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Qualitative and Quantitative Phytochemical Screening of Cleome Viscosa Linn. (Cleomaceae) leaves, Root and stem extract

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Abstract - The prime objective of this investigation is to evaluate phytochemical constituent of root, stem and leaves of Cleome viscose by qualitative and quantitative assay. The phytochemical screening revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins and tannins which have tremendous medicinal values.

Keywords - Cleome viscosa Linn. qualitative & quantitative screening phytochemical.

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INTRODUCTION

All angiospermic plants are confined with treasure house of potential drugs. Drugs from plants are easily available, less expensive, safe, efficient and have no side effects. (Dewick, PM, 1996)). The curative properties of medicinal plants are mainly due to the presence of various complex chemical compounds in varied composition which occur as secondary metabolites. (Trease, GE *et al.,* 1989). The existing secondary metabolites form the backbone of modern medicine (Karthikeyan *et al.,* 2009). The phytochemical research based on ethnopharmacology is considered an effective approach in the finding of new anti-infective agents from higher plants (Edeoga, HO *et al.*, 2005). In the present study, the prime focus is to evaluate the phytochemical constituents present in root, stem and leaves of *Cleome viscosa* Linn. (CLEOMACEAE).

MATERIALS AND METHODS

Materials: Root, Stem, Leaves of *Cleome viscosa*

- Test tube, Beaker, Conical flask.
- Ethanol
- Methanol
- Whatman filter paper no. 1
- Centrifuge
- Chloroform
- Distilled water
- Digital balance
- Incubator
- Water bath
- $FeCl_3$
- Measuring cylinder 10ml capacity

Methods:

- **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.

- **Qualitative evaluation of phytochemical :**

The extracts were subjected to preliminary qualitative phytochemical screening following standard methods (Mali RG, 2010; Harborne, JB, 1998)

1. **Test for alkaloids:** 2 ml filtrate was taken in a clean test tube and added 1% HCL. The mixture solution was changed to cream colour which confirmed the test of alkaloid.
2. **Test for cardiac glycosides:** 2 ml filtrate was taken in a clean test tube and added 1 ml glacial acetic acid, $FeCl_3$ and conc H_2SO_4. A brown ring confirmed the test of cardiac glycosides.
3. **Test for phenols :** 2 ml filtrate was taken in a clean test tube and added 1 ml of 1% $FeCl_3$. Brown haziness appeared which confirmed the test of phenols.
4. **Test for saponins:** 1 ml filtrate was mixed with 5 ml distilled water and shaken vigorously for getting a stable froth. The persistence of frothing confirmed the test of saponin.
5. **Test for steroids:** 2 ml of filtrate was taken in a clean test tube and added 2 ml acetic analydride and conc. H_2SO_4. Blue-green ring confirmed the test of steroids.

6. **Test for terpenoids:** 5 ml of each extract was mixed in 2 ml of $CHCl_3$ and conc. H_2SO_4 (.3 ml) was added carefully to form a layer. A reddish-brown colouration at the interface was formed which showed the +ve result of the presence of terpenoids. The results were presented in table-1.

Table 1: Qualitative screening of phytochemical present in root, stem leaves of *Cleome viscosa.*

Phytochemical Constituents	Aqueous extract			Ethanolic extract			Methanolic extract		
	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	Leaves
Alkaloids	+	++	++	++	++	+	++	+	-
Phenols	+	+	+	++	+	+	-	+	-
Cardiac glycosides	-	-	-	+	+	+	++	++	+
Steroids	-	-	-	-	-	-	-	-	-
Saponins	+	++	+	+	++	+	+	++	+
Terpenoids	++	-	++	+	+	+	-	-	+

\+ = Detected

++ = Detected in higher amount

\- = Not detected

Quantitative evaluation of phytochemical:

1. **Quantification of total alkaloids:** Total alkaloid content was estimated by following the method reported by Singh *et al.,* (2004). For estimation of alkaloids, 100 mg powdered plant sample was placed in 10 ml of 80% ethanol overnight and filtered by whatman filter paper and then centrifuged at 5000 rpm for 10 minutes. The supernatant obtained was used for the estimation of total alkaloids. The reaction mixture contained 1 ml plant extract + 1 ml of 0.025 M $FeCl_3$ in 0.5 M HCl and 1 ml of 0.05 M of 1, 10-Phenanthroline in ethanol. The mixture was warmed in hot water bath. The absorbance of red colour complex was measured at 510 nm against blank. Alkaloid content was estimated using the standard curve of colchicines.
2. **Quantification of total cardiac glycosides:** The plant sample was suspended in 25 ml distilled water. Then 2 ml conc. H_2SO_4 was added to it and was soon refluxed for 6-8 hours, cooled and extracted with chloroform (2x25ml). The chloroform layer was then washed with distilled water unit it become acid-free. The chloroform extract was then transferred to a pre-weighed beaker and dried on a water bath and then in oven upto a constant weight. The amount of dried extract represented the amount of cardiac glycoside in the plant sample (Pradhan *et al.,* 2013).

