ORIGINAL ARTICLE

Qualitative and quantitative evaluation of secondary metabolites in leaves, roots, and stem of *Cleome viscosa* L.

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Abstract

Objectives: The aim of present investigation is to evaluate secondary metabolites in leaves, roots, and stem of *Cleome viscosa* L. qualitatively and quantitatively. **Materials and Methods:** The leaf, root, and stem powder samples were subjected to methanolic, ethanolic, and aqueous extraction using Soxhlet apparatus. The extracts were evaluated for major phytochemicals qualitatively and quantitatively using standard methods. **Results:** The phytochemical screening revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, and tannins. Anthocyanides and terpenoids were not detected in any of the extracts. Quantitative evaluation of leaves, roots, and stem extracts showed the alkaloids, cardiac glycosides, flavonoids, phenols, and saponins in significant amounts. **Conclusion:** The extracts of leaves, roots, and stem showed the presence of important secondary metabolites which have tremendous medicinal values. Thus, the use of this plant in ayurvedic system of medicines can be correlated with the modern-day science which is based on phytoconstituents.

Key words: *Cleome viscosa,* phytochemical screening, quantification, secondary metabolites

INTRODUCTION

The importance of plants is well known to us. The plant kingdom is a treasure house of potential drugs, and in the recent years, there has been an increasing awareness about the utilization of medicinal plants. Drugs from the plants are easily available, less expensive, safe, efficient, and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining for therapeutically effective new drugs.[1] The curative properties of medicinal plants are mainly due to the presence of various complex chemical compounds in different compositions which occur as secondary metabolites.[2] These secondary metabolites produce specific physiological action on human body.[3] Herbal drugs or their extracts are prescribed widely by the experts, even when their biological active compounds have not been investigated.[4] Secondary metabolites (alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds, cardiac glycosides, etc.) form the backbone of modern medicine[5] and play an important role in plant defense against herbivory[6-8] and other interspecies defenses. Many medicinal plants contain a large amount of antioxidant phytochemicals other than Vitamins C and E and carotenoids.[9] Humans use these secondary metabolites as medicines, flavorings, and recreational drugs. The phytochemical research based on ethnopharmacology is generally considered an effective approach in the discovery of new anti-infective agents from medicinal and higher plants.[10] The results indicate the need for further research into traditional health systems in terms of phytochemical and pharmacological investigation.[11]

Cleome viscosa L., commonly known as wild or dog mustard, belongs to the caper family (Capparidaceae). It is an annual, sticky herb found as a common weed all over the plains of India

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and throughout the tropics of the world. 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.[12] A wide variety of clinical constituents have been isolated from various parts of *C. viscosa* plant. The seeds of *C. viscosa* are reported to have nutritive value while juice of leaves is applied to the skin as counterirritant.[13] In the present study, the main focus is towards the phytochemical evaluation of leaves, roots, and stem of *C. viscosa* through systematic studies.

MATERIALS AND METHODS

Collection and Processing of Plant Samples

The plant samples of *C. viscosa* were collected from Bargi block of Jabalpur district in Madhya Pradesh. The plant parts (leaves, roots, and stem) were separated and washed in tap water to remove foreign and dust particles. The cleaned materials were cut into pieces and dried in shade. The dried materials were powdered using grinder and stored in polythene bags for further chemical analysis.

Extraction of Plant Materials

About 100 mg of dried and powdered plant materials was kept overnight in 25 ml of different solvents, namely aqueous, ethanol, and methanol. The extracts were filtered using Whatman filter paper no.1 and used for phytochemical analysis.

Qualitative Evaluation of Plant Extracts for Phytochemicals

The extracts were subjected to preliminary qualitative phytochemical screening for the presence of various phytoconstituents following the standard methods.[2,14]

Test for alkaloids

2 ml of filtrate was taken and followed by addition of 1% HCl. Creamish color indicated the presence of alkaloids.

Test for anthocyanides

1 ml of filtrate with 5 ml of 10–12% HCl showed the presence of pale pink colors, indicating the presence of anthocyanides.

Test for cardiac glycosides

2 ml of filtrate was taken followed by addition of 1 ml glacial acetic acid, $FeCl_3$, and concentrated H_2SO_4. A brown ring indicated the presence of cardiac glycosides.

Test for flavonoids

5 ml of diluted NH_3 solution was added to a portion of the filtrate of plant extract followed by addition of concentrated HCl. Yellow color indicated the presence of flavonoids.

Test for phenols

2 ml of filtrate was taken in test tube and 1 ml of 1% $FeCl_3$ added to it. Brown haziness indicated the presence of phenols.

