

## Experimental Evaluation of Anti-Allergic, Antioxidant & Diuretic Activity in *Vetiveria Zizanoides* (Cleome Viscose) Roots in Rats

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### Abstract

**Background:** *Cleome viscosa* Linn. (Capparidaceae), popularly known as the “Asian spider flower”, is a native tree and found abundant in India. This study aimed to evaluate the antioxidant, anti-inflammatory, anti-allergic, and diuretic activity of the root extracts obtained from *Cleome viscosa* using in vitro and in vivo experimental models.

**Methods:** The ethanolic extracts and its fractions obtained from *Cleome viscosa* roots through Soxhlet extraction was analyzed by various activities. Different extracts were tested for total phenolic and flavonoid contents and in vitro antioxidant (ABTS & DPPH assay), anti-inflammatory (protein denaturation), anti-allergic (milk-induced eosinophilia), and diuretic (Lipschitz test) activities.

**Results:** Ethanolic extract exhibited the total phenolic (4.51±0.4) and flavonoid (5.34±0.93) content and highest antioxidant activity [ {in ABTS (at 0.1 mg/mL; 33.62±0.76)} and {in DPPH (at 0.02 mg/mL; 29.30±0.56)} comparable to ascorbic acid, a standard compound {(0.04 mg/mL; 47.45±0.19) and (0.06 mg/mL; 97.14±0.53)} % inhibition] respectively. Whereas the 150 µg/mL dose of EECV shows 51.07±0.92 % inhibition and viscosity of 0.79±0.75 cp as compared to 625 µg/mL dose of Diclofenac sodium shows 56.23±1.98 % inhibition and viscosity 0.92±0.61 cp. Additionally, the total leucocyte count (at 100 mg/kg; 2483.34±148.14 per cu mm) and Eosinophils count (at 100 mg/kg; 66.6±10.54 per cu mm) was observed in anti-allergic activity when compared to the standard (Milk as 4 ml/kg; 3180.21±136.0 and 84.1±42.90) respectively. Also, diuretic activity was found significant in the petroleum ether fraction (200 mg/kg) as compared to the tested standard drug (Furosemide).

**Conclusions:** It can be suggested that *Cleome viscosa* a great potential source of antioxidant, anti-inflammatory, anti-allergic and diuretic compounds useful for new diuretic drugs from the natural basis.

**Keywords:** *Cleome viscosa*, anti-allergic, anti-inflammatory, diuretic, ABTS, DPPH

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### Introduction

In the rich biodiversity of India, various traditional medicinal and culinary herbs, among some endemic species are of particular interest because they may be used

for the production of raw materials or preparations containing phytochemicals with remarkable pharmacological properties and health benefits. Many plant species and

herbs show antioxidants and anti-allergic activities as secondary metabolites. The plant *Cleome viscosa* Linn. (Family: Capparidaceae), used throughout the research is also known to possess varied medicinal properties[1]. It is widely distributed all over the tropics of world and has been used as medicinal herb due to its various biological activities like stomachic, laxative, anthelmintic, diuretic, skin diseases: itching, urticaria and leprosy. It is good in malarial fever and blood disease, the seeds are anthelmintic; the juice of leaves is used in ear pain; the roots are used as vermifuge and stimulant[2]. Herbs, spices, condiments and phytochemicals are often used for the treatment and prevention of various anomalous disorders.[3]

*Cleome viscosa* Linn. (Family: Capparidaceae) is an annual erect, branched, viscid pubescent herb in 30-90 cm height with 3-7 foliate leaves, white, yellow, pink flowers, stems grooved, densely clothed with glandular and simple hairs found in waste grounds and grassy places. The natives and traditional healers of India called this plant as 'Hul-hul' and Asian spider flower.[4] Traditionally the leaves, bark, root and seeds of the plants of *Cleome* genus are used as stimulant, anti-scorbutic, anthelmintic,[5] rubifacient, carminative, stomachic, laxative, diuretic,[6] anti-tumor, antiseptic and antileprotic.[7] The plant is used for uterine complaints and leaves are used for wounds and ulcers. *Cleome viscosa* leaves and young shoots are used sort of a vegetable, which has sharp mustard like flavor. The pungent seeds and seed pods are used as a mustard substitute in curries. The analgesic[8] antibacterial,[9] antimicrobial,[10] anti-diarrhoeal,[11] antipyretic, hepatoprotective,[12] hypolipidemic,[13] anti-bacterial, anti-fungal, contact insecticidal and nematicidal[14] and antiulcer[15] activities of the aerial parts have been reported. Ethanol extract of *Cleome viscosa* has showed several biological activity as anthelmintic activity,[16] while ethanol

extract of *Cleome viscosa* has showed good antioxidant activity,[17] antipyretic activity,[18] analgesic activity[19] anti-diarrhoeal activity.[20] Coumarinolignoids from seeds of *Cleome viscosa* has showed the immunomodulatory activity.[21]

Plant and leaves have antiseptic, anti-inflammatory activity and wound healing properties. No scientific data have been reported to substantiate the normal uses of this plant for anti-allergic and diuretic activity till now. In the present study, we investigated the anti-allergic, antioxidant and diuretic activity of the ethanol extracts of *Cleome viscosa* roots in experimental animal models. Various constituents of this plant are utilized for different activities with a scientific justification. Still, majority of the activities are without any scientific backing. The present work is an attempt to justify the use of the plant in ethnopharmacology with a scientific study along with characterization parameters.

Indigenous herbal medicines are a very rich source of polyphenolic compounds as well as ubiquitous in origin, and it also comprises an integral part of the human diet. Recent trends towards estimation of polyphenols have greatly inclined, since these phytochemicals have been implicated in suppressed rates to oxidative stress, which are the root of various disorders and cancer also. Diuretics are used in many clinical conditions including the edematous disorders and hypertension. All diuretics increase renal excretion of sodium and water, but they are differing in chemical derivation, efficacy, sites and mode of action. Diuretics are capable of increasing the flow of urine and are useful in the treatment of diseases with the retention of fluids.[22] An ideal diuretic clinically relies on the objective of therapy and the pathophysiology of the disease. Patients with renal dysfunction needs loop diuretics because they do not respond to other agents to a clinically relevant degree. Patients with cirrhosis are reported for having secondary hyperaldosteronism as a cause of sodium retention and diuretic treatment in such

patients is initiated with an inhibitor of aldosterone, spironolactone. Other uses include the treatment of hypertension and the treatment of cerebral edema.[23] An alternative system of medicine for chronic and acute disorders resulted in an intense area of research and discovery of a number of herbs with potential health benefits. In a previous study, a sufficient number of herbs have been reported for the modulation of the immune system from Ayurvedic formulations either alone or in combination.[24]

## 2. Materials and methods

### 2.1. Plant collection

The dried roots of *Cleome viscosa* were collected in the month of June from local market of Varanasi (Uttar Pradesh) and further processed according to WHO quality control methods for herbal materials[42]. The roots were authenticated as *Cleome viscosa* (Capparidaceae) by pharmacogenetic evaluation and a voucher specimen was deposited at Taxonomic Division of National Botanical Research Institute (Lucknow), for future reference. (Voucher No: NBRI/CIF/173/2010 dated 06-08-2010).

