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## DETERMINATION OF PRELIMINARY PHYTOCONSTITUENTS, TOTAL PHENOLIC AND FLAVONOIDS CONTENTS IN THE ROOTS, LEAVES AND STEMS OF *CLEOME VISCOSA* LINN.

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### ABSTRACT

*Cleome viscosa* Linn, belongs to family Capparidaceae, it is widely distributed sultry herb with yellow flowers and long slender pods containing seeds. It is widely distributed throughout the tropics of world and has been used as medicinal herb due to its various biological activities like stomachic, laxative, diuretic, anthelmintic, skin diseases: itching, leprosy. In the present study, three different parts: roots, stems, and leaves of *C. viscosa* were extracted with Pet. ether, Chloroform, methanol, and water and all these extracts were screened for the presence of various plant metabolites (primary and secondary) including proteins, alkaloids, steroids, flavonoids, carbohydrates, and tannins. Further, these primary metabolites were quantitatively estimated, the results showed that maximum amount of soluble sugars and starch was found in the root and the alkaloid are absent in all three plant parts. Total flavonoid and phenolic contents were also determined in all three parts of *C. viscosa*, the results showed that.

**Key Words:** Flavonoid, Phytoconstituents, Methanol extract.

### INTRODUCTION

*Cleome viscosa* Linn, an annual herb, belongs to family Capparidaceae, it is also known as Hulhul and Jangliharra. It is 30-90 cm in height, stems have simple hairs; leaves are 3-5 in number and foliolate. The flowers are yellow in color, axillary and seeds are brown-black with subglobose. It is widely distributed throughout the tropics of world and has been used as medicinal herb due to its various biological activities like stomachic, laxative, diuretic, anthelmintic, skin diseases: itching, 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 (Aline M *et al.*, 2005). Phytochemical investigation showed that *C. viscosa*

contains diverse class of chemical constituents like fatty acids (Aparadh VT, Karadge BA, 2010), volatile oils (Bawankule *et al.*, 2007) diterpenoid (Chauhan JS *et al.*, 1979) triterpenoids (Devi BP *et al.*, 2002), coumarinolignoids glycoflavanones (Gupta *et al.*, 2009) naringenin glycoside (Gupta *et al.*, 2011), etc. Ethanolic extract of *C. viscosa* has showed several biological activity as Hepatoprotective activity (Jayaraman J, 1981), Antimicrobial activity (Jente R *et al.*, 1990), Antifibrotic effect (Kirtikar KR, Basu BD, 1956), anthelmintic activity (Kokoshi CJ *et al.*, 1949), while methanol extract of *C. viscosa* has showed good antioxidant activity (Kumar SV *et al.*, 2009), antipyretic activity (Lowry OH *et al.*, 1952), analgesic activity (Merekar *et al.*, 2011) anti-diarrheal activity (Olatunji G *et al.*, 2005). Coumarinolignoids from seeds of *C. viscosa* has showed the immunomodulatory activity (Olatunji G *et al.*, 2005; Parimaladevi B, 2003). In present work we have carried out the detail analysis of primary and secondary metabolites in the leaves, stem and roots of

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*C. viscosa*, for that total flavonoid and total phenolic contents were determined by the well known method (Dowd method and Folin–Ciocalteu method, respectively) (Srivastava *et al.*, 1979).

## MATERIALS AND METHODS

### Plant material

The roots, leaves and stems of *C. viscosa* were collected separately from the local campus of Rajasthan University in the month of August–September and shade dried. The plants material was identified by the Botanist, Department of Botany and the voucher specimens (RUBL211339) were deposited in the herbarium, Department of Botany, Rajasthan university, India.

### Extraction

Extracts of dried and pulverized plant material (roots, stems, and leaves) were prepared by two methods (maceration and successive Soxhlet extraction). Maceration: pulverized plant materials (0.5 kg) were subjected to maceration with methanol for 24 × 3 h, the extract solutions were filtered and dried under vacuum. Successive Soxhlet extraction: pulverized plant materials (0.8 kg) were subjected to successive extraction for 24 h, with increasing polarity solvents (Pet. ether, chloroform, methanol, and water), the extract solutions were filtered and dried under vacuum and for each extract the extractive value was calculated.

