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RESEARCH ARTICLE

ANTIOXIDANT POTENTIAL, ANTIDEPRESSANT ACTIVITY AND HPTLC FINGERPRINTING OF PHENOL & FLAVONOID COMPOUNDS OF *CLEOME VISCOSA* SEED EXTRACTS

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ABSTRACT

Objective: The present study is design to evaluate preliminary phytochemical constituents, physiochemical evaluation, free radical scavenging activity, HPTLC analysis and Antidepressant activity of *Cleome Viscosa* seed oil.

Methods: Total phenol content, Total flavonoid content, Total tannin content, DPPH radical scavenging method, HPTLC analysis for phenols and flavonoids compounds, Tail suspension test(TST) and Forced swimming test (FST).

Result: Phytoconstituents like alkaloids, flavonoids, saponins, fixed oil etc were assessed by preliminary phytochemical screening. Oil contains an acid value, saponification value, iodine value of 36.57 mg KOH/g, 257 mg KOH/g and 117.1 mgI_2/100g respectively. Phenol content, flavonoid content and tannin content is 63.36 ± 0.32 mg gallic acid equivalent/g, 153.47 ± 0.56 mg rutin equivalent/g, 19.4 ± 0.40 mg tannic acid equivalent/g respectively and also having a very good radical scavenging activity. The HPTLC analysis assessed that, the methanolic extract has phenol and flavonoid compounds and also possess significant antidepressant activity.

Conclusion: Our results suggest that *Cleome Viscosa* seeds are may be proved to be a natural antioxidant and antidepressant with various bioactive compounds used for the treatment of various other diseases.

INTRODUCTION

The plant *Cleome Viscosa* is locally known as "wild mustard" belonging to the family Capparaceae and is distributed throughout India growing as a weed in planes. The oil from the plant is used for biodiesel production[1] along with various medicinal uses like anthelmintic, carminative, rubefacient, vesicant etc. Whole plant used as folk remedy for wounds, ulcers, inflammations and skin infections[2].

Now a day there is an increase in pollution which results in consumption of a lot of oxygen species by our body. These oxygen species like superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide[3] results in various physical and chemical changes in our body which may results in various types of diseases like cancer, liver diseases, renal failure, stress etc[4]. So it is important to discover the materials which may stop these ill effects. Plant materials like vitamin C, vitamin E etc are very good source for antioxidant property.

Depression is a heterogeneous disorder that affect a person mood, physical health and behaviour. Suicidal tendency remains one of the common outcomes of depression, with depressive illness being responsible for 60% of the death to all.

So here by these studies we are going to discover the antioxidant activity and antidepressant activity of the plant *Cleome Viscosa* seed extract by different models along with its phytochemical investigation and physiochemical studies.

MATERIALS AND METHOD

Chemicals

The entire chemicals used were of analytical grade and purchased commercially. Folin-Ciocalteus reagent, gallic acid, tannic acid, rutin, DPPH and other chemicals were purchased from S D Fine.

Plant Material

The seed of *Cleome viscosa* were collected from the local area of Lucknow. The seed of *C.viscosa* were authenticated by Dr. A.K.S Rawat (Head of Department Pharmacognosy & Ethanopharmacology Div) CSIR- National Botanical Research Institute (NBRI), Lucknow, having specification no. of NBRI/PH/6/3.

Preparation of Seed Oil and extract

50 gm *Cleome viscosa* seeds were powdered and the coarse powder was packed in soxhlet apparatus and extracted with hexane and methanol, several cycles were run at 70ºC. Mixture obtained was filtered and the oil content was collected. The extract obtained was evaporated and dried with rota evaporator at temperature < 50º C. The fixed oil obtained was weighed and solubilised in DMSO for further experimentation.[5]

In-Vitro Antioxidant Activity

Estimation of Total Phenolic Content

Phenolics were determined using gallic acid as standard. A 100µg/ml stock solution of gallic acid was prepared. From the above stock a 0.5ml aliquot was pipette out into 25ml volumetric flask. 10ml distilled water and 1.5ml Folin ciocalteus reagent was added. Then after 10 min 4 ml 20% sodium carbonate was added and volume is makeup up to 25ml using distilled water.

A stock solution of 1mg/ml in methanol of methanolic extract was prepared. From the above stock 0.5ml of extract was taken in 25ml volumetric flask. 10ml distilled water and 1.5ml Folin ciocalteus reagent was added. Then after 10 min 4 ml 20% sodium carbonate was added and volume is makeup up to 25ml using distilled water.

After 30min the absorbance of both test and standard solution was taken at 765nm. Percentage of total phenolics was calculated using the equation based on calibration curve of gallic acid:

$Y = 0.3716X + 0.0114$
$R^2 = 0.9985$

Estimation of Total Flavonoid Content

Flavonoids were determined using rutin as standard. A 100µg/ml stock solution of rutin was prepared. From the above stock a 0.5ml aliquot was pipette out. 0.5ml 2% methanolic aluminium chloride, few drops of distilled water and 4ml methanol was added.

A stock solution of 1mg/ml in methanol of methanolic extract was prepared. From the above stock 0.5ml of extract was taken, add 0.5ml 2% methanolic aluminium chloride, few drops of distilled water and 4ml methanol was added.

After 20min the absorbance of both standard and test solution was taken at 420nm. Percentage of total flavonoids was calculated using the equation based on calibration curve of rutin.

$Y = 0.0206X – 0.0078$
$R^2 = 0.999$

Estimation of Total Tannin Content

Tannins were determined using tannic acid as standard. A 100µg/ml stock solution of tannic acid was prepared. From the above stock a 1ml aliquot was pipette out in 100ml volumetric flask, 5ml Folin ciocalteus reagent, few ml distilled water, 10ml saturated sodium carbonate solution were added and volume was make up by distilled water up to 100ml.

2gm powdered seed material were extracted and 1ml aliquot was pipette out in 100ml volumetric flask, 5ml Folin ciocalteus reagent, few ml distilled water, 10ml saturated sodium carbonate solution were added and volume was make up by distilled water up to 100ml.

Absorbance of both standard and test solution was taken at 760nm. Percentage of total tannin was calculated using the equation based on calibration curve of tannic acid.

$Y = 0.2515X + 0.0563$
$R^2 = 0.9979$

DPPH (1, 1-Diphenyl, 2-picryl-hydrazyl) Antiradical Activity

Methanolic solution of methanolic extract (20, 40, 60, 80, 100 µg/ mL) was mixed with 400 µM DPPH methanol solution at a ratio 1:3. Gallic acid is taken as standard. A control was prepared using methanol and DPPH solution. The mixture was mixed well and set in dark at room temperature for 30min. The change of colour from violet to yellow of DPPH was determined by measuring the absorbence at 517nm. The percentage of inhibition was calculated by the given formula and also IC_{50} Value is calculated.

(%) Scavenging activity = Control absorbance - Test absorbance x 100 / Control absorbance

Chromatographic Estimation

Development of TLC fingerprint

Thin layer chromatography (TLC) studies were done as per conventional one dimensional ascending method using preparative plates of silica gel G, plates were marked by a pencil and spotted by using simple glass capillaries at a distance of 1cm. The mobile phase used were A. Hexane: Ethyle Acetate: Acetic Acid (4:4:2) v/v, B. Propanol: Ethyl Acetate: Water (7:2:1) v/v, C. Toluene: Ethyl Acetate: Formic Acid (7:3:0.5) v/v, D. Chloroform: methanol: acetic acid (14: 2.9: 1) v/v. The plates were dried and detected using Iodine chamber and UV chamber. Retention factor of different spots were calculated by the formula:

$$Rf = \frac{\text{distance spot moved}}{\text{distance solvent moved}}$$

Development of HPTLC method for analysis of phenols and flavonoids

HPTLC analysis was carried out using Camag HPTLC system equipped with Linomat -V applicator and 100 µl syringe. The samples were spotted in the form of bands using microlitre syringe on pre-coated silica gel 60 F_{254} HPTLC plates and development of the applied plate was carried out in pre-saturated Camag twin-trough chamber. The developed plates were dried and analysed at 254nm and 366nm. The mobile phase used for phenols is choloroform: etylacetate: methanol: formic acid in a ratio of 3.5:5:0.5:1 and for flavonoids is toluene: ethyl acetate: formic acid in a ratio of 3:6:1.

Antidepressant Activity

30 swiss albino mice (25-35 gms body weight) of either sex were randomly selected and grouped into 5 groups (n=6). They were acclimatized and housed in animal house with 12hr: 12hr light-dark cycle at 27±2°c temperature and 45-55% relative humidity. Food and water supplied ad libitum. The work was approved by the Institutional Animal Ethical Committee (IAEC). Control animals were treated with distilled water. Drugs like imipramine (10mg/kg), test drug *Cleome viscosa* (100 mg, 200mg, 400mg/kg) were dissolved in distilled water

and administered orally once daily for seven days. On 8th day tests were repeated.