### 2.2. Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl radical (DPPH<sup>\*</sup>), 2, 2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulphate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>), ascorbic acid, and FeCl<sub>3</sub> were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.). Folin-Ciocalteu's phenol reagent, sulfuric acid, petroleum ether, chloroform, ethyl acetate, ethanol, and sodium carbonate were from Merck Chemical Supplies (Merck KGaA, Darmstadt, Germany), Diclofenac sodium was procured from Organic Chemical Industries Pvt. Ltd., Kolkata 70001, West Bengal, India. All the other chemicals used,

including solvents, were of analytical grade.

### 2.3. Experimental animals

Male Albino mice (30-40 gm) were obtained from the animal house, Faculty of Pharmacy, Northern India Engineering College (NIEC), Lucknow (U.P.) the animals were housed in polypropylene cage with steel net and maintained under standard conditions of temperature 25±5 °C and 55±5 % relative humidity with a regular 12 hours light and 12 hours dark cycles and allowed free access to standard laboratory food and water. The animals were treated strictly according to the CPCSEA guidelines and the study was performed humanely in accordance with guidelines from case of animals as set by IAEC (Registration no. 319/01/ab/CPCSEA).

### 2.4. Preparation of drug extract

The plant materials were dried in the shade and size reduction of the dried roots of *Cleome viscosa* was done by a mechanical grinder. The (100g) powder of *Cleome viscosa* was initially defatted with petroleum ether (40-60 °C) followed by 1000 mL of chloroform, ethyl acetate and ethanol respectively by using a soxhlet extractor until the crude drug become exhausted (up to 72 hours) at a temperature not exceeding the boiling point of the solvent. The extract was filtered using Whatman filter paper (No.10) and then combined ethanol extracts were then rotary evaporated at 40 °C to dryness and kept in the dark at 2-8 °C until tested for a short period of time (1-2days). The extracts (petroleum ether, chloroform, ethyl acetate and ethanol extract) yields (% dry weight) of the samples were 3.10%, 7.10%, 11.75% and 21.30% (w/w), respectively and further dried at 45 °C and to obtain a concentrated mass (Table 1). The extracts were kept in a sterile bottle under refrigeration conditions of about 2-8 °C for further studies.

**Table 1: Characteristics of extracts from root extracts of *Cleome viscosa*.**

Extracts	Colour	Consistency	% Yield (w/w)
Pet Ether (40-60°C)	Yellow	Semisolid	3.10
Chloroform	Greenish–Brown	Semisolid	7.10
Ethyl acetate	Dark Brown	Semisolid	11.75
Ethanol	Brown	Semisolid	21.30

### 2.5 Route of administration

The route of administration was by oral gavage in accordance with the main route of intake of *Cleome viscosa* decoction to the animals for medicinal purposes.

### 3. Phytochemical screening procedure

Qualitative tests for alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, terpenoids, proteins and anthraquinone were performed according to

the procedure described by [13]. Mayer's test, Wagner's test for Alkaloids, Shinoda's test for flavonoids, Benedict's test, Molisch's test for carbohydrates, Keller-Killani test for cardiac glycosides, Froth test for saponins, Lead acetate test for tannins, Salkowski tests for triterpenoids, Liebermann-Burchard test for steroids (L-B test), Ninhydrin and Biuret test for protein and Ammonia test for anthraquinone were performed (Table 2).

**Table 2: Qualitative and quantitative screening of root extracts of *Cleome viscosa*.**

Qualitative phytochemical analysis			
S. No.	Tests	Petroleum ether extract	Ethanol extract
1.	<b>Alkaloid</b>		
	Mayer	–	++
	Wagner	–	+
	Dragendorff	–	–
2.	<b>Anthraquinones</b>		
	Ammonia	–	+
3.	<b>Carbohydrates</b>		
	Benedict	–	++
	Molisch	–	–
4.	<b>Flavonoids</b>		
	Shinoda	–	++
	Alkaline reagent	–	–
5.	<b>Glycosides</b>		
	Borntrager	–	+
	Keller Killani	–	++
6.	<b>Proteins</b>		
	Ninhydrin	–	++
	Biuret	–	–
7.	<b>Saponins</b>		
	Froth	–	+
	Lead acetate	–	+
	Haemolysis	–	+
8.	<b>Steroids</b>		
	Liebermann-Burchard	+	+
9.	<b>Tannins</b>		

	Ferric chloride	–	+
	Lead acetate	–	++
<b>10.</b>	<b>Terpenoids</b>		
	Salkowski test	–	+
	<b>Quantitative phytochemical analysis</b>		
	Phenolic content (g GAE/100 g d.w.)	Flavonoid content (g RE/100 g d.w.)	
	4.51±0.4*	5.34±0.93*	

‘+’ Present, ‘++’ Strongly Present ‘-’ Absent, \* Values are given as mean±SD where (n=3)

### 3.1. Determination of total phenolic content (TPC)

The absolute phenolic content was evaluated utilizing the changed Folin-Ciocalteu photometric technique. [25] The proper measure of separated ethanol extracts was oxidized with Folin-Ciocalteu's reagents and following 5 minutes was the response killed with soaked sodium carbonate. The arrangement

was then promptly weakened to the volume of 50 mL with refined water. The absorbance was estimated at 750 nm after 90 minutes of brooding at room temperature against the blank. The alignment bend was readied utilizing diverse grouping of Gallic acid. The total phenolic content is here communicated as Gallic acid counterparts (GAE) per 100 g of dry weight (d.w.) (Table 2 and Fig. 1).

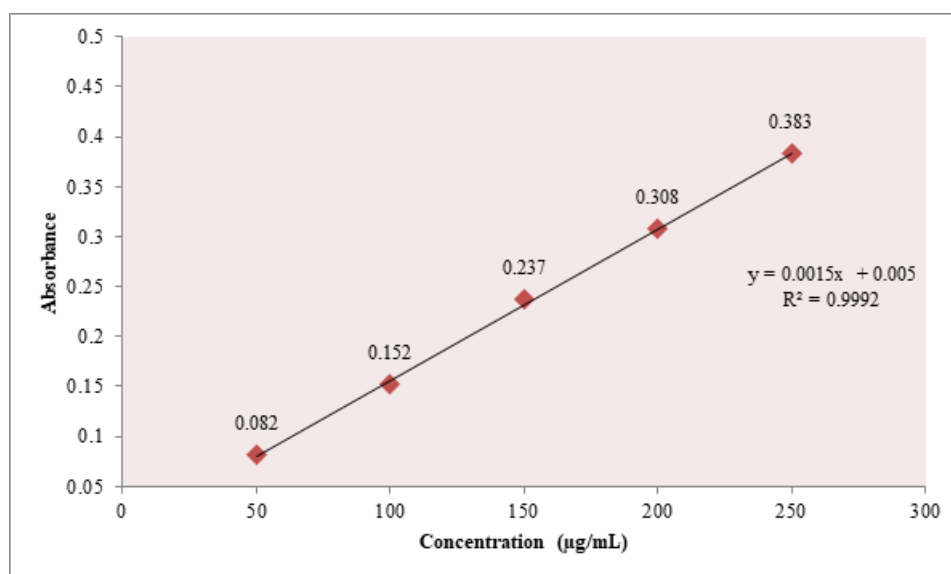
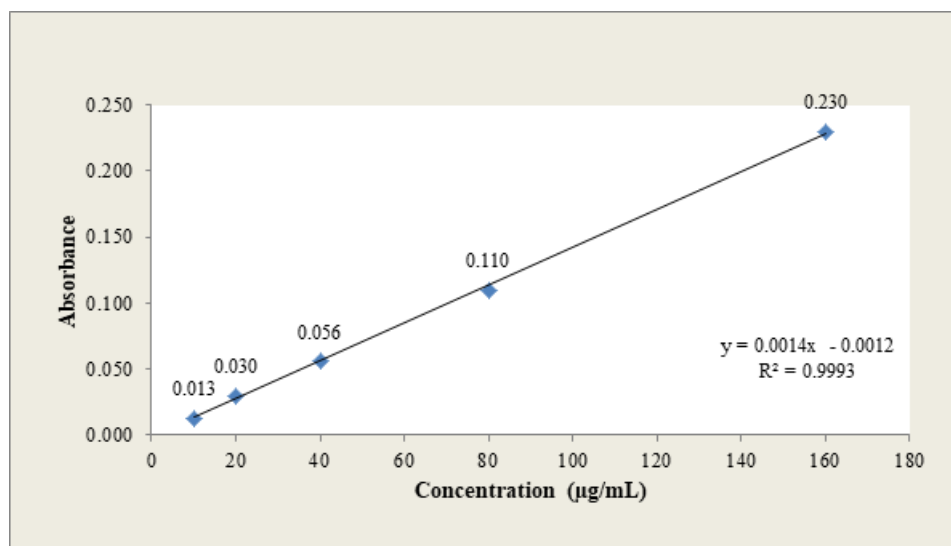


Figure 1: Calibration curve of Gallic acid.

### 3.2. Determination of total flavonoid content (TFC)

The total flavonoid content was estimated utilizing an adjusted colorimetric technique. [26] The appropriate amount of extract was added to a test-tube together with distilled water. Then was added 5 % NaNO<sub>2</sub>, after 5 minutes 10 % AlCl<sub>3</sub> and

after another 5 minutes 1 M NaOH followed by the addition of distilled water. The absorbance was estimated against the clear at 510 nm following 15 minutes. The alignment curve was readied utilizing distinctive concentrations of Rutin. The flavonoid content was communicated as Rutin equivalents (RE) per 100 g of d.w. (Table 2 and Fig. 2).



**Figure 2: Calibration curve of Rutin.**

### 3.3. Acute toxicity and gross behavioral study

The animals were abstained overnight, splitted into groups ( $n = 3$ ) and were dispensed orally with expanding portions (250, 500, 750, and 1000 mg/kg body weight) of petroleum ether, chloroform, ethyl acetate and ethanol extracts. After administration of the concentrates, the creatures were seen during initial 2 hours for their gross conduct changes and once in 30 min for next 4 hours and afterward once in 24 hours for next 72 hours to discover rate mortality of creatures.[27]

### 3.4. Antioxidant activity (*in vitro*)

#### 3.4.1. ABTS radical scavenging assay

The ABTS assay was performed as described by[35]. The stock solutions of ABTS and potassium persulfate were prepared at a concentration of 7 mM and 2.4 mM respectively. Then, the working solution was prepared by taking the 2 stock solutions in 1:1 ratio and was kept for incubation at room temperature for 12 h in dark condition. The solution was further diluted by mixing 1 mL ABTS<sup>+</sup> solution with 60 mL of 80% ethanol to obtain an

absorbance of  $0.706 \pm 0.001$  units at 734 nm using spectrophotometer (SHIMADZU, UV 1800). Plant extracts (1 mL) were allowed to react with 1 mL of the ABTS<sup>+</sup> solution and the absorbance was taken at 734 nm after 7 min. The ABTS<sup>+</sup> scavenging capacity of the extract was compared with that of ascorbic acid. The percentage inhibition was calculated as follows: (Eq. 1)

$$\text{ABTS radical scavenging capacity (\%)} = \left\{ \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \right\} \times 100$$

where  $\text{Abs}_{\text{control}}$  is the absorbance of ABTS radical + 80% ethanol;  $\text{Abs}_{\text{sample}}$  is the absorbance of ABTS radical with sample extract or standard.

The inhibitory concentration ( $\text{IC}_{50}$ ), effective concentration ( $\text{EC}_{50}$ ), and antiradical power (ARP) were estimated and calculated as described by[29]. The results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC)[28] using the following equation: (Eq. 2) and presents as in (Table 3).

$$\text{AEAC} = \left[ \frac{\{\text{IC}_{50(\text{reference})}\}}{\{\text{IC}_{50(\text{sample})}\}} \right] \times 10^5$$

**Table 3: Effect of ethanol extract on different radical-scavenging activities.**

<b>(%) Inhibition</b>				
	<b>ABTS radical scavenging assay</b>		<b>DPPH radical scavenging assay</b>	
<b>Concentration (mg/mL)</b>	<b>EECV</b>	<b>AA</b>	<b>EECV</b>	<b>AA</b>
0.02	6.26±2.48	43.21±1.64	29.30±0.56	95.98±1.07
0.04	14.41±0.38	47.45±0.19	32.22±1.05	96.36±0.76
0.06	17.69±0.38	48.97±2.49	33.98±0.63	97.14±0.53
0.08	20.01±1.03	51.47±1.61	35.47±1.41	97.36±0.17
0.1	33.62±0.76	53.20±0.94	37.60±0.45	97.96±0.19
IC <sub>50</sub> values	0.29	0.16	0.21	0.02
EC <sub>50</sub> values	0.06	0.03	1.93	0.07
ARP values	1956.27	5367.39	29.03	1120.45
AEAC values	7866.21	--	3033.09	--

Values are given as Mean±SD where (n=3)

EECV = Ethanol extract of *Cleome viscosa*; AA = ascorbic acid.

### 3.4.2. DPPH radical scavenging assay

The impact of target extract on DPPH radical was resolved utilizing the strategy for [30]. A solution of 0.135 mM DPPH in 80 % ethanol was readied and 1 mL of this solution was blended in with 1 mL of plant extract. Plant extract in 80 % ethanol contained 0.02 to 0.10 mg of the dried blend. The reaction mixture was vortexed thoroughly and was incubated at room temperature for 30 min in dark condition. The absorbance of the mixture was determined spectrophotometrically at 517 nm by comparison with reference (Ascorbic acid). Ascorbic acid was used as reference. The free radical scavenging property of DPPH radical was calculated by the following equation: (Eq. 3).

$$\text{DPPH radical scavenging capacity (\%)} = \left\{ \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \right\} \times 100$$

where  $\text{Abs}_{\text{control}}$  is the absorbance of DPPH radical + 80% ethanol;  $\text{Abs}_{\text{sample}}$  is the absorbance of DPPH radical with sample extract or reference. The IC<sub>50</sub>, EC<sub>50</sub>, ARP and AEAC were estimated and calculated as described in (Table 3).

### 3.5. Anti-inflammatory activity (*in vitro*)

The reaction mixture (5 mL) comprised of 0.2 mL of egg whites (gathered from new hen's egg), 2.8 mL of phosphate buffered

saline (PBS, pH 6.4) and 2 mL of fluctuating convergences of plant extract with the goal that last focuses become 50, 100, 150, 200, 250, 300 µg/mL. Comparative volume of twofold refined water (DDW) filled in as control. At that point the mixtures were brooded at (37±2) °C in a BOD hatchery (Lab line Technologies) for 15 min and afterward heated at 70 °C for 5 min. In the wake of cooling, their absorbance was estimated at 660 nm (SHIMADZU, UV 1800) by utilizing vehicle as clear and their thickness was controlled by utilizing Ostwald viscometer. Diclofenac sodium at the last grouping of (78.125, 156.25, 312.5, 625, 1250, 2500 µg/mL) was utilized as standard medication and rewarded likewise for assurance of absorbance and consistency. The rate restraint of protein denaturation was determined by utilizing the accompanying formula (Eq. 4).

$$\% \text{ inhibition} = \left\{ \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \right\} \times 100$$

where  $\text{Abs}_{\text{control}}$  is the absorbance of DDW;  $\text{Abs}_{\text{sample}}$  is the absorbance of reaction mixture with sample test concentrate or standard. The concentrate/medicate fixation for 50 % restraint (IC<sub>50</sub>) was dictated by plotting percentage inhibition as

for control against treatment focus (Table 4).

**Table 4: Effect of plant sample extract on protein denaturation.**

Sample extract			Diclofenac sodium		
Concentration ( $\mu\text{g/mL}$ )	% Inhibition	Viscosity (cp)	Concentration ( $\mu\text{g/mL}$ )	% Inhibition	Viscosity (cp)
Control	-	1.12 $\pm$ 0.29	Control	-	1.12 $\pm$ 0.86
50	22.6 $\pm$ 1.03	0.85 $\pm$ 0.56	78.125	10.65 $\pm$ 1.02	0.79 $\pm$ 0.51
100	39.6 $\pm$ 1.31	0.92 $\pm$ 0.69	156.25	13.09 $\pm$ 1.52	0.82 $\pm$ 0.23
150	51.07 $\pm$ 0.92	0.79 $\pm$ 0.75	312.5	27.37 $\pm$ 2.01	0.93 $\pm$ 0.38
200	62.67 $\pm$ 2.09	0.98 $\pm$ 0.51	625	56.23 $\pm$ 1.98	0.92 $\pm$ 0.61
250	92.36 $\pm$ 2.45	1.03 $\pm$ 0.88	1250	209.91 $\pm$ 3.24	0.83 $\pm$ 0.33
300	152.09 $\pm$ 3.23	1.12 $\pm$ 0.83	2500	789.33 $\pm$ 5.23	0.99 $\pm$ 0.50

Values are given as Mean $\pm$ SD where (n=3)

### 3.5. Anti-allergic activity (Milk induced eosinophilia)

Eosinophilia was brought about in mice utilizing healthy bovine-milk (bubbled and cooled) according to the convention with some adjustment[31]. Mice were separated into six gatherings of six creatures in each gathering. Blood was gathered from retro-orbital plexus. group I received vehicle, 5 % polyethylene glycol (5 mL/kg, i.p.); group II filled in as negative control and considered with milk (4 mL/kg, i.p.); test groups III–VI were treated with various aggregation of *Cleome viscosa* extract (50-200 mg/kg, i.p.) accordingly and following 1 hour of medication treatment every

creature was infused with bubbled and cooled milk (4 mL/kg, i.p.). The eosinophils tally was done in each gathering before treatment and 24 hours after milk infusion and the blood tests were gathered from retro-orbital plexus. The eosinophils count was done in each group before drug dispensation and 24 hours after milk infusion. The blood was sucked in WBC pipette and weakened with eosin stain. The eosin solution encourages annihilation of all corpuscles with the exception of eosinophils. Neubauer's chamber was accused of above liquid and eosinophils were tallied (Table 5). The distinction in eosinophils tally when 24 hours of the treatment was determined[32].