### Solvents/Chemicals/Instrumentation

LR grade Methanol was purchased from Merck specialties private limited, Worli, Mumbai, HPLC grade methanol was purchased from Merk KGaA, Germany, aluminium trichloride was purchased from Spectrochem, Mumbai, quercetin was obtained from Sigma–Aldrich, Switzerland, sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) was purchased from LOBA Chemie, Mumbai, UV-Vis Absorption was measured on UV spectrophotometer (Perkin Elmer, USA),

### Preliminary Screening of primary metabolites

All the above extracts were then subjected to preliminary phytochemical screening for the detection of various plant primary metabolites (soluble sugars, starch, proteins and lipids). The roots, stem, and leaves of *C. viscosa* were evaluated quantitatively to determine the total levels of soluble sugars, starch, proteins and lipids following the established methods for the sugars, starch (Dubois *et al.*, 1951), lipid (Jayaraman, 1981), and protein (Lowry, 1951). All experiments were repeated three times for precision and values were expressed in mean  $\pm$  standard deviation in terms of air dried material.

### Preliminary Screening of secondary metabolites

The secondary metabolites (alkaloids, terpenoids, steroids, flavonoids) was estimated in the all three part of *C. viscosa* using well define chemical test and a well-established protocol by Kokoshi *et al.*, 1949. For that, the powder was treated with acids: 1N hydrochloric acid, sulfuric acid, nitric acid, and acetic acid; and alkaline solutions: 1N sodium hydroxide and ammonia. Further, these secondary metabolites were confirmed by the well define chemical test or by TLC profiling and derivatization of TLC plate with different derivatization reagents like vanillin sulfuric acid reagent (for steroid and terpenoids), NP-PEG reagents (for flavonoids), dragendorff reagent (for alkaloids), and alcoholic KOH (for coumarins) etc.

### Determination of total flavonoid content

The total flavonoids content was determined using the Dowd method as established by Arvouet-Grand *et al.*, 1994. Briefly, 5 ml of 2% aluminium trichloride in methanol was mixed with the 5 mL of extract solution (1 mg/mL). UV-Vis Absorption readings was measured at 415 nm after 10 min against a blank sample consisting of 5 mL extract in methanol without aluminium trichloride ( $\text{AlCl}_3$ ). The total flavonoid content was determined using a standard curve (linear regression curve) of quercetin (1–50mg/L) as the standard. The data are expressed as mg of quercetin equivalents per 100 g plant material.

### Determination of total phenolic content

The Folin–Ciocalteu method (Singleton *et al.* 1999) was applied to establish total phenolic content in the three parts of *C. viscosa* L. for that each sample (1 g methanol extract of each part) was diluted to 10 ml with distilled water and filtered through Whatman filter paper. This filtered solution (0.5 ml) was then mixed with 2.5 ml of 0.2 N Folin–Ciocalteu reagent (Sigma–Aldrich, Switzerland) for 5 min and then added 2 ml of 75 g/L  $\text{Na}_2\text{CO}_3$ . After incubation for 2 h at room temperature, the absorbance was measured at 760 nm against a methanol blank (excluding the  $\text{Na}_2\text{CO}_3$ ). The total phenolic contents were determined with respect to gallic acid equivalent, for that a linear curve was plotted using different concentration of gallic acid in methanol (0–200 mg/L). The data are expressed in %w/w of gallic acid equivalents.

## RESULTS AND DISCUSSION

### Extraction

The pulverized plant materials were subjected to extraction by two methods: maceration and Soxhlet extraction, the results showed that Soxhlet extraction is the highly efficient as compared to the maceration. Further, the maximum % yield was observed for methanol extracts (by

Soxhlet extraction), followed by the aqueous and chloroform extract (Table 1).

#### Preliminary Screening of primary and secondary metabolites

The results showed that pet. ether extract of all three parts contains only plant acids, volatile oils and some terpenoids; chloroform extract contains some part of terpenoids, flavonoids and steroids. The methanol extract contains mainly flavonoids and their glycosides, some part of tannins are also there; and the aqueous extract contains tannins, some highly polar glycoside, sugars, carbohydrates and proteins (Table 3).

#### Determination of total flavonoids and phenolic content

The total flavonoid and total phenolic contents were determined by using the Dowd method and Folin-Ciocalteu method, respectively. For that the linear curves were determined using different concentration of quercetin and gallic acid. The slope of linear curve was calculated by the following equation  $y = 0.06x + 0.057$  ( $r^2 = 0.998$ ) for total flavonoid content determination  $y = 0.096x + 0.034$  ( $r^2 = 0.999$ ) for total phenolic content determination

The result showed that leaves contain maximum amount of flavonoids and phenolic contents (0.019 and 0.057% w/w, respectively) as compared to roots and stems.

