthworms of nearly equal size (motion less) and complete death of earthworms was noted. The experiment was repeated thrice and confirmed the readings

Statistical Analysis

All the data are expressed as mean±S.E.M. (standard error of the mean). The significance level was determined using the Student's't' test. A p value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The results of the above studies demonstrated that the aqueous, ethanolic extracts, Petroleum ether Fraction, Diethyl ether Fraction, Ethyl acetate Fraction and n-butanol Fraction of *Cloeme hassleriana* shows potent anthelmintic activity with varying magnitudes. But the extract of *Cloeme hassleriana* showed highest activity, which is almost equal in effectiveness to standard Piperazine citrate. The difference in the time taken for induction of paralysis in both Piperazine citrate and *Cloeme hassleriana* was insignificant or almost same.

Table 3: Anthelmintic activity of Aqueous and Ethanolic (95%) extracts of *Cloeme hassleriana* and Piperazine citrate.

S. No.	Treatment	Concentration (mg/ml)	Paralysis Time (min)	Death time (min)
01	Normal Saline	0.9% NaCl	No paralysis	No death
02	Piperazine Citrate	01	043.66 ± 1.071	063.33 ±0.838
03	Piperazine Citrate	02	030.00 ± 0.881	066.66 ± 1.071
04	Piperazine Citrate	04	021.33± 0.509	036.33 ± 1.895
05	Powder Drug	04	031.23± 0.463	037.65 ± 1.876
06	Ethanolic Extract	04	022.57 ±0.765	043.77 ±1.315
07	Petroleum ether fraction	04	016.76 ± 0.876	029.66 ± 1.432
08	Diethyl Ether Fraction	04	013.54±0.453	017.23±1.423
09	Ethyl Acetate Fraction	04	027.88 ± 0.675	021.43 ± 1.213
10	n-butanol fraction	04	031.32±0.675	028.45 ± 1.564
11	Aqueous Extract	04	0.25.53 ± 0.764	018.32±1.564

However, significant difference was observed when compared the induction of paralysis time of Piperazine with aqueous extracts. The mode of action for the Piperazine is generally by paralyzing parasites, which allows the host body to easily remove or expel the invading organism. The preliminary phytochemical observations of the ethanolic extracts of *Cloeme hassleriana* and its plant ingredients have shown the occurrence of Carbohydrates, Fixed Oils and Fats, Phytosterols and Triterpenoids, Saponins Tannins and Phenolic Compounds, Flavonoids. It indicates that, the *Cloeme hassleriana* is a mixture of all these phytoconstituents and interaction of all these chemicals might be resulted in synergistically enhanced therapeutic efficacy of anthelmintic activity.

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