ISSN: 2347-6567

International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

IJAMSCR |Vol. 14 | Issue 3| Jul - Sep 2026

www.ijamscr.com

DOