International Journal of Innovative Pharmaceutical Sciences and Research
www.ijipsr.com

STUDIES ON ANTIOXIDANT, ANALGESIC, ANTI-INFLAMMATORY AND CNS DEPRESSANT ACTIVITIES OF THE PLANT *CLEOME VISCOSA* LINN.

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Abstract

Cleome viscosa Linn. (Family: Capparaceae) is an annual and sticky herb and commonly known as wild or dog mustard. It has various ethnomedicinal values as various traditional communities find diverse medicinal properties. This study aimed to investigate the antioxidant, analgesic, anti-inflammatory and CNS depressant activities of crude methanolic extract, chloroform fraction as well as ethyl acetate fraction of Cleome viscosa Linn. In DPPH free radical scavenging activity, in total antioxidant activity and in reducing power capacity tests the chloroform fraction showed stronger activity than that of the crude methanolic extract and its ethyl acetate fraction with an IC50 value 4.1 which is almost equal to that of the standard ascorbic acid (3.8), absorbance of 1.139 and 1.495 respectively. But total phenolic content and total flavonoid content were found to be significantly higher in ethyl acetate fraction (264.11±16.2595 and 224.79±7.1686) than that of the crude methanolic extract and its chloroform fraction. In analgesic activity study using acetic acid induced writhing test chloroform fraction showed highest activity (writhing inhibition 81.25%, 50 mg/kg b.w). In anti-inflammatory activity study following carrageenan induced paw edema test ethyl acetate fraction showed potent anti-inflammatory activity, on the other hand crude methanolic extract and chloroform fraction showed moderate activity. In CNS depressant activity using hole cross and open field test it was observed that all the extract showed moderate activity in hole cross method while in open field method the crude methanolic extract showed the highest activity.

Keywords: *Cleome viscosa* Linn., antioxidant activity , analgesic activity, anti-inflammatory activity, CNS depressant activity.

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INTRODUCTION

Cleome viscosa Linn. commonly known as wild or dog mustard, belongs to the caper family (Capparaceae). It is an annual, sticky herb found as a common weed all over the plains of India, Pakistan and throughout the tropics of the world. The whole plant and its parts (leaves, seeds, and roots) are widely used in traditional and folkloric systems of medicine. The leaves, seeds and roots of the plant are widely used in traditional and folkloric systems of medicine as an anthelmintic, antiscorbutic, antiseptic, cardiac stimulant, carminative, febrifuge and sudorific [1], anticonvulsant [2], antidiarrheal [3,4], and also for treating skin diseases [5]. Pharmacological studies have shown that Cleome viscose possesses analgesic, antidiarrheal, anti-inflammatory, antimicrobial, antipyretic, anthelmintic, hepatoprotective and immunomodulatory activities [6]. The seeds contain 18.3% oil, a mixture of amino acids, fatty acids and sucrose [7]. Linoleic, palmitic, stearic, oleic and linolinic acids are present in the oil [8, 9, and 10]. Aqueous and alcoholic extracts of seeds showed analgesic [11], anthelmintic [12] and hepatoprotective activity [13]. Seed oil possesses mutagenic properties[14].The crude methanol extract of C. viscosa showed significant analgesic activity using acetic acid induced writhing, tail flick, tail clip and tail immersion methods in mice[15]. A wide variety of chemical constituents have been isolated from various parts of C. viscose. A novel umbeliferone derivative, designated as cleosandrin, series of coumarino-lignans (cleomisconsins) from the seeds and a new glycoside eriodictyal-5-rhamnoside have been isolated from the whole plant [16]. From the previous data it is evident that various biological activities have been carried out with this plant. As a continuation of the previous work on Cleome viscosa Linn, this attempt has been taken to investigate the antioxidant, analgesic, anti-inflammatory and CNS depressant activities of different extracts of leaves of the plant. CNS depressant activity was first time investigated from this plant.

MATERIALS AND METHODS

Plant Material

The whole plant of *Cleome viscosa* Linn. was collected from the adjoining area of Rajshahi University campus, Bangladesh, in the month of February, 2013. Taxonomist Mahabubur Rahman, Department of Botany, University of Rajshahi, botanically identified the plant *Cleome viscosa* Linn. and a voucher specimen had also been deposited under a voucher number of 0128 in the department of Botany, Rajshahi University. The plant was then preserved in Phytochemistry lab, Department of Pharmacy, University of Rajshahi, Bangladesh.

Preparation of Plant Material

The fresh plants are thoroughly washed with water and dried in shade. Then plants are cut into small pieces to make it suitable for grinding purpose and finally dried in an oven at 40-45^{o}C for 36 hrs. The materials are grinded into coarse powder with the help of a grinder and store in an air tight container for further use.

Plant Extract Preparation

Extraction is the process in which the plant materials are treated with specific solvents whereby, the medicinally active constituents are dissolved out and most of inactive components remain undissolved [17]. Extraction can be performed in two ways-Cold extraction and hot extraction. In this study, cold extraction method is used to extract the active components. Powdered plant materials having a weight of about 1kg were taken in an amber colored reagent bottle and soaked in 5 liter of methanol. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No.1 filters paper and was concentrated with a rotary evaporator under reduced pressure at 50°C temperature to afford crude methanolic extract, CME (25 gm). Distilled water was mixed to crude extract to obtain a satisfactory volume which was then partitioned according to increasing polarity using chloroform, ethyl acetate and petroleum ether sequentially for separation of the compounds according to their partition co-efficient. We got three different fractions from partitioning and these were chloroform fraction, CHF (3.2 gm), ethyl acetate fraction, EAF (2.5 gm) and petroleum ether fraction, PEF (1.8 gm).

Animals

Long Evans mice of both sexes, aged 4-5 weeks, weighting about 20-25gm were purchased from the animal research branch of the international center for diarrheal disease and research, Bangladesh (ICDDRB). Before initiating the experiment, the mice were kept in standard environmental conditions (temperature: (23.0 ± 2.0º), relative humidity: 55 - 65% and 12 h light/12 h dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiments. For CNS depressant activity the experiments were done in an isolated and noiseless room. All protocols for animal experiment were approved by the institutional animal ethical committee.

Preparation of the Test Materials and Standard

50 mg of CME, CHF and EAF fractions are triturated by the addition of small amount of suspending agent (Tween-80). Normal saline (0.9%NaCl) is slowly added to make the final

volume up to 2.5 ml. To prepare the standard, Diclofenac sodium 10 mg was dissolved into 0.9% normal saline and made the volume up to 10 ml. For preparing control sample, tween-80 (1%) was mixed properly in the normal saline to make the volume up to 5 ml.

In-Vitro Antioxidant Assays

DPPH Free Radical Scavenging Activity

The free radical scavenging activity of the crude methanolic extract (CME), chloroform fraction (CHF) and ethyl acetate fraction (EAF) was detected based on the method described by Braca, A., Tommasi, Nunziatina De, Bari, Lorenzo Di, Pizza, Cosimo, Politi, Mateo & Morelli, Ivano., (2001) [18]. Sample (2 ml) will be added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm will be determined after 30 min, and the percentage inhibition activity was calculated from $I\% = [(A_0 - A_1)/A_0] \times 100$ (I% is the percentage of scavenging activity, A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard).

Total Antioxidant Capacity Assessment

The assay is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound present in the sample and subsequent formation of a green phosphate/Mo (V) complex at acidic P^H described by Prieto, P., Pineda, M., Aguilar, M., (1999) [19]. The sample (0.5mL) was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95^0C for 90 min. The mixture was cooled to room temperature, and then the absorbance of the solution was measured at 695nm against blank. A typical blank solution contained 3 mL of reaction mixture and the same volume of solvent used for the sample, and it is incubated under the same conditions as the rest of the sample solution. The total antioxidant activity was expressed as compared with ascorbic acid.

Determination of Total Phenolics

Total phenolic content of the different fractions of *Cleome viscosa* was determined employing the method as described by Singleton, V. L., & Rossi, J. A. (1965) [20] involving Folin-Ciocalteu reagent as oxidizing agent and catechin as standard. The amount of total phenolics in extract was determined according to the Folin-cio calteu procedure. Samples (500µl) were introduced into test tubes. 2.5mL of Folin- ciocalteu reagent and 2.5 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorbance at 760 nm was measured. The total phenolic content was expressed as Gallic acid equivalents (GAE) in milligrams per gram extract.

Determination of Total Flavonoids (TF)

Total flavonoid content of the different fractions of *Cleome viscosa* was determined by aluminium chloride colorimetric method as described by Dewanto, Wu, Adom, and Liu, (2002) [21]. Catechin was used as standard and the flavonoid content of the extractives was expressed as mg of catechin equivalent/gm of dried extract. One milliliter of extract containing 0.1 g/mL of dry matter was placed in a 10 mL volumetric flask, and then 5 mL of distilled water added followed by 0.3mL of 5% $NaNO_2$. After 5 min, 0.6 mL of 10% $AlCl_3$ was added. After another 5 min 2 mL of 1M NaOH was added and volume made up with distilled water. The solution was mixed and absorbance measured at 510 nm. TF amounts were expressed as catechin equivalents per dry matter. All samples were analyzed thrice and result averaged.

Reducing Power Capacity Assessment

The reducing power of different fractions of *Cleome viscosa* was evaluated by the method of Oyaizu, M. (1986) [22]. A 2.5 mL fraction of Piper *betle* was mixed with 2.5 mL of phosphate buffer (200mM, p_H 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was placed in a water bath for 20 min at 50^0C. The resulting solution was cooled rapidly, mixed with 2.5 mL of 10% trichloro acetic acid and centrifuged at 3,000 rpm for 10 min. A 5.0 mL fraction from the supernatant was mixed with 5mL of distilled water and 1mL of ferric chloride. Absorbance of the resultant mixture was measured at 700nm after 10 min. The higher the absorbance value the stronger the reducing power.

Analgesic Activity Determination

Acetic Acid Induced Writhing Method

The assay is performed following the method of Sharma *et. al*. [23]. The experimental animals were randomly divided into five groups consisting of five mice in each group. The groups were denoted from group-I to group-V.

Analgesic activity of Crude methenolic extract(CME),Chloroform fraction (CHF) and ethyl acetate fraction (EAF) were carried out with group-III to group-V whereas group-I to group-II were employed to evaluate the analgesic activity of the control and standard respectively. Each group of mice received a specific treatment.

Prior administering the drugs, each mouse was weighed properly and the doses were adjusted accordingly.

Anti-Inflammatory Activity Determination

Carrageenan Induced Paw *EDEMA* Method

The assay is performed following the method of. Elisabetsky, T.A. Amador, R.R. Albuquerque, D.S. Nunes and C. Carvalho Ado (1995) [24]. Experimental animals were randomly selected and divided into five groups consisting of five mice in each group for control, standard and 2 test samples group respectively. Each group received a particular treatment i.e. control (1% tween-80 in water) standard (Indomethacin, 10 mg/kg, p.o.) and the test sample (CME of 100 and 200 mg/kg, p.o for each sample). Here, 1% carrageenan was injected to the left hind paw of each animal for creating inflammatory response. The right hind paw served as a reference non-inflamed paw for comparison.

Central Nervous System (CNS) Depressant Activity

Hole Cross Method

The experiment was carried out as described by Takagi *et al*. [25]. The animals were randomly divided into five groups & each group consisting of five mice. The test groups received crude methanolic extract(CME), chloroform fraction (CHF)and ethyl acetate fractions (EAF)of methanolic extract of *Cleome viscosa* at the doses of 50 mg/kg while positive control was treated with diazepam (4 mg/kg) and control with vehicle (1% Tween 80 in water). The aim of this study is to characterize the emotional behavior of mice using the hole-board test. The different fractions were administered to the mice. Then their spontaneous movement of the animals through the hole from one chamber to another chamber of a wooden box is counted for 5 minutes in this test. The observations are made on 0, 30, 60, 90 minutes after intraperitoneally injection of the test drugs.

Open Field Method

Field Test (OFT) is the most frequently used of all behavioral tests in pharmacology and neuroscience. This experiment was carried out as described by Gupta *et al*. [26]. The animals were randomly divided into five groups & each group consisting of five mice.

The test groups received crude methanolic extract(CME), chloroform fraction (CHF)and ethyl acetate fractions (EAF)of methanolic extract of *Cleome viscosa* at the doses of 50 mg/kg while positive control was treated with diazepam (4 mg/kg) and control with vehicle (1% Tween 80 in water).

STATISTICAL ANALYSIS

All results of In-vitro antioxidant assays were presented as mean ± STD (n=3). All measurements were replicated three times. Statistical significance of results of analgesic, anti-inflammatory and

CNS depressant activity determination were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnet test. Five animals were included in each group. $P < 0.05$ was considered statistically significant. The data are reported as the mean ± SEM (n=5). *$P < 0.05$ as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac Na (analgesic activity), Indomethacin (anti-inflammatory activity), diazepam (CNS depressant activity) 10 mg/kg body weight, Group III, Group IV and Group V were treated with 50 mg/kg body weight (p.o.) of the *Cleome viscose.*

RESULTS AND DISCUSSION

Results of Investigation Of Different Fractions

From three different fractions TLC analysis of a portion of the chloroform fraction (CHF) of *Cleome viscosa* Linn. showed several prominent spots with small tailing at the base when developed with n-hexane: ethyl acetate (1:14) and either sprayed with vanillin/sulfuric acid spray reagent or exposed in the iodine chamber.

This fraction showed spots deep blue color at R_f value 0.63, light violet color at R_f value 0.71 and deep violet color at R_f value 0.83. On the other hand, TLC examination of acetate fraction (EAF) showed three prominent dark spots along with some minor spots when checked by TLC with solvent system chloroform: ethyl acetate (7:1) and detected under UV light , iodine chamber and spray reagent.

This fraction showed yellow color at R_f value 0.8, light violet color at R_f value 1.4 and deep violet color at R_f value 3.4.

In-Vitro Antioxidant Activity Study

The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function or it is the concentration of an inhibitor that is required for 50-percent inhibition of an enzyme in vitro. In DPPH radical scavenging assay IC_{50} values of the crude methanolic extract (CME), chloroform fraction (CHF) and Ethyl acetate fraction (EAF) were evaluated.

From the results it was observed that the scavenging activity of crude methanolic extract was higher as compared with that of chloroform fraction (CHF) and ethyl acetate extract.

The IC_{50} values of chloroform fraction, methanolic extract, ethyl acetate fraction and ascorbic acid were 4.1µg/ml, 19.4µg/ml, 12.8µg/ml and 3.8µg/ml, respectively(Tab-1, Fig:1.a and 1.b).

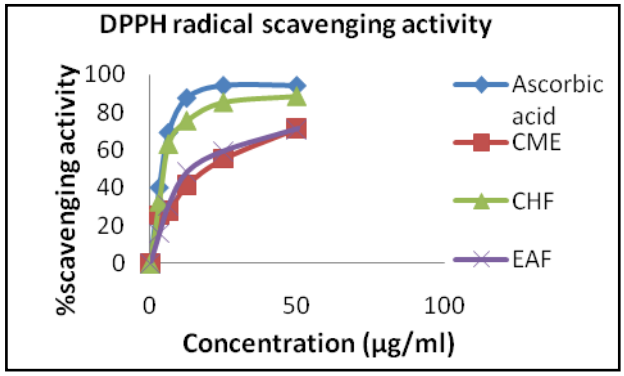

Fig:1(a)

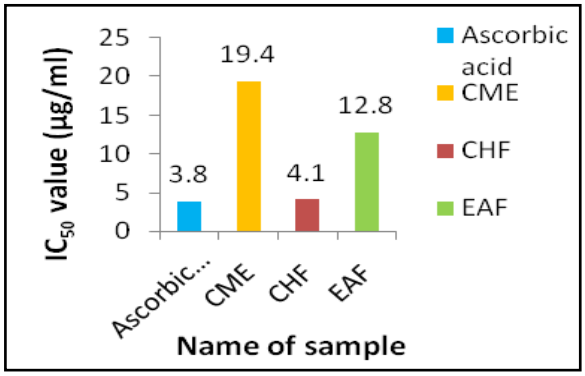

Fig:1(b)

Fig-1(a): DPPH radical scavenging activity of crude methanol extract (CME), chloroform fraction (CHF), ethyl acetate fraction (EAF) of *Cleome viscosa* Linn. and ascorbic acid (standard).Fig-1(b): IC_{50} (µg/ml) values of ascorbic acid (standard), crude mehanolic extract (CME) of *Cleome viscosa* Linn, its chloroform fraction (CHF) and ethyl acetate fraction (EAF).

Table: 1 DPPH free radical scavenging activity of *Cleome viscosa* leaf extracts, its different fractions and Standard.

Name of sample	Conc. (µg/ml)	% of scavenging Mean ± STD	IC_{50} (µg/ml)
Ascorbic acid	3.125	40.41 ± 0.3115	3.8
	6.25	69.55 ± 0.031	
	12.5	87.86 ± 0.02	
	25	94.393 ± 0.0058	
	50	94.393 ± 0.0058	
	100	94.393 ± 0.0058	
CME	3.125	25.52 ± 0.663	19.4
	6.25	27.78 ± 0.226	
	12.5	41.52 ± 0.467	
	25	55.44 ± 0.0321	
	50	71.61 ± 0.6027	
	100	91.52 ± 0.1939	
CHF	3.125	32.86 ± 0.1124	4.1
	6.25	63.26 ± 2.757	
	12.5	75.58 ± 0.811	
	25	85.30 ± 1.109	
	50	88.55 ± 1.017	
	100	89.30± 0.6305	
EAF	3.125	16.04 ± 0.66	12.8
	6.25	30.97 ± 0.55	
	12.5	48.48 ± 0.1709	
	25	59.48 ± 0.3677	
	50	71.27 ± 0.7	
	100	84.14 ± 0.0754	

Here, the antioxidant activity of the samples was expressed as IC_{50} (µg/ml) which meant the concentration of the sample while the scavenging rate of DPPH was 50% and was determined by regression analysis. Results were presented as mean ± STD (n=3).

From the Total phenol content experiment it was observed that the total phenolic content of the ethyl acetate fraction (264.11 ± 16.2594) was more than two times that of the crude methanolic extract (112.17 ± 5.1209) and chloroform fraction (110.5 ± 3.9242) (**Tab-2 and fig-2.a**). From the Total flavonoid content experiment it was found that the total flavonoid content of the ethyl acetate fraction was exceptionally high (224.79 ± 7.1686) in comparison with that of the crude methanolic extract (17.75 ± 3.40189) and chloroform fraction (89.57 ± 6.6274) (**Tab-3 and Fig-2.b**).

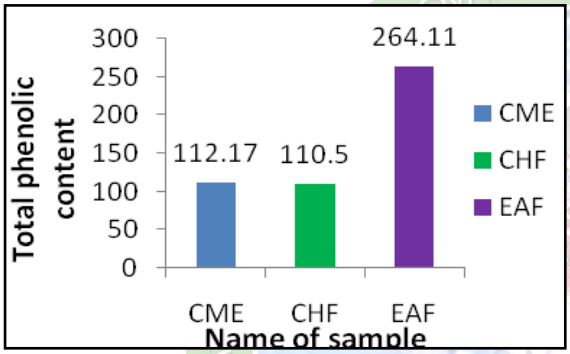

Fig. 2(a)

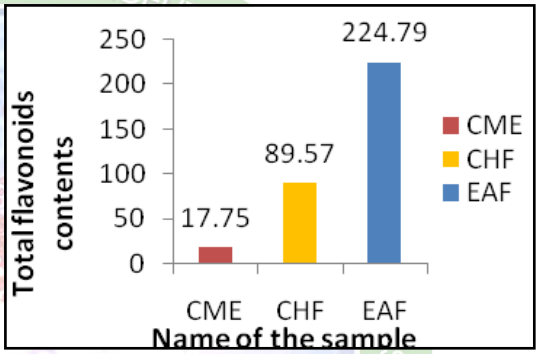

Fig. 2(b)

Fig-2(a): Total phenol content (mg/gm plant extract in gallic acid equivalent) of crude methanol extract (CME) of *Cleome viscosa* Linn, its chloroform fraction (CHF) and ethyl acetate fraction (EAF). Fig-2(b): Total flavonoid content (mg/gm plant extract in catechin equivalent) of crude methanol extract (CME), its chloroform fraction (CHF) and ethyl acetate fraction of *Cleome viscosa* Linn. Values are mean of triplicate experiments and represented as mean ± STD

Table: 2 Determination of total phenolic content of *Cleome viscosa* leaf extracts and its different fractions.

Sample	Conc. (µg/ml)	Absorbance	GAE/gm of dried sample	GAE/gm of dried Sample (Mean±STD) (n=3)
CME	100	1.028	117.24	112.17 ±5.1209
	100	0.985	112.29	
	100	0.939	107.00	
CHF	100	0.937	106.00	110.50 ± 3.9242
	100	0.985	112.29	
	100	0.993	113.21	
EAF	100	2.440	279.72	264.11 ± 16.2595
	100	2.315	265.33	
	100	2.158	247.27	

Table: 3 Determination of total flavonoid content of *Cleome viscosa* leaf extracts and its different fractions

Sample	Conc. (µg/ml)	Absorbance	CE/gm of dried sample	CE/gm of dried Sample (Mean±STD) (n=3)
CME	500	0.095	14.23	17.75 ± 3.40189
	500	0.105	18.00	
	500	0.113	21.02	
CHF	500	0.278	83.28	89.57 ± 6.6274
	500	0.293	88.94	
	500	0.313	96.49	
EAF	500	0.632	216.87	224.79 ±7.1686
	500	0.658	226.68	
	500	0.669	230.83	

In total antioxidant activity test the results demonstrated that the total antioxidant activity of chloroform fraction (CHF) is higher than that of the crude methanolic extract and ethyl acetate fraction. However, the activity was less than that of ascorbic acid. All the samples were concentration dependent and it found that the total antioxidant activity was increased with the increase of the concentration. At 100µg/ml, the absorbance of methanolic extract, chloroform fraction, ethyl acetate fraction and ascorbic acid were 0.419, 1.139, 0.552 and 2.778, respectively **(Tab-4 and Fig-3.a**). From the results of reducing power capacity test it was found that the reducing activity of CHF was higher than CME and EAF but less than that of ascorbic acid. CHF, EAF and CME increased the reducing activity with the increase of concentration. At 100 µg/ml concentration, the absorbance of CME, CHF, EAF and the reference standard ascorbic acid were 1.088, 1.495. 0.888 and 2.808 respectively **(Tab-5 and Fig-3.b**).

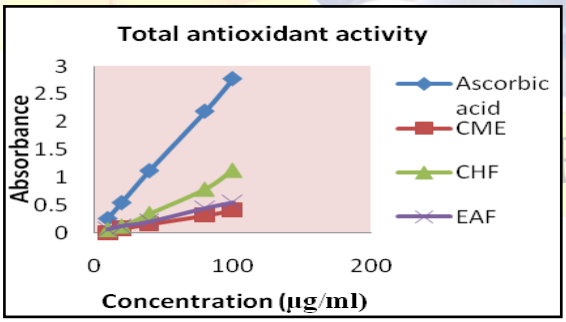

Fig. 3(a)

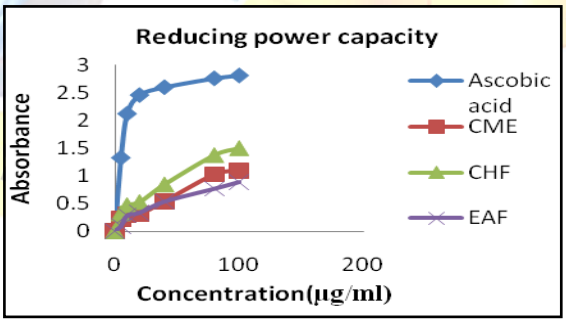

Fig. 3(b)

Fig-3(a): Total antioxidant activity of the crude methanol extract (CME), its chloroform fraction (CHF), ethyl acetate fraction (EAF) of *Cleome viscosa* Linn and ascorbic acid (Standard). Fig-3(b): Reducing power capacity of the crude methanol extract (CME) of Cleome viscosa Linn, its chloroform fraction (CHF), ethyl acetate fraction (EAF) and ascorbic acid (Standard)

Table: 4 Total antioxidant capacities of Cleome viscosa leaf extracts, its different fractions and standard

Name of sample	Conc. (µg/ml)	Absorbance Mean ±STD
Ascorbic acid	10	0.266 ± 0.00808
	20	0.553 ± 0.01311
	40	1.127 ± 0.02066
	80	2.194 ± 0.03023
	100	2.778 ± 0.02112
CME	10	0.008 ± 0.0045
	20	0.086 ± 0.01137
	40	0.170 ± 0.01171
	80	0.326 ± 0.00953
	100	0.419 ± 0.01833
CHF	10	0.058 ± 0.01014
	20	0.124 ± 0.02113
	40	0.353 ± 0.010016
	80	0.795 ± 0.003
	100	1.139 ± 0.1266
EAF	10	0.066 ± 0.0104
	20	0.128 ± 0.0085
	40	0.206 ± 0.00985
	80	0.450 ± 0.01682
	100	0.552 ± 0.01343

Table: 5 Reducing power capacity of *Cleome viscosa* leaf extracts, its different fractions and standard

Name of sample	Concentration (µg/ml)	Absorbance Mean ± STD
Ascorbic acid	5	1.326 ± 0.116
	10	2.119 ± 0.116
	20	2.455 ± 0.116
	40	2.5973 ± 0.1160
	80	2.756 ± 0.1160
	100	2.808 ± 0.0485
CME	5	0.227 ± 0.0531
	10	0.301 ± 0.0257
	20	0.310 ± 0.0269
	40	0.538 ± 0.054
	80	1.019 ± 0.033
	100	1.088 ± 0.0816
CHF	5	0.324 ± 0.0148
	10	0.47 ± 0.0195
	20	0.526 ± 0.0450
	40	0.842±0.1270
	80	1.369 ± 0.0580
	100	1.495 ± 0.1187

EAF	5	0.097 ± 0.01457
	10	0.285 ± 0.00873
	20	0.335 ± 0.0085
	40	0.535 ± 0.0132
	80	0.767 ± 0.01457
	100	0.888 ± 0.03122

DETERMINATION OF ANALGESIC ACTIVITY

ACETIC ACID-INDUCED WRITHING TEST

In acetic acid induced writhing test, the three fractions of methanolic extract of *Cleome viscosa* linn. Significantly and dose dependently suppress the frequency of acetic acid-induced writhing in mice after oral administration. At 50 mg/kg body weight, crude methalonic extract(CME), chloroform fraction(CHF) and ethyl acetate fraction (EAF) showed 79.16%, 81.25% and 70.83% writhing inhibition, respectively. At 10 mg/kg body weight, the standard drug diclofenac sodium shows 83.33% writhing inhibition. Among the three fractions, it is found that chloroform fraction showed highest writhing inhibition that is close to the standard drug used in the experiment (Fig-4, Tab-6).

Table: 6. The effect of crude methanolic extract (CME) of *Cleome viscosa,* chloroform fraction (CHF) and ethyl acetate fraction (EAF) in acetic acid induced writhing test

Groups	Treatment	Dose mg/kg(p.o.)	No. of writhing Mean± SEM	% of inhibition of writhing
Group I	1% Tween 80 in water (control)	1ml/10gm	12.00±2.16025	--
Group II	Diclofenac sodium(Standard)	10mg/kg	2.00±0.95743	83.33*
Group III	CME	50mg/kg	2.50±0.57735	79.16*
Group IV	CHF	50mg/kg	2.25±0.95742	81.25*
Group V	EAF	50mg/kg	3.50±0.57735	70.83*