Tail suspension test (TST): A mouse was hung on a wire in an upside down posture so that its nostrils just touch the water surface in container. After initial vigorous movement, the mouse assumes an immobile posture and the period of immobility during 5 min observation noted. This test is reliable and rapid screening method for antidepressants, including those involving the serotonergic system. [6]

Forced swimming test (FST): The rats were placed a cylinder (45x20cm) containing 38 cm water (25±2°C), so that the rat could not touch bottom of cylinder with its hind limb or tail or climb over the edge of the chamber. Two swim sessions were conducted, an initial 15 min pre-test, followed by 5 min test 24 h later. Drug was administered after pre-test. The period of immobility (remained floating in water without struggling and making only those movements necessary to keep its head above water) during 5 min test period was noted.[7]

RESULTS AND DISCUSSION

Table 1 Preliminary phytochemical screening of seed extracts of *Cleome viscosa* done according to C. K Kokate. "Practical Pharmacognosy.[8]

Constituents	Hexane	Methanol
Alkaloids	+	+
Carbohydrates	+	+
Caumarins	++	+
Flavonoids	_	+
Fixed oil	+++	+
Glycosides	_	+
Gums and resins	+	-
Mucilage's	_	-
Proteins and amino acid	_	_
Saponins	_	+
Steroids	++	+
Tannins	+	_
Triterpenoids	++	+

Note: +++ High, ++ Moderate: + Slight: -Negative

Table 2 Physicochemical properties of seed oil of *Cleome viscosa* done according to AOAC (1990).[9]

S.No.	Oil Property	Unit	Value
1.	State of oil	-	Brown Yellow
2.	Colour of oil	-	Brownish Black
3.	Oil content	%	38.2
4.	Acid value	mgKOH/g	36.57
5.	Saponification value	mgKOH/g	257
6.	Iodine value	mgI2/100g	117.1
7.	Refractive index	dimensionless	1.462
8.	Free fatty acids	%	18.39
9.	Ester value	mgKOH/g	220.43
10.	Glycerin	%	12.04
11.	Moisture content	%	8.1

Table 3 Total phenolics, flavonoids & tannins content of extracts of *Cleome viscose*

Plant extracts	Total phenolics (mg gallic acid equivalent/g)*	Total flavonoid (mg rutin equivalent/g)*	Total tannin (mg tannic acid equivalent/g)*
Methanolic extract	63.36 ± 0.32	153.47 ± 0.56	19.4 ± 0.40

(*mean ± S.D, n=3)

RESULTS OF DPPH MODEL

In DPPH Model lower is the absorption of mixture at 517nm greater is the radical scavenging activity and same is obtained by the methanolic and hexane extract of *Cleome viscosa* seeds.

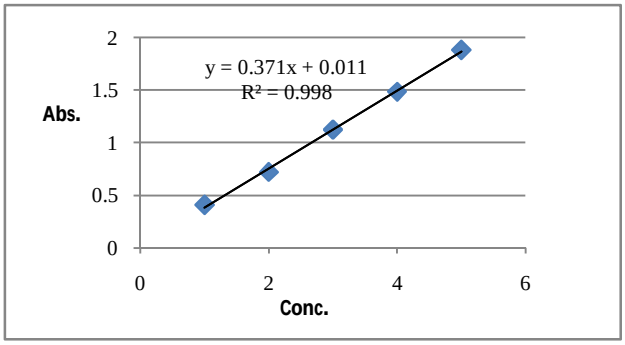


Fig 1 Calibration curve of gallic acid

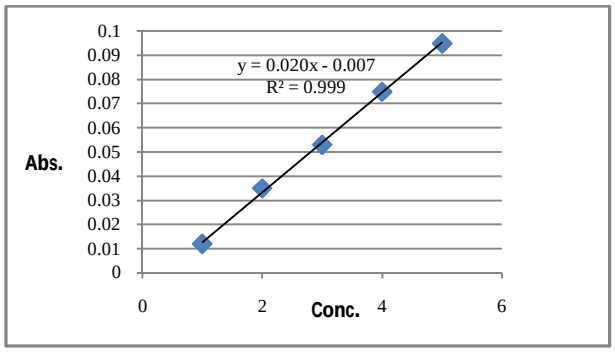


Fig 2 Calibration curve of rutin

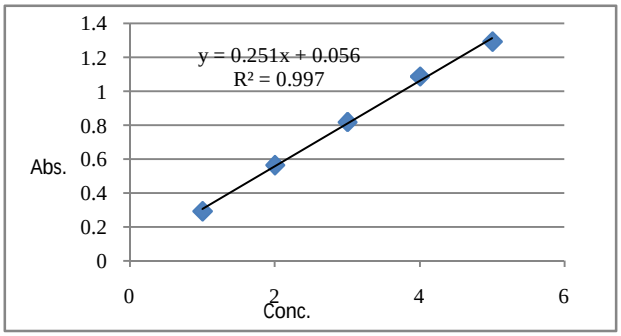


Fig 3 Calibration curve of Tannic acid

The methanolic extract shows better radical scavenging activity than the hexane extract. The results of % inhibition were shown in table 4 and represented in Fig 4 by graph and the IC_{50} value calculated is tabulated in table 5.

