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Full Proceeding Paper

PHYTOCHEMICAL AND *IN-VITRO* SCREENING OF AERIAL PARTS OF *CLEOME VISCOSA* LINN. EXTRACTS (CAPPARIDACEAE)

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ABSTRACT

Hexane, Chloroform and Ethanol extracts from aerial parts of *Cleome viscosa Linn.* were evaluated for their antioxidant and antimicrobial potential. Antioxidant activity is determined, employing DPPH and NO method. Extracts were studied for their IC_{50} values. Antimicrobial activity was determined by Kirby-Bauer agar diffusion method. Terpenes, phenol carboxylic acid and flavanoids were detected in TLC. HPTLC fingerprinting was also performed using Toluene: Ethyl acetate (7:3). Present work will contribute to the chemical identification of this plant extract as a potential antioxidant and antimicrobial agent.

Keywords: IC_{50} value, DPPH, NO, Kirby-Bauer agar diffusion, HPTLC

INTRODUCTION

Herbal medicines are becoming popular in modern world as people resort to natural therapies. Natural products isolated from higher plants and microorganisms have been providing novel clinically active drugs. *Cleome viscosa* is a weed distributed throughout the tropics of the world and plains of India and is known as wild mustard and dog mustard. A wide variety of clinical constituents have been isolated from various parts of *Cleome viscosa.* The seeds of *Cleome viscosa* are reported to have nutritive value while the juice of leaves is applied to the skin as counter irritant[1].

In the present study the main focus is towards the identification of phyto constituents from *Cleome viscosa* through systematic phytochemical studies. Hexane, chloroform and methanol fractions were subjected to in-vitro antioxidant studies and antibacterial screening[2].

MATERIALS AND METHODS

Materials

Cleome viscosa was collected from Orthanadu near Thanjavur. The aerial portions were identified and authenticated using Flora of Presidency of Madras and confirmed with the specimens deposited at Raphinet Herbarium St. Joseph's college, Trichy.

Methodology

The Physico- chemical studies were done to determine P^H, extractive values, total ash, acid insoluble ash, sulphated ash, loss on drying[3], Major constituents and TLC profile were determined for the test drug as per standard procedures[4].

Extraction of plant material

A weighed quantity of powdered aerial portions of *Cleome viscosa* were subjected to successive solvent extraction using Soxhlet apparatus using Hexane, Chloroform and Ethanol successively. The extracts were separately concentrated in rotary vacuum evaporator not exceeding 45^0C. The yields of extracts were determined. The dried extracts were subjected to loss on drying, residue on ignition, HPTLC fingerprinting profile, antioxidant and antimicrobial activity.

HPTLC analysis

HPTLC finger printing profile was determined for all the three extracts.

Sample preparation 1.0 g of the extract was dissolved in respective solvents and is taken for TLC analysis.

Instrumentation and Chromatographic conditions

Hexane, Chloroform, Ethanol extracts of *Cleome viscosa* were spotted on a 10x10cm Silica gel precoated 60 F254 TLC plate (E.Merck) of thickness (0.2mm),was used were sample was applied as a wide band (7mm) 8mm from the bottom using automatic TLC applicator Linomat V. The plates were eluted with Ethyl acetate: Methanol: Toluene (7:2:1 v/v) as the eluent. The plates were saturated with the mobile phase vapour for 20 min and developed to a distance of 8. The plated were dried in hot air oven and scanned at 254 nm and 366 nm using CAMAG Scanner 3 with WINCAT software. The plates were photographed at 254 nm, 366 nm and visible light using CAMAG Reprostar 3 (Fig 3 & 4).

Antioxidant activity studies

DPPH Radical scavenging activity[5]

Stock solution of extract/ascorbic acid (1mg/mL) was prepared in ethanol and then serial dilutions were carried out.

Radical scavenging activity of *Cleome viscosa* extract was measured according to the method of Blois (1958). Briefly, 1mL each of different concentrations (100-500µg/mL in ethanol) of extracts and standard (ascorbic acid) were added to 2.5 mL of a 0.3 mM DPPH-ethanol solution. After shaking the mixture it was allowed to stand at ambient temperature in the dark for 30minutes.The colour developed in the mixture was measured at 517nm.

Nitric oxide method[5]

The extract was tested for its Nitric oxide scavenging activity using Griess reagent. Reaction mixture containing 3 mL of Sodium nitroprusside(3mM) in phosphate buffer saline and test extract of different concentration were incubated at 25^0C for 150 minutes. Control is also prepared using 3 mL of Sodium nitroprusside in Ethanol which is used as solvent to dissolve the extracts and allowed for incubation. After incubation, absorbance was measured at 546 nm using colorimeter with 0.5 mL of Griess reagent.