Test for saponins

1 ml of filtrate was mixed with 5 ml of distilled H_2O and shaken vigorously for a stable froth. The persistence of frothing indicated the presence of saponins.

Test for steroids

2 ml of filtrate was taken followed by addition of 2 ml of acetic anhydride and concentrated H_2SO_4. Blue-green ring indicated the presence of steroids.

Test for tannins

2 ml of filtrate was taken followed by addition of 2 ml of $FeCl_3$. Brownish color indicated the presence of tannins.

Test for terpenoids

5 ml of each extract was mixed in 2 ml of $CHCl_3$ and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration at the interface was formed to show the positive result for terpenoids.

Quantitative Evaluation of Plant Extracts for Phytochemicals

Quantification of total alkaloids

Total alkaloid content was estimated by following the method reported by Singh *et al*. (2004).[15] For estimation of alkaloids, 100 mg powdered plant sample in 10 ml of 80% ethanol was kept overnight. This was then filtered through Whatman filter paper no. 1 and then centrifuged at 5000 rpm for 10 min. The supernatant obtained was used for the estimation of total alkaloids. The reaction mixture contained 1 ml of plant extract + 1 ml of 0.025M FeCl3 in 0.5 M HCl and 1 ml of 0.05 M of 1,10-Phenanthroline in ethanol. The mixture was incubated for 30 min in hot water bath (70 ± 2°C). The absorbance of red color complex was measured at 510 nm against blank. Alkaloid content was estimated using the standard curve of colchicine [Figure 1].

Quantification of total cardiac glycosides

Cardiac glycoside content was assessed by the method of Pradhan *et al*., (2013).[16] 1 g of plant sample was suspended in 25 ml of distilled H_2O. To this solution, 2 ml of concentrated H_2SO_4 was added. It was then refluxed for 6–8 h, cooled and extracted with chloroform (2 × 25 ml). The chloroform layer was then washed with distilled H_2O until it is acid-free. The chloroform extract was transferred to a pre-weighed beaker and dried on a water bath and then in oven up to a constant weight. The amount of dried extract represented the amount of cardiac glycosides in the plant sample.

$$\% \text{ of Cardiac glycoside} = \frac{B - A \times 100}{\text{Weight of sample}}$$

Where
B = weight of beaker with extract
A = weight of empty beaker

Quantification of total phenols

Phenolic content was quantified by the standard method.[17] 0.5 g powdered sample was taken in mortar and pestle and grinded with 10 times volume of 80% methanol. The homogenate was centrifuged at 1000 rpm for 20 min. The supernatant was evaporated to dryness. The residue was dissolved in 20 ml of distilled water. A volume of 0.2 ml was taken into a test tube and was made up to 3 ml with distilled water. 0.5 ml of Folin–Ciocalteu reagent was then added. After 3 min, 2 ml of 20% Na_2CO_3 solution was added. The reaction mixture was then placed in boiling water bath for 1 min. Cooled and the absorbance was taken at 650 nm against the blank. The phenolic content was calculated using a standard curve of gallic acid [Figure 2].

$$\text{Percentage of Phenol} = \frac{\text{Amount of standard}}{\text{O.D of standard}} \times \frac{\text{O.D of sample}}{\text{Weight of sample}} \times \frac{\text{Total volume makeup}}{\text{Volume taken}} \times 100$$

Quantification of total flavonoids (Chang et al., 2002)

Total flavonoids were determined by $AlCl_3$ calorimetric method.[18] 0.5 g sample in 20 ml of 95% ethanol was kept for 24 h. It was then filtered and the volume was made up to 25 ml with 80% ethanol. 0.5 ml of filtrate was then mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of potassium acetate, and 2.8 ml of distilled water. The tubes were then incubated at room temperature for 30 min. Observations were recorded at 415 nm. The flavonoid content of the samples was calculated from the standard graph of quercetin [Figure 3]. The percentage of flavonoids was calculated using the same formula as in case of phenols.

Quantification of total saponins

Saponin content was estimated using the method reported by Obdoni and Ochuko (2001).[19] 1 g sample was taken into a conical flask and 25 ml of 20% methanol was added. The mixture was heated over a water bath for 4 h at 55°C. The mixture was filtered and the residue was re-extracted with another 25 ml of 20% methanol. The combined extracts were reduced to 15 ml over a water bath at 90°C. The concentrate was transferred to a separating funnel, and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was retained while the ether layer was discarded. The aqueous layer was further separated by 60 ml (2 ml × 30 ml) of butanol. The combined butanol layer was washed with 10 ml of 5% NaCl (twice). The extract was transferred to a pre-weighted beaker and dried in an oven to a constant weight. The weight of the extract was the saponin content.