Table 4 Results of DPPH radical scavenging activity

S. No.	Extract	Concentration (µg/ml) and % inhibition				
		20	40	60	80	100
1	Std. Gallic acid	91.11	91.44	92.44	92.95	93.61
2	Methanolic	85.36	86.95	88.9	89.82	91.13
3	Hexane	64.96	65.78	66.9	68.3	69.1

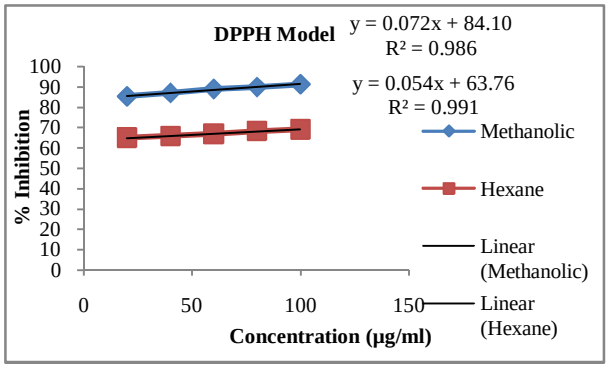


Fig 4 Radical scavenging activity by DPPH method

Table 5 IC_{50} value for DPPH radical scavenging assay

S No.	Plant Extract	IC_{50} Value ((µg/ml)*
1	Methanolic	254.96±0.53
2	Hexane	473.74 ±0.18

(*mean ± S.D, n=3)

Table 6 Rf values of TLC analysis of antioxidants in different mobile phase

S. No	Extract	Mobile Phase A		Mobile Phase B		Mobile Phase C		Mobile Phase D	
		Spot No.	R_f	Spot No.	R_f	Spot No.	R_f	Spot No.	R_f
1.	Hexane extract	2	0.23 0.89	1	0.34	2	0.42 0.54	1	0.31
2.	Methanol extract	2	0.4 0.56	4	0.3 0.67 0.81 0.90	3	0.12 0.35 0.82	3	0.42 0.61 0.75

HPTLC Analysis

Table 7 HPTLC- Phenols profile of methanolic extract of seeds of *Cleome viscose*

Peak	R_f	Height (AU)	Area (AU)	Assigned substances
1	-0.05	43.0	382.4	Unknown
2	-0.0	228.2	3043.4	Unknown
3	0.05	27.3	842.5	Unknown
4	0.47	18.7	196.7	Unknown
5	0.67	38.4	959.1	Gallic Acid
6	0.71	72.3	1855.7	Unknown
7	0.77	167.8	6291.6	Unknown
8	0.83	192.6	10855.4	Unknown
9	0.94	14.4	346.5	Unknown

Table 8 HPTLC- Flavonoids profile of methanolic extract of seeds of *Cleome viscosa*

Peak	R_f	Height (AU)	Area (AU)	Assigned substances
1	-0.05	23.9	485.0	Unknown
2	-0.01	351.8	4052.7	Unknown
3	0.01	67.8	1332.7	Unknown
4	0.05	25.1	422.8	Rutin
5	0.62	47.9	1361.0	Unknown
6	0.68	51.8	1841.2	Unknown
7	0.73	117.4	5038.0	Unknown
8	0.85	128.0	7026.7	Unknown