Anti microbial activity[6]

10 sterile test tubes were taken, placed in 2 test tube racks and labelled as antibiotic sensitive and antibiotic resistance. 2 mL of BHI broth were added in both sets of tubes. Both sets of tubes were mixed with 2 mL of different concentrations of antibiotic solution. Using sterile pipette, 2 mL of sample from 1^{st} tube was transferred to the 2^{nd} tube and serial dilutions were made up to 9^{th} tube. 10^{th} tube will act as positive control and similar tests were followed for another series of tubes which is incubated at 37^0C for 18 hours.

RESULTS AND DISCUSSION

Table 1: Tests for Identity and Purity

1	PH	6.32
2	Loss on drying at 105ºC	4.50
3	Total ash	4.34
4	Acid insoluble ash	1.28
5	Sulphated ash	0.84

Table 2: Preliminary Phytochemical screening of Powder[7]

Phytochemicals	Ethanol	Chloroform	Hexane
Carbohydrates	+	-	-
Proteins	+	-	-
Fixed oil	-	-	-
Steroids	-	-	+
Alkaloid	+	+	-
Glycoside	-	-	-
Flavonoids	+	+	-
Tannins	+	+	-
Triterpenoids	-	-	+
Saponin	+	+	-
Volatile oil	-	-	-

Table 4: Yield of various extracts

S. No	Extracts	Percentage Yield (%)
1.	Hexane	3.89
2	Chloroform	9.57
3	Ethanol	11.41

Table 3: TLC profiles for powder

Possible Phytochemical	Hexane	Chloroform	Ethanol	Solvent system used
	No of spots & R_f Values			
Terpenes Sesiqui	3 spots	3 spots	1 spot	100% Chloroform
Phenol carboxylic acid	2 spots	5 spots	3 spots	C_6H_6:CH_3OH:CH_3COOH
Flavonoids	1 spot	5 spots	4 spots	Toluene :EA (95:5)
Polyphenols	1 spot	5 spots	1 spots	C_6H_6:acetone (60:40)

Table 5: Preliminary characteristics of Extracts

S. No	Extracts	Loss on	Residue on
1.	Hexane	15.48	1.98
2	Chloroform	11.26	4.34
3	Ethanol	18.42	2.16

HPTLC Profiles

Stationary Phase: TLC precoated plate with Silica gel 60 F_{254}

Mobile Phase used: Toluene: ethyl acetate (95:5)

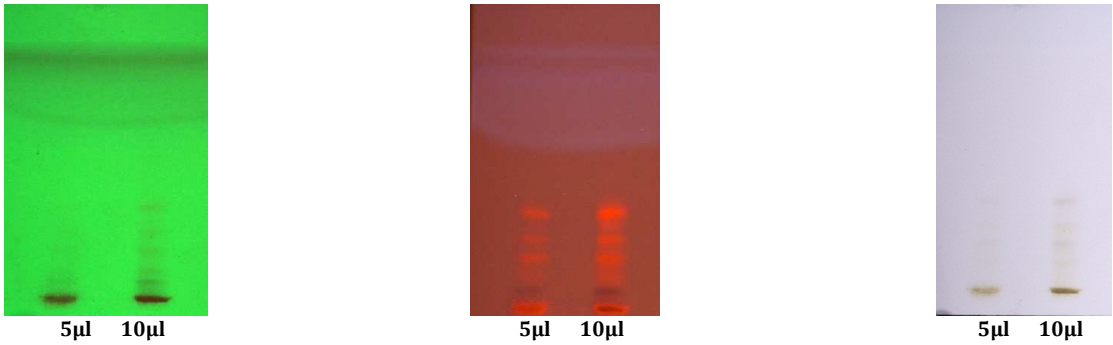

Fig. 1: HPTLC profile of Hexane Extract

	R_f values		
Hexane extract	**254 nm**	**366 nm**	**Visible Region (400-800)**
	0.08,0.18,0.26,0.43 (all blackish brown)	0.08,0.18,0.26,0.43 (all red)	0.08,0.18,0.26,0.43 (all Light brown)

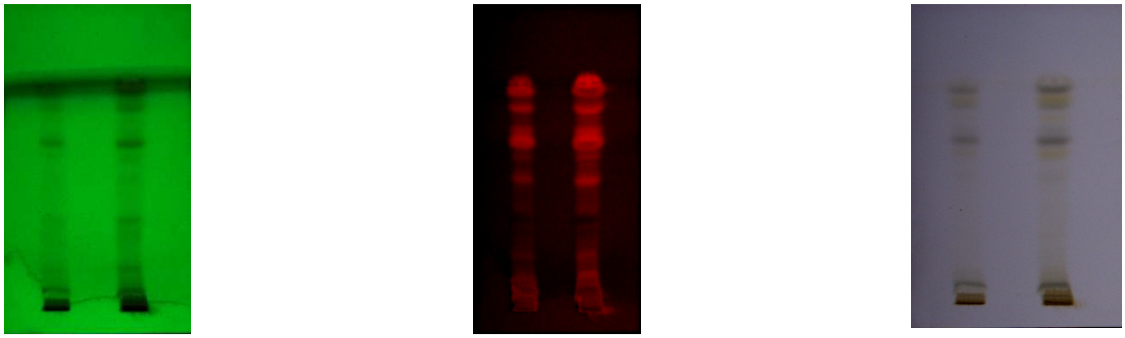

Fig. 2: HPTLC finger print of chloroform extract

Chloroform extract	R_f values		
	254 nm	366 nm	Visible Region (400-800)
	0.13, 0.21, 0.46, 0.53,	0.13, 0.21, 0.46,	0.13(Yellow), 0.21(Dark blue), 0.46(Dark blue),