$$\% \text{ of cardiac glucoside} = \frac{B-A \times 100}{\text{Weight of sample}}$$ where,

B = Weight of beaker with extract

A = Weight of empty beaker

3. **Quantification of total saponins:** 1 gm sample was taken into a conical flask and added 25 ml 20% methanol. The mixture solution was warmed in water bath for 4 hours at 35^{O}C. The mixture solution 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^{O}C. The concentrate was then 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 (2ml x 30 ml) of butanol. The combined butanol layer was washed with 10 ml of 5% NaCl twice. The extract was then transferred to a pre-weighed beaker and dried in a oven to a constant weight. The weight of the extract was the saponin content.

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

4. **Quantification of total phenols:** 0.5 gm powdered sample was taken in a mortar and grinded with pestle and added 5 ml 80% methanol. The homogenate was centrifuged at 1000 rpm for 20 minutes. The supernatant was evaporated to dryness. The residue was dissolved in 20 ml distilled water. A volume of 0.2 ml was taken in a clean test tube and was made 3 ml by adding distilled water. Again 0.5 ml Folin-ciacaten reagent was added. Just after 3 minutes, 2 ml of 20% NaCl was added. The reaction mixture was then placed in water bath for 1 minute. Cooled and the absorbance was taken at 650 nm against the blank. The phenolic content was calculated by applying following formula –

$$\% \text{ phenol} = \frac{\text{Amount of standard}}{\text{O.D.of standard}} \times \frac{\text{O.D.of sample}}{\text{Weight of sample}} \times \frac{\text{Total volume make up}}{\text{Volume taken}} \times 10$$

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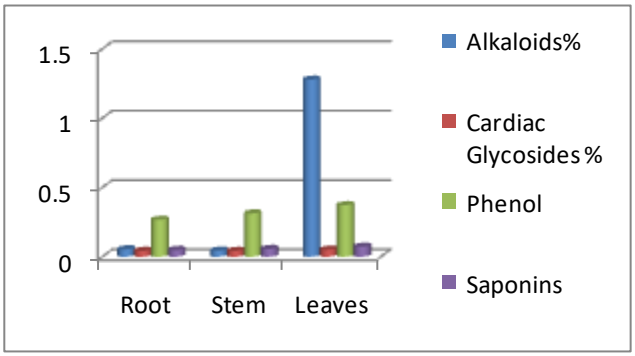
RESULT AND DISCUSSION

The qualitative phytochemical profile of root, stem and leaves of *Cleome viscosa* is provided in table-1. The result showed the presence of alkaloids in all the extracts except methanolic extract of leaves. Cardiac glycosides were detected in ethanolic and methanolic extracts of all plant parts but not in aqueous extracts. Steroids were not detected in any

of the extracts. Terpenoids were detected in ethanolic exctracts of all plant parts as well as aqueous and methanolic extracts of leaves which was not detected in aqueous and methanolic extract of root and stem.
Quantification of alkaloids and phenols are given in figures 1-2. The secondary metabolite contents were measured in root, stem and leaves of *Cleome viscosa.* It can be observed that leaves contained the maximum contents of alkaloids (1.278%), cardiac glycoside.

Table 2: Showing content of secondary metabolite present in the leave, stem and root of *Cleome viscosa*

Plant parts	Alkaloids %	Cardiac glycosides %	Phenol %	Saponins %
Root	0.0535±.008	0.042±0.020	0.267±0.174	0.049±0.002
Stem	0.044±0.007	0.042±0.176	0.312±0.205	0.056±0.001
Leaves	1.278±0.074	0.051±0.004	0.372±0.013	0.073±0.001



(0.051%), Phenol (0.372%) and saponins (0.073%), Preliminary phytochemical screening actually helps in isolating and characterizing the chemical constituents present in the plant extracts which is necessary to understand herbal drugs and their preparations.

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