$$\% \text{ of saponins} = \frac{B - A}{\text{Weight of sample}} \times 100$$

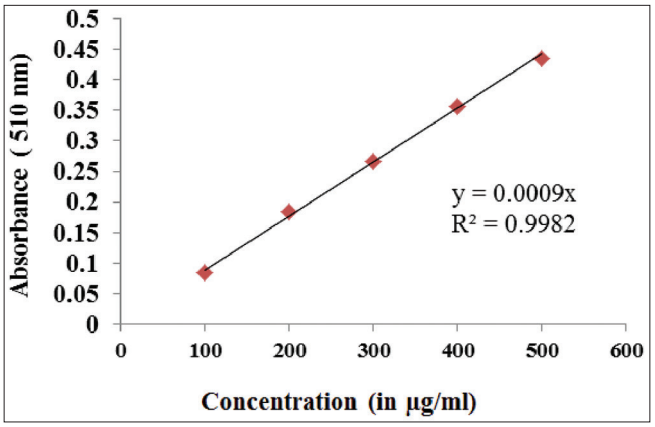

Figure 1: Standard calibration curve of colchicine used for the quantitation of alkaloids

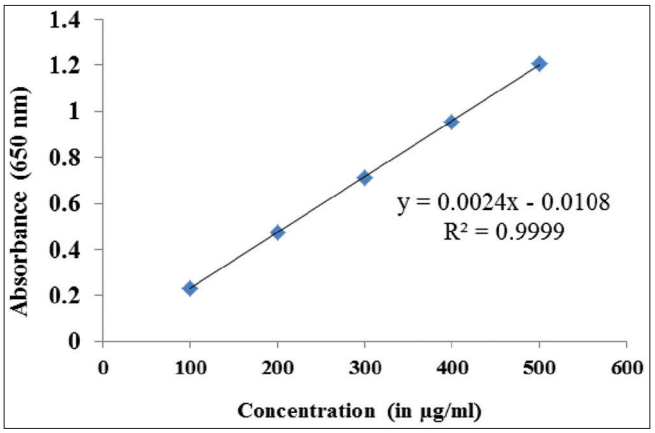

Figure 2: Standard calibration curve of gallic acid used for the quantitation of phenols

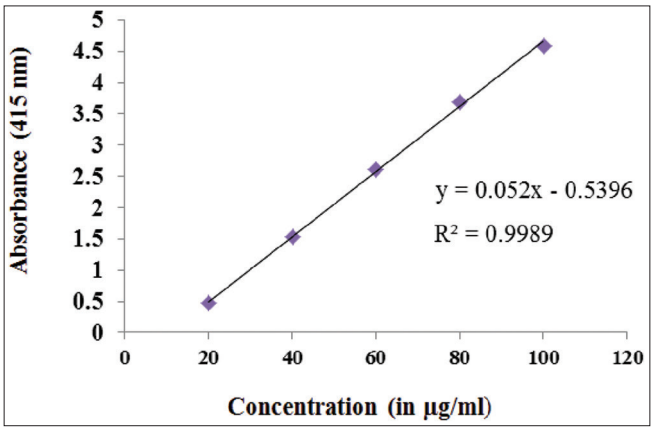

Figure 3: Standard calibration curve of quercetin used for the quantitation of flavonoids

Where
B = weight of beaker with extract
A = weight of empty beaker.

Statistical Analysis

Each experiment for the quantification of secondary metabolites was carried out in triplicate, and the results are expressed as mean ± standard deviation (n = 3).

RESULTS AND DISCUSSION

The qualitative phytochemical profile of leaves, roots, and stem of *C. viscosa* is given in Table 1. The results showed the presence of alkaloids in all the extracts of leaves, roots, and stem expect the methanolic extract of leaves. Tannins and phenols were present in all extracts, except in methanolic extracts of leaves and roots. Cardiac glycosides were detected in ethanolic and methanolic extracts of all three plant parts but not detected in all aqueous extracts. Steroids and anthocyanides were not detected in any of the extracts. Saponins were present in all the extracts. Flavonoids were not observed in aqueous extract of roots and methanolic extract of leaves. Terpenoids were detected in ethanolic extracts of all plant parts as well as aqueous and methanolic extracts of leaves. These were not detected in aqueous and methanolic extracts of roots and stem.

The calibration curves for the quantification of alkaloids, phenols, and flavonoids are given in Figures 1-3. The secondary metabolite contents measured in the leaves, roots, and stem of *C. viscosa* are presented in Table 2 and Figure 4.