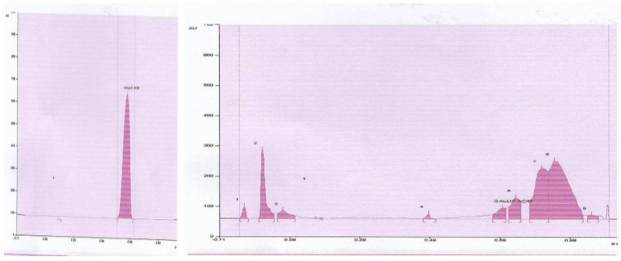


Fig a

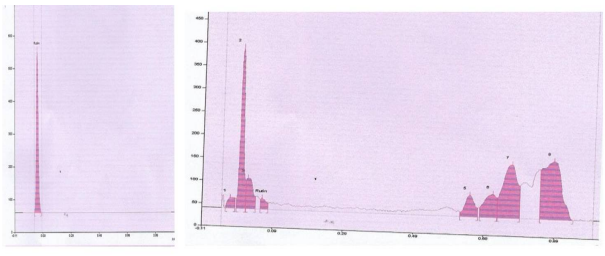
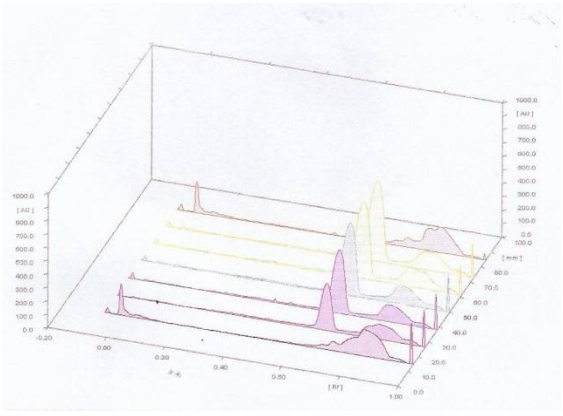
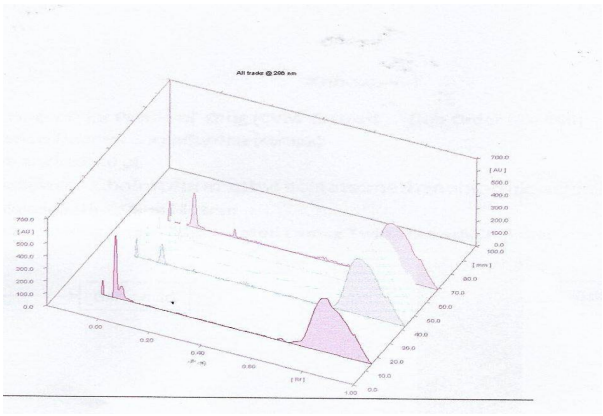


Fig b

Fig 5 HPTLC densitogram for metanolc extract with their respective standards



A For phenols



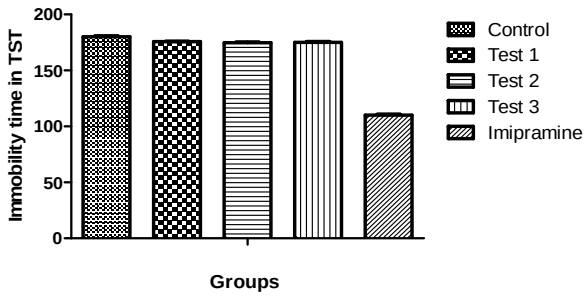
B For flavonoids

Fig 6 3D diagram of HPTLC densitograms

Antidepressant Activity

Table 9 Effect of methanolic extract of Cleome viscosa on immobility time in tail suspension test

Treatment	Dose (mg/Kg)	Immobility Time(sec)
Vehicle	-	180.00±0.83
C.Viscosa	100	175.67±0.25**
C.Viscosa	200	174.67±0.51**
C.Viscosa	400	175.00±0.76**
Imipramine	10	110.00±0.67

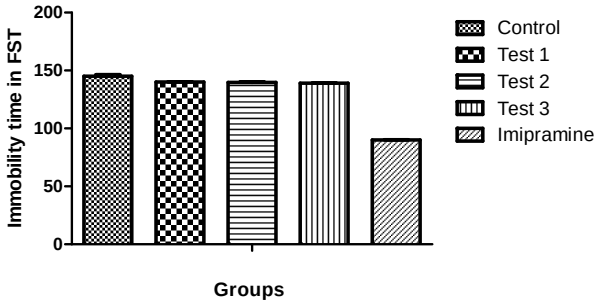


N=6 in each group ** indicates significant difference as compared to vehicle treated group at p<0.001.

Values are given as mean ±SEM for groups of six animals each.

Table 10 Effect of methanolic extract of C.viscosa on immobility time in FST test

Treatment	Dose (mg/Kg)	Immobility Time(sec)
Vehicle	-	145.00±1.48
C.Viscosa	100	140.00±0.33
C.Viscosa	200	139.67±0.54
C.Viscosa	400	139.00±0.17
Imipramine	10	90.00±0.33



N=6 in each group ** indicates significant difference as compared to vehicle treated group at p<0.0001.
Values are given as mean ±SEM for groups of six animals each.

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