**Table 5: Effect of root extract of *Cleome viscosa* on milk-induced leukocytosis and eosinophils in mice.**

Group	Treatments (mL/kg)	Total leukocytes count (per cu mm)	Eosinophils count (per cu mm)
I	5% PEG	121.23 $\pm$ 13.15	53.0 $\pm$ 19.34
II	Milk	3180.21 $\pm$ 136.0	84.1 $\pm$ 42.90
III	50 mg/kg	2025.03 $\pm$ 272.87	66.07 $\pm$ 16.67*
IV	100 mg/kg	2483.34 $\pm$ 148.14*	66.6 $\pm$ 10.54*
V	150 mg/kg	1766.67 $\pm$ 316.14	50.0 $\pm$ 12.91*
VI	200 mg/kg	1545.89 $\pm$ 732.49	37.8 $\pm$ 74.11

Data is presented as mean  $\pm$  SEM (n = 6); \*p<0.05 compared with vehicle (5% PEG) + milk group (group II; negative control).

### 3.6. Diuretic activity

To evaluate the diuretic activity of petroleum ether extracts of *Cleome viscosa*,

chloroform fraction of *Cleome viscosa*, ethyl acetate fraction of *Cleome viscosa*, ethanol fraction of *Cleome viscosa*, and



standard (Furosemide) was carried out by using *in-vivo*, Lipschitz test method[33]. The rats were randomly classified into six groups each comprising of six animals which were required for individual dosing and deprived of food and water for 18 hours. All the rats derived priming dose of normal saline (25mL/kg) orally. The two extracts and standard were fluxed in a normal saline (Table 6). Directly later application, the rats (one in each cage) were allocated in metabolic cages uniquely constructed to detach urine and faeces and

kept at room temperature of  $25 \pm 0.5^\circ\text{C}$ . The urine was gathered in an estimating chamber up to 24h hours. During this period, no food or water was made accessible to creatures. The volume of urine gathered was estimated for all the lot. The variable taken for every individual rat were body weight when trial, urine volume (concentrated for water consumption during the trial), and concentration of  $\text{Na}^+$  in urine. The substance of  $\text{Na}^+$  in the urine was evaluated by flame photometry.

**Table 6: Effect of administration of plant extract(s) of *Cleome viscosa* and Furosemide on sodium excretion in urine and urine output**

S. No.	Groups	Dose (mg/kg)	Urine vol. (mL/kg)	Conc. of excreted $\text{Na}^+$ ions (mEq/L)
1.	Control (normal saline)	25 mL/kg	23.85 $\pm$ 0.423	78.36 $\pm$ 0.637
2.	Standard (Furosemide)	100 mg/kg	33.64 $\pm$ 0.433	150.70 $\pm$ 0.958
3.	PE <sub>1</sub>	100 mg/kg	34.83 $\pm$ 0.523 <sup>a</sup>	99.34 $\pm$ 0.928 <sup>b</sup>
4.	PE <sub>2</sub>	200 mg/kg	34.51 $\pm$ 0.474 <sup>a</sup>	93.08 $\pm$ 0.581 <sup>b</sup>
5.	CE <sub>1</sub>	100 mg/kg	22.30 $\pm$ 1.669	118.30 $\pm$ 1.464
6.	CE <sub>2</sub>	200 mg/kg	20.86 $\pm$ 0.198	123.60 $\pm$ 2.891
7.	EAE <sub>1</sub>	100 mg/kg	22.72 $\pm$ 0.259	129.10 $\pm$ 1.506
8.	EAE <sub>2</sub>	200 mg/kg	22.19 $\pm$ 0.252	135.10 $\pm$ 2.627
9.	EE <sub>1</sub>	100 mg/kg	23.01 $\pm$ 0.396	112.10 $\pm$ 1.398
10.	EE <sub>2</sub>	200 mg/kg	32.75 $\pm$ 0.359 <sup>a</sup>	98.07 $\pm$ 1.701 <sup>b</sup>

Values are expressed as mean  $\pm$  SEM (n = 6), <sup>a</sup>p<0.05 when compared with control (F = 87.03, df = 9) (one way ANOVA followed by Newman-Keuls Multiple Comparison Test); <sup>b</sup>p<0.05 when compared with control (F = 177.04, df = 9) (one way ANOVA followed by Dunnett's Multiple Comparison Test) respectively. PE<sub>1</sub> and PE<sub>2</sub>= Different doses of petroleum ether extract (E<sub>1</sub>: 100 mg/kg and E<sub>2</sub>: 200 mg/kg); CE<sub>1</sub> and CE<sub>2</sub>= Different doses of chloroform extract; EAE<sub>1</sub> and EAE<sub>2</sub>= Different doses of ethyl acetate extract and EE<sub>1</sub> and EE<sub>2</sub>= Different doses of ethanol extract, respectively.

### 3.7. Statistical analysis

In the *in vitro* antioxidant and anti-inflammatory, the data were expressed as mean  $\pm$  standard deviation (SD), using Microsoft excel where (n = 3). And in anti-

allergic activity the data were expressed as mean  $\pm$ SEM; \*p<0.05 compared with vehicle (5% PEG) + milk group (group II; negative control). Where the 100 mg/kg dose of EECV shows total leukocytes count values 2483.34 $\pm$ 148.14 per cu mm and eosinophil count 66.6 $\pm$ 10.54 per cu mm. Though in diuretic activity, trial results are communicated as mean  $\pm$  (SD) (n = 6) utilizing measurable investigation with SPSS 20.0 (Statistical Program for Social Sciences, SPSS Corporation, Chicago, III., U.S.A.) variant. Analyses of variance (ANOVA) in a completely randomized design, by Newman-Keuls multiple comparison test, and Dunnett's multiple comparison test were performed to compare the data. P-values p<0.05 were considered as significant. Results were expressed as mean  $\pm$  SEM (n = 6) in each group. Among all test compounds used, the

petroleum ether fraction was found to be most active which shows  $34.83 \pm 0.523^a$  mL/kg (Urine vol.);  $99.34 \pm 0.928^b$  mEq/L (Conc. of excreted  $\text{Na}^+$  ions) at 100 mg/kg dose and  $34.51 \pm 0.474^a$  mL/kg (Urine vol.);  $93.08 \pm 0.581^b$  mEq/L (Conc. of excreted  $\text{Na}^+$  ions) at 200 mg/kg dose and ethanol extract was also active which shows  $32.75 \pm 0.359^a$  mL/kg (Urine vol.);  $98.07 \pm 1.701^b$  mEq/L (Conc. of excreted  $\text{Na}^+$  ions) at 200 mg/kg dose, which is significant from the control group, at 0.05 % probability.