It can be observed that among all plant parts, the leaves contained the maximum contents of alkaloids (1.279%), cardiac glycosides (0.051%), phenols (0.372%), flavonoids (0.17%), and saponins (0.073%). Besides, roots were found to contain alkaloid content (0.0535%) next to leaves, while both roots and stem contained the same amount of cardiac glycosides (0.042%). Stem extract was observed with higher content of phenols (0.312%), flavonoids (0.068%), and saponins (0.056%) than root extracts. Our results showed higher content of phenols and flavonoids as communicated earlier by Bainiwal *et al*. (2013), who reported the total phenolic content as 0.057% (leaves), 0.007% (roots), and 0.024% (stem) and flavonoid content as 0.019% (leaves), 0.012% (roots), and 0.012% (stem) in *C. viscosa*.[20] Gupta *et al*. (2011) reported a higher phenolic content in leaves (6.638%) and stem (5.846%) while lower flavonoid content in leaves (0.054%) and stem (0.048%) of *C. viscosa*[21] in comparison to our results. The differences in results of the present and previous studies may be due to variations in environmental factors which have been described to affect the secondary metabolite concentrations in plants of different regions by many researchers from time to time.[22-24]

Preliminary phytochemical screening actually helps in isolating and characterizing the chemical constituents present in the plant extracts, and the knowledge of the chemical constituents of plants is necessary to understand herbal drugs and their preparations and finally in discovering the

Table 1: Qualitative phytochemical screening of leaves, roots, and stem of *C. viscosa*

S. No	Phytochemical constituents	Aqueous extract			Ethanol extract			Methanol extract		
		Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem
1.	Alkaloids	++	+	++	+	++	++	-	++	+
2.	Tannins	+	+	+	+	++	++	-	-	+
3.	Phenols	+	+	+	+	++	+	-	-	+
4.	Cardiac glycosides	-	-	-	+	+	+	+	++	++
5.	Steroids	-	-	-	-	-	-	-	-	-
6	Saponins	+	+	++	+	+	++	+	+	++
7.	Flavonoids	++	-	+	+	++	+	-	+	++
8.	Terpenoids	++	-	-	+	+	+	+	-	-
9.	Anthocyanides	-	-	-	-	-	-	-	-	-

+: detected, ++: detected in higher amount, and -: not detected, *C. viscosa: Cleome viscosa*

Table 2: The contents of secondary metabolites in leaves, roots, and stem of *C. viscosa*

Plant parts	Alkaloids (%)	Cardiac glycosides (%)	Phenols (%)	Flavonoids (%)	Saponins (%)
Leaves	1.279±0.074	0.051±0.004	0.372±0.013	0.170±0.001	0.073±0.001
Roots	0.0535±0.008	0.042±0.020	0.267±0.174	0.067±0.002	0.049±0.002
Stem	0.044±0.007	0.042±0.176	0.312±0.205	0.068±0.001	0.056±0.001

C. viscosa: Cleome viscosa

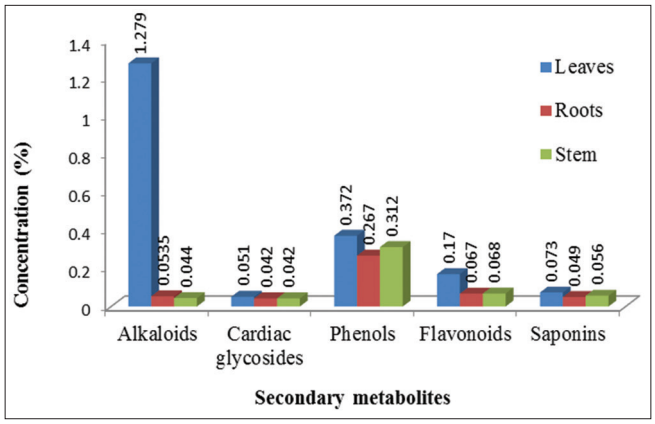

Figure 4: Concentration of secondary metabolites in plant samples

actual value of folkloric remedies.[25] Phytochemicals such as alkaloids, flavonoids, terpenoids, cardiac glycosides, phenols, and saponins present in different extracts exhibit a number of biological activities and protect from most of the chronic diseases.[26,27]

CONCLUSION

The study exhibited the presence of therapeutically important secondary metabolites, namely alkaloids, cardiac glycosides, flavonoids, phenols, and saponins in leaves, roots, and stem of *C. viscosa*. The leaves were found to contain their maximum concentrations, but roots and stem were also found to have a considerable amount. Thus, it can be concluded that the leaves, roots, and stem of *C. viscosa* are a potential source of biologically active chemical constituents. Further studies can also be taken up to investigate the lead compounds of pharmaceutical importance from the plant.

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