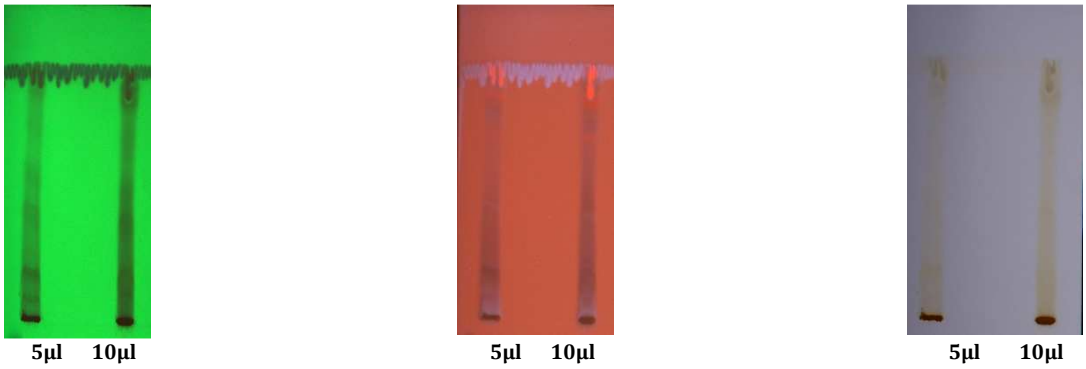

Fig. 2: HPTLC finger print of ethanol extract

Ethanol extract	R_f values		
	254nm	366nm	Visible Region(400-800)
	0.14,0.22,.0.42,0.62,0.91	0.14,0.22,.0.42,0.62,0.91	0.14,0.22,.0.42,0.62,0.91(all Yellow)

Table 6: Anti-oxidant activity by DPPH method

S. No	Concentration (µg/mL)	Standard Ascorbic	*C.viscosa extract*		
			Hexane	Chloroform	Ethanol
1	100	51.85	17.65	7.60	25.92
2	200	59.25	29.41	26.92	37.03
3	300	62.96	41.18	42.30	55.55
4	400	77.77	58.82	57.69	62.96
5	500	92.59	76.47	61.53	70.37

Table 7: Anti-oxidant activity by Nitric oxide method

S. No	Concentration (µg/mL)	Standard Ascorbic	*C.viscosa extract*		
			Hexane	Chloroform	Ethanol
1	100	15.38	37.03	22.22	25.92
2	200	38.46	51.85	33.33	37.03
3	300	61.53	62.96	51.85	55.55
4	400	62.23	77.77	59.25	62.96
5	500	76.92	92.59	77.77	70.37

Table 8: Anti microbial activity by Kirby-Bauer agar diffusion method

Test Organism	Zone of Inhibition in mm		
	Ethanol	Chloroform	Hexane
Klebsiella	12	10	-
Shigella	14	11	-
Streptoccous mutans	-	-	-
Proteus	11	9	-
Pseudomonas	16	10	-
Staphylococcus	12	8	-
Listeria	-	-	-
Salmonella	15	12	-

DISCUSSION

The powders were subjected to solvent extraction using soxhlet apparatus using solvents such as hexane, chloroform and ethanol. Each extract had been subjected to preliminary phytochemical screening and analytical studies. HPTLC profile reveal the presence of blue spots in chloroform and ethanol extracts suggesting the presence of flavanoids in the test drug.

The presence of yellow spot in chloroform and ethanol extract depict the presence of flavanoids. Presence of pinkish red spots in hexane and chloroform extracts indicates the various types of flavanoids. The anti-oxidant activity found in hexane extract of *Cleome viscosa* revealed that more potent antioxidant compounds are present in this extract. The activity is due to the presence of non polar compounds present in hexane extract. The presence of polar compounds in ethanol extract of *cleome viscosa* is responsible for the better antimicrobial activity compared to other extracts.

CONCLUSION

The present study proved the antimicrobial potential of ethanolic extract of *Cleome viscosa* probably due to the presence of more polar compounds. While hexane extracts containing non polar compounds showed better antioxidant activity. Terpenes and flavones of *Cleome viscosa* identified by chemical tests, TLC and confirmed by HPTLC. This may be responsible for the antibacterial and antioxidant action respectively.

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