## 4. Results

### 4.1. Preliminary qualitative and quantitative screening of *Cleome viscosa* (Phytochemical analysis)

The outcome of the fundamental phytochemical screening of ethanol concentrates and its parts are given (Table 2).

### 4.2. Acute toxicity studies

In acute toxicity study, all the animals were found to be surviving after 72 hours. This indicates that the extracts were found to be safe up to the dose levels studied. Since all the animals survived at a dose of 1000 mg/kg body weight, the  $\text{LD}_{50}$  of the extracts will be  $>1000$  mg/kg body weight. No major complications and behavioral changes were observed during the period of acute study. The animals showed mild

sedative effect upon administration of all the extracts.

## 4.3. Antioxidant activity (*in vitro*)

### 4.3.1 ABTS radical scavenging

The reduction potential of  $\text{ABTS}^{+\cdot}$  radical was determined by decrease in its absorbance at 734 nm which was induced by antioxidants. The  $\text{ABTS}^{+\cdot}$  radical scavenging capacity (%) of the ethanol extract of *Cleome viscosa* (EECV) with comparison to ascorbic acid are depicted in (Table 3 and Fig. 3). At a concentration of 0.02 to 0.1 mg/ml of ethanol extract of *Cleome viscosa* scavenged the ABTS radical in a dose-dependent manner. The positive control (ascorbic acid) at a concentration of 0.02 to 0.1 mg/mL were also found to produce dose-dependent inhibition of ABTS radical. The quantity of EECV extract required to produce 50% inhibition ( $\text{IC}_{50}$ ) of ABTS radical was found to be 0.29 mg/mL, whereas in ascorbic acid ( $\text{IC}_{50}$ ) values were found to be 0.16 mg/ml. The  $\text{EC}_{50}$  and ARP values of EECV extract were also compared to the standards and as a result  $\text{EC}_{50}$  was found to be 0.06 mg/mL and 0.03 mg/mL respectively. The ARP and AEAC value of the EECV extract were  $1.956 \times 10^3$  and  $7.866 \times 10^3$ , whereas the ARP value of Ascorbic acid depicted as  $5.3 \times 10^3$  respectively (Table 3).

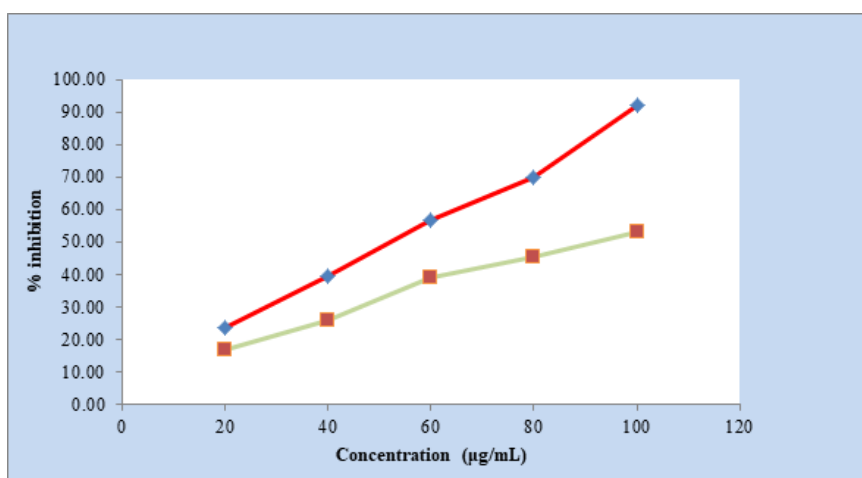


Figure 3: Percentage inhibition with ABTS radical scavenging assay.

#### 4.3.2. DPPH radical scavenging

DPPH radical, a stable-free radical was used to determine the antioxidant capacity of natural compounds[34]. The free radical scavenging capacity of EECV extract was compared to the positive control (ascorbic acid). The IC<sub>50</sub> values were found to be 0.21, and 0.02 mg/mL for EECV extract and ascorbic acid, respectively. The EC<sub>50</sub> value of DPPH scavenging activity of the EECV extract and the positive control was found to be 1.93, and 0.07 mg/mL whereas the ARP and AEAC values were found to

be  $2.903 \times 10^3$  and  $3.033 \times 10^3$  respectively. The DPPH activity of EECV extract was found to increase in a dose-dependent manner. The EECV extract, at the used concentrations, displayed potential effect of DPPH activity as percentage of free radical inhibition (Table 3) and (Fig.4). A higher DPPH radical scavenging activity is linked with a lower IC<sub>50</sub> value. Therefore, these results suggested that the EECV extract contained strong free-radical scavenging capacity but was less potent than the positive control, ascorbic acid (Table 3).

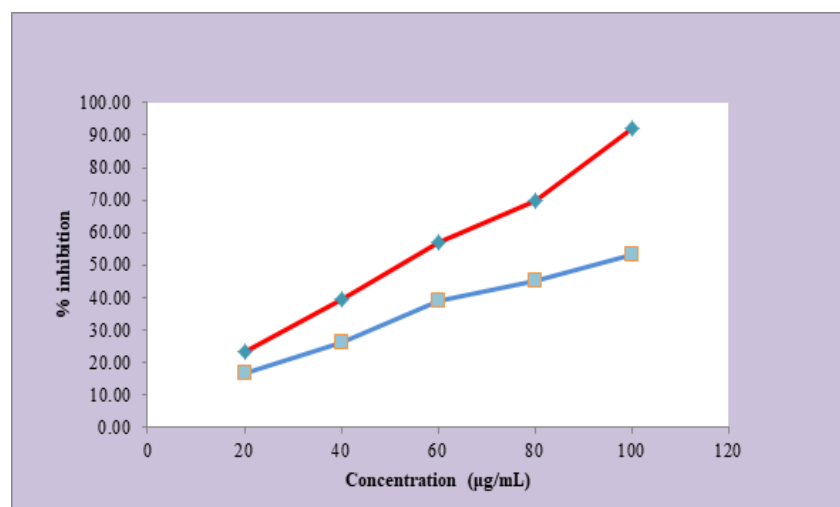


Figure 4: Percentage inhibition with DPPH radical scavenging assay.