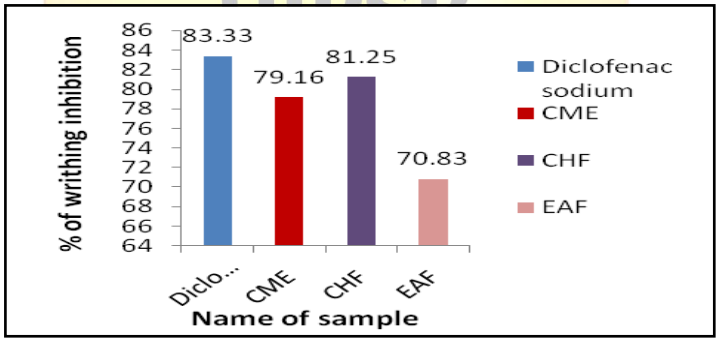

Fig-4: % of writhing inhibition value in analgesic activity of crude methanolic extract (CME), chloroform fraction (CHF), ethyl acetate fraction (EAF) and diclofenac sodium (standard). Here, values are mean ± SEM, (n = 5); *p<0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test)

Determination of Anti-Inflammatory Activity

1% Carrageenan Induced Paw Edema Test

From the results it was observed that plant extract as well as all other fractions have significant activity as an anti-inflammatory agent. In 4th hour all the fractions have activity almost close to the standard Indomethacin **(Fig-5, Tab-7).**

Table 7: The effects of the *Cleome viscosa* Linn crude methanolic extract (CME), chloroform fraction (CHF) and ethyl acetate fraction (EAF) on carrageenan induced paw edema test

Group	Dose(mg/kg b.wt.p.o)	Paw edema diameter in mm (% inhibition)			
		1 hr	2 hr	3 hr	4 hr
Group-I (Control)	1% CMC	14.75 ± 0.35	15 ± 0.71	15.25 ± 0.35	15.25 ± 0.35
Group-II (Indomethacin)	10	13 ± 0.11* (38.48)	12.5 ± 0.18* (57.14)	12 ± 0.18* (73.33)	11 ± 0.05* (100)
Group-III (CME)	50	12.75±2.12 (23.08)	12±1.4142 (42.86)	12±1.4142 (46.67)	11.25±1.06 (66.67)
Group-IV (CHF)	50	13.5±0.707 (38.46)	12.75±0.353 (64.29)	12.25±0.35 (80)	11.9±0.1414 (89.33)
Group-V (EAF)	50	12.5±0.707 (61.54)	12.25±0.3535 (71.43)	11.5±0.707 (93.33)	11.25±0.3535 (100)

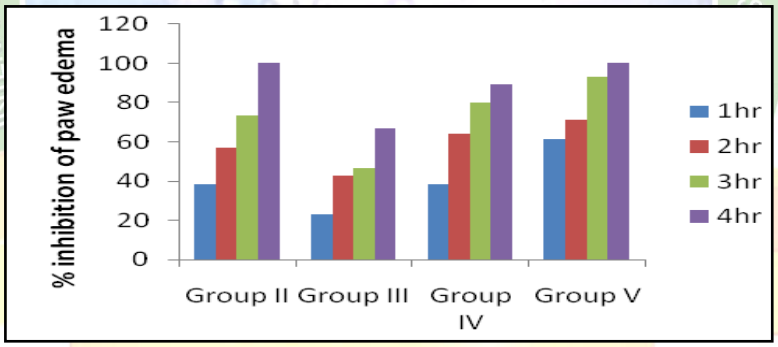

Fig. 5: The % inhibition of paw edema value of standard indomethacin, crude methanolic extract (CME), chloroform fraction (CHF) and ethyl acetate fraction (EAF) of *Cleome viscosa* Linn. Group I animals received vehicle (1% Tween 80 in water), Group II received Indomethacin 10 mg/kg body weight, Group III, Group IV and Group V were treated with 50 mg/kg body weight (p.o.) of the *Cleome viscosa* Linn. Here, values are mean ± SEM, (n = 5); *p<0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test)

Central Nervous System (CNS) depressant activity determination Hole cross test

In the animal treated with different fractions of methanolic extract of *Cleome viscosa* Linn. at doses (50 mg/kg) showed dose dependent reduction in the locomotor activity and it was comparable with that of standard drug diazepam. The extract produced reduction in spontaneous motor activity, and this effect may be attributed to CNS depression, as depression of locomotor

activity is common to most neuroleptics. From the results it was observed that test animals showing significant decrease in number of movement in the dosages of 50 mg/kg (CME:2.5 ± 0.7071, CHF: 2.5 ± 2.1213 and EAF: 2 ± 0) respectively, as compared to 12 ± 1.4142 in the control group and 1.5 ± 0.7071 in the standard group) at 120 min of administration of the extract **(Tab-8, Fig-6.a).**

Table 8: Comparison of mean movement of hole cross before and after drug administration