**Table 1. Extractive values**

Sample code	Extractive value (%w/w) <sup>a</sup>	Extractive values (%w/w) <sup>b</sup>			
	Methanol extract	Hexane extract	Chloroform extract	Methanol extract	Aqueous extract
Roots	5.5	0.7	1.8	2.4	1.8
Stems	6.1	0.5	1.9	2.6	2.2
Leaves	9.7	1.6	3.3	4.3	2.8

Extract was prepared by: <sup>a</sup>maceration method, <sup>b</sup>successive Soxhlet extraction method

**Table 2. Fluorescence Analysis of Dry Powder of *C. viscosa* L.**

S. No	Chemical Test	Color
1	Powder + Acetic Acid	Radish blue
2	Powder + Hydrochloric Acid	Yellowish green
3	Powder + 50% Sulfuric Acid	Greenish
4	Powder + 5% Iodine Solution	Yellowish green
5	Powder + 50% Ferric chloride	Radish-orange
6	Powder + Acetic Acid + Sulfuric Acid	Radish blue
7	Powder + 10% NaOH + Cauper sulphate	Greenish
8	Powder + conc. Nitric acid + excess ammonia	Yellowish green
9	Powder + 40% NaOH + few drops of Lead Acetate	Orange
10	Powder + Acetic Acid + Ferric chloride + Sulfuric Acid	Greenish blue

**Table 3. Preliminary phytochemical screening in different extracts of *C. viscosa* L., Obtained by Successive Soxhlet extraction**

Metabolites	Pet. ether extract			Chloroform Extract			Methanol extract			Aqueous extract		
	R	S	L	R	S	L	R	S	L	R	S	L
Carbohydrates	–	–	–	–	–	–	–	–	–	+++	++	++
Protein	–	–	–	–	–	+	+	+	+	++	+	++
Tannins	–	–	–	–	–	–	–	–	–	+++	++	+++
Flavonoids	–	–	–	–	+	++	+	++	+++	+	+	++
Alkaloids	–	–	–	–	–	–	–	–	–	–	–	–
Terpenoids	+	+	++	+	+	++	–	–	–	–	–	–
Steroids	–	+	+	+	+	+	–	–	–	–	–	–

+, ++, +++ relative measure; – Not detected

**Table 4. Concentration of primary metabolites in roots, stems, and leaves of *C. viscosa* L.**

S. No.	Primary metabolites	Contents of Primary metabolites (mg/g, in dried extract)* of		
		Roots	Stems	Leaves
1	Lipids	10.5 ± 1.6	15.2 ± 1.9	37.1 ± 2.3
2	Proteins	29.4 ± 2.4	19.2 ± 3.2	23.8 ± 2.1

3	Starch	46.4 ± 3.8	29.8 ± 4.1	26.1 ± 2.4
4	Sugars	51.4 ± 4.3	34.3 ± 2.8	31.6 ± 4.2

\* Primary metabolite contents were determined in the methanol extract, which was prepared by maceration method

**Table 5. Total flavonoids and phenolic contents in roots, stems, and leaves of *C. viscosa* L.**

Sample code	Total flavonoids content (% w/w)*		Total phenolic content (% w/w)*	
	In Extract	In Plants	In Extract	In Plants
Roots	0.194	0.012	0.136	0.007
Stems	0.206	0.013	0.397	0.024
Leaves	0.223	0.019	0.589	0.057

\* Total flavonoid and phenolic contents were determined in the methanol extract, which was prepared by maceration method

## CONCLUSION

Plants are rich and useful source of primary and secondary metabolites like proteins, lipids, carbohydrates, alkaloids, flavonoids, terpenoids, tannins etc. These metabolites are useful for the plants as well as for the human being for the treatment of various illnesses. Determinations of these metabolites are helpful to know the medicinal as well as food value of respective plants. In conclusions, in the present work, we have determined the content level of primary and secondary metabolite in the

roots, stems, and leaves of *C. viscosa* by using various chemical test as well as by applying the well-developed analytical methods. The described method is reliable and applicable for the quantitation of primary and secondary metabolite in the respective plant.

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