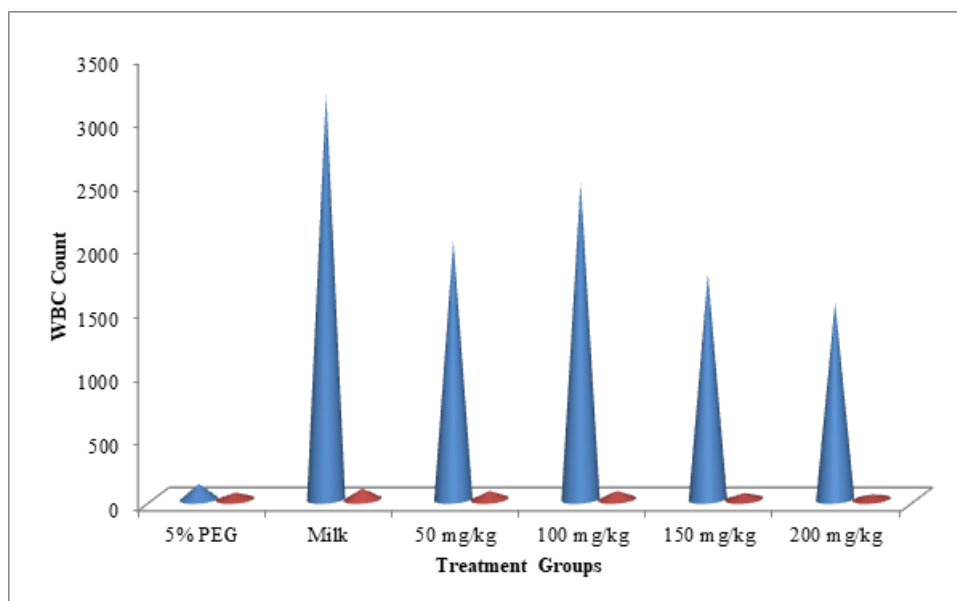
#### 4.4. Anti-inflammatory activity

In the current examination, the *in vitro* anti-inflammatory impact of plant extract (ethanol) was assessed against denaturation of egg albumin method. Discoveries uncovered a focus subordinate hindrance of protein (egg albumin) denaturation by plant extract all through the fixation scope of 50 to 300 µg/mL. Diclofenac sodium (at the fixation scope of 78.125 to 2500 g/mL) was utilized as a standard which additionally showed focus subordinate restraint of protein denaturation. In any case, the impact of diclofenac sodium was seen as less when contrasted and EECV. This was additionally affirmed by looking at their IC<sub>50</sub> esteems. Plant extract had esteem 148 µg/mL while that of diclofenac sodium was seen as 625 µg/mL. The outcomes are summed up in (Table 4).

#### 4.5 Anti-allergic activity

The eosinophils are accounted for to intervene incendiary and cytotoxic occasions related with allergic issues, including bronchial asthma, rhinitis and urticaria. As shown in (Table 5), the administration of boiled and cooled bovine milk in group (II) exhibited an irregular upregulation in eosinophils count associated as group (I) [41] stated that, escalation in apparent eosinophils count greater than 4% of total leucocyte count hasin-lineconnection with respirational ailments and are frequently allergic in nature. The mice pre-treated with various *Cleome viscosa* extracts (group III-VI) showed significant (\*p < 0.05) reduction in eosinophils count induced by milk. Similarly, the *Cleome viscosa* extract has the higher phenolic content which could be

the compound which induces the anti-allergic effects (Table 5 and Fig. 5).



**Figure 5: Effect of root extract of *Cleome viscosa* on milk-induced leucocytosis and eosinophils in mice.**

#### 4.5. Diuretic activity

The petroleum ether extract was found to create noteworthy diuretic movement by expanding in urinary yield and discharge of sodium when contrasted with control. The impact of petroleum ether extract was found to be in dose dependent way; for example, higher portion indicated significant impact as tried (100 < 200 mg/kg i.p.). The request for action of increment in urinary yield and urinary electrolyte discharge was seen as petroleum ether > ethanol > chloroform > ethyl acetate fraction (Table 6).

#### 5. Discussion

There are firm dilemmas behind use of experimental animals in phyto-pharmacological investigation, likewise ethical matters and be short of grounds in comparison with other appropriate procedures which are accessible or possibly to be explored. The current study expresses that plant *Cleome viscosa* exhibits the occurrence of abundant primary as well as secondary metabolites like alkaloids, anthraquinones, carbohydrates, flavonoids,

glycosides, proteins, saponins, tannins and terpenoids in different extract fractions. Occurrence of these important metabolites in our plant alone or in conjunction could be responsible for the said diuretic and antioxidant activity.

Furthermore, it was successfully supported from phytochemical screening of different extract fractions. The TPC and TFC were found to be  $4.51 \pm 0.4$ ,  $5.34 \pm 0.93$  and  $48.16 \pm 0.46$  respectively (Table 2). Assessment of antioxidant activity was supported with *in vitro* examination. Present study explored the EECV extract, contained outstanding phenolic profile and was accomplished of constraining and subsiding the free radicals to alleviate the radical cascade, and working as reducing agents.

At a glance, accountability of phenolic constituents are proven facts for antioxidant potential in most of the herbal medicinal plants (HMP). Consequently, a significant and in line association to be found between the phenolic profile and antioxidant potential, indicative of phenolic compounds could be foremost contributors

in lieu of antioxidant activity. The EECV extract exhibited a sturdy antioxidant potential as seeming through DPPH and ABTS models, against ascorbic acid. Also, EECV extract was found with noticeable quantity of total flavonoids, and ascorbic acid, which imparts vital role behind antioxidant activity.

Estimation of antioxidant potential via *in vitro* methods were only suggestive of prospective health aids, so these findings could be very helpful for preliminary screening of antioxidant activity of said extract. Additional descriptive studies resolve its antioxidant potential likewise *in vitro* and *in vivo* systems and the EECV extract could be used as an accessible foundation of expected antioxidants with subsequent health profits in current life-threatening ground and stressful conservational circumstances.

For evaluation of anti-inflammatory activity, *in vitro* protein denaturation examination is selected for evaluation throughout various concentration of plant extract. Distortion of membrane proteins is a distant and notable stand of inflammatory and allergic clauses. Substance which can put off denaturation of tissue protein therefore, would be appreciated for advancement of anti-inflammatory remedy. A very plausible absorbances of test samples against control stipulates protein equilibrium i.e., inhibition of heat-induced protein distortion by said extract and standard drug (diclofenac sodium). From the resultant IC<sub>50</sub> values of plant extract, showed enhanced outcomes in contrary to standard drug in lower concentration.

It was strongly hypothesized that viscosity of the drug also plays a significant role in anti-denaturation effect, which becomes increased in denaturation of protein. From our studies, it could be a proven fact as the control dispersion had very high viscosity as compared to extract samples. Plant extract counteracted this and despise the protein denaturation though, lower concentration in viscosities of test sample

(plant extracts) and standard drug were found. However, the viscosities of the test samples of all aliquots were always not as much of that of control samples. The decrement in viscosities may be due to concentration shift of test extract in reaction mixture due to vague physicochemical aspects. On the other hand, alteration in viscosities provoked inhibition of protein denaturation, while the effect of concentration of test sample and its behavior on viscosity needs reconsideration. Polyphenols are renowned natural entities known to acquire several notable pharmacological responses. In existing screening of *in vitro* anti-inflammatory activity of HMP can be accredited to aforementioned polyphenolics. As previously documented, stabilization of heat-treated albumin protein by rendering of physiological pH (pH: 6.4) is the key component in several existed non-steroidal anti-inflammatory drugs. Thus, from the present study it can be supposed that *Cleome viscosa* acquired noticeable *in vitro* anti-inflammatory activity against the inhibition of heat induced inflammatory responses. Future prospective studies are necessary to criticize its mechanisms and overall behavior beneath their anti-inflammatory potential.

From the assessment of anti-allergic action of the plant drug extract(s) from *Cleome viscosa* may be due to the presence of total phenols (4.51±0.4 g GAE/100 g d.w.) and flavonoids (5.34±0.93 g RE/100 g d.w.). As reported earlier [35] the most significant nature of an anti-allergic action, is its capability to upsurge competition against adverse stimuluses of an extensive range of factors (i.e. physical, chemical and biological); irrespective of the pathologic states, in this situations *Cleome viscosa* extracts significantly diminished eosinophils, which shows that these extracts are useful in allergic circumstances. In spite of it the root extracts of *Cleome viscosa* has anti-allergic activity and potential to stabilize mast cells by

antagonizing the milk-induced eosinophilia. As reported by [36] the phenolic compounds have the anti-allergic effects could be partly mediated through the reduction of  $\text{Ca}^{2+}$  influx and elevation of cAMP in the mast cells.

For assessment of diuretic activity, Lipschitz test model was utilized to justify the potential of plant extracts and its fractions (petroleum ether, chloroform, ethyl acetate and ethanol) as well as standard drug (Furosemide). The outcomes revealed that, the 100 mg/kg and 200 mg/kg dose of petroleum ether extract and 200 mg/kg dose of ethanol extract possesses significant diuretic activity. A parallel framework of diuresis is testified with some other plant diuretics[37] as reported previously. It is supposed that, if this inferences to be applicable for further studies on human volunteers then this is an impeccable achievement both locally and globally as it provides scientific evidence in favor of its claimed diuretic potential in strict and controlled environment.

Undoubtedly, this would open the new era of broad opportunities of future potential diuretics obtainable to traditional experts. The subsequent diuresis remained strong and was not accompanied with a reduction in  $\text{K}^+$  concentration in excreted urinary output. Continuous efforts revealed that, there was not any chances of urinary alkalization. For instance, we had concluded that the Chloroform extract did not impart in acting as potassium-sparing diuretics[38] The cold-water extract(CWE) is also uncertain to be substituted thiazide diuretics: these only upsurge the concentration of urinary  $\text{K}^+$  output and amended the urinary  $\text{Na}^+/\text{K}^+$  ratio[39] During current study, both urinary  $\text{Na}^+$  and  $\text{K}^+$  levels were augmented without any variation in the  $\text{Na}^+/\text{K}^+$  ratio. Alternatively, the urinary outcome encouraged by the Chloroform extract was forcefully comparable to that of standard drug and accompanied by noticeable rises in both urinary  $\text{Na}^+$  and  $\text{K}^+$  level.

Instantaneously the urine became somewhat acidified. Thus, we conclude that the current study strongly proposed that the Chloroform extract stands as a loop diuretic. Loop diuretics were the utmost influential of all diuretics and inhibit the  $\text{Na}^+/\text{K}^+/\text{Cl}^-$  co-transporter channel in the dense ascending loop of the Henle, thereby collectively induced natriuresis and kaliuresis[39] In spite of the heavy loss of  $\text{Na}^+$  and  $\text{K}^+$  in urinary outflow, there was a significant drop in the osmolarity of urine in Chloroform extract treated subjects. Thus, the diuresis action of the Chloroform extract supposed accredited phenolic constituents of the plant itself. Loop diuretics were clinically utilized in patients with excess salt and water because of pulmonary edema, heart failure ascites, hypertension[40]. Loop diuretic action of the Chloroform extract designates that it might be valuable as an innocuous natural remedy in the management of such circumstances by traditional practitioners. [41-45] The onset of the diuretic action of the Chloroform extract was enormously quick besides it also had an impartially long extent. The aforementioned diuretic profile of the HMP's profound that, as it would curtail the frequency of administration according to severity of ailments. Though, there is a foremost constraint as it had an augmented risk of hypokalemia as with further intensely used loop diuretics. In conclusion, the current study revealed that, warranted drug has initial scientific indication in favor of appealed diuretic potential of Chloroform extract. It additionally profound that the Chloroform extract primarily behave as a loop diuretic as it induces diuresis.

## 6. Conclusion

The present study suggests that ethanol extract of *Cleome viscosa* roots possess powerful antioxidant activity, diuretic and anti-allergic activity. Therefore, it is suggested that *Cleome viscosa* could be a potential source of natural antioxidants, diuretic and anti-allergic activity that could

have great importance as therapeutic agent in preventing or slowing down the progress of ageing, age associated oxidative stress related degenerative diseases, near future in treatment of hypertension, renal failure, congestive heart failure and clinical conditions leading to edematous state of body. The preliminary phytochemical investigation reveals the presence of alkaloids, flavonoids and terpenoids in ethanol extract which can be responsible for diuretic and anti-allergic activity as well as antioxidant activity but need to be confirmed by a clinical study. Further research is recommended for better characterization of important active constituents, responsible for antioxidant activity, diuretic and anti-allergic activity. The revealed antioxidant, diuretic and anti-allergic properties of extracts may provide potential therapeutic intervention against oxidative threats and degenerative disorders.

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