Group	Mean movement				
	0 min	**30 min**	**60 min**	**90 min**	**120min**
Control	10.5 ± 0.7071	11.5 ± 0.,7071	14.0± 2.8284	12.0 ±5.6568	12.0± 1.4142
Standard	11.0 ± 1.4132	5.0 ± 1.4142	5.0 ± 2.8284	2.0 ± 00	1.5 ± 0.7071
CME	7.5 ± 2.1213	7.0 ± 1.4142	5.5 ± 0.7071	3.5 ± 0.7071	2.5 ± 0.7071
CHF	7.5 ± 0.7071	6.0 ± 00	5.5 ± 0.7071	4.0 ± 1.4142	2.5 ± 2.1213
EAF	8.0 ± 1.4142	5.5 ± 2.1213	3.5 ± 0.7071	2.5 ± 0.7071	2.0 ± 00

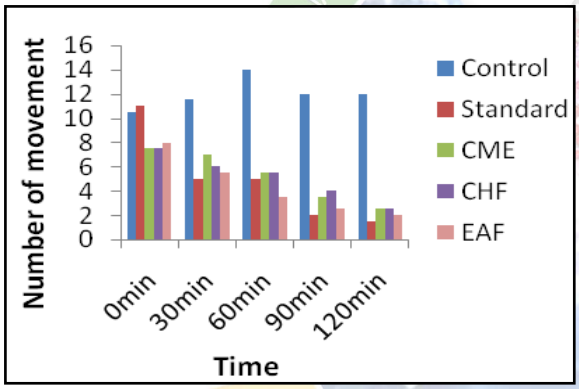

Fig. 6(a)

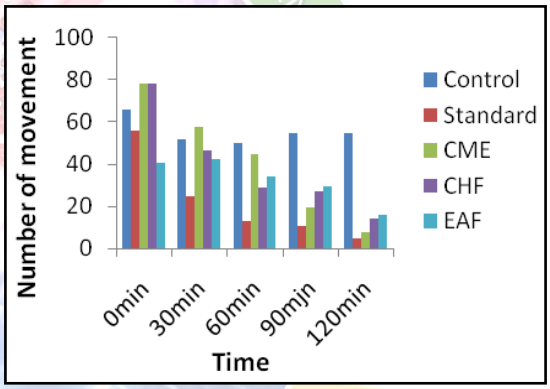

Fig. 6(b)

Fig-6(a): CNS depressant activity by hole crosses method in mice. Fig-6(b): CNS depressant activity by open field method in mice. Here, Control= 1% tween 80 in water, standard = diazepam, CME =Crude methanolic extract, CHF= Chloroform fraction, EAF= ethyl acetate fraction and values are mean ± SEM, (n = 5)

Open Field Test

Open field test was carried out to determine the depressive action of the test drugs on CNS in mice. In the test, the extract shows a noticeable decrease in locomotion in the test animals from the second observation period to last study period at dose level (50 mg/kg body weight). The effect observed is increasing with time and a noticeable result is found at 120 min of test sample administration. Results evidenced that test animals were showing significant decrease in number of movement in the dosages of 50 mg/kg (CME:7.5 ± 7.7781, CHF: 14 ± 1.4142 and EAF: 16 ±

5.6568) respectively, as compared to 55 ± 4.2426 in the control group and 4.5 ± 0.7071 in the standard group) at 120 min of administration of the extract (**Tab-9, Fig-6.b**).

Table-9: Comparison of mean movement of open field before and after drug administration

Group	Mean movement				
	0 min	**30 min**	**60 min**	**90 min**	**120min**
Control	66 ± 2.8284	52 ± 2.8284	50± 2.8284	55 ± 1.4142	55 ± 4.2426
Standard	56 ± 5.6568	25 ± 1,4142	13 ± 1.4142	10.5 ± 2.1213	4.4 ± 0.7071
CME	78 ± 31.112	57.5 ± 24.748	44.5 ± 7.7781	19.5 ± 21.920	7.5 ± 7.7781
CHF	78 ± 25.455	46.5 ± 9.1923	29 ± 2.8284	27 ± 2.8284	14 ± 1.4142
EAF	40.5 ± o.7071	42.5 ± 4.9497	34 ± 5.439	29.5 ± 7.7781	16± 5.6568

CONCLUSION

Free radicals are known to play a definite role in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, alzheimer, hepatic damage etc. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. The present study was aimed at investigating the antioxidant and biological activities of the methanolic extracts of *Cleome viscosa* Linn. and it's different fractions. From the present work on the whole plant *Cleome viscosa* Linn. it can be concluded that the plant contents some bioactive principle that possess strong antioxidant activity along with strong anti-inflammatory activity, analgesic activity and moderate CNS depressant activity. Therefore, the plant *Cleome viscosa* Linn. could be considered as an important source of antioxidant activity and could be possible to extend its use in folk medicine as an analgesic and anti-inflammatory agent.

AKNOWLEDGEMENT

Authors would like to give thanks to Rajshahi University for successfully conducting the whole work and the staffs of the department for their technical support and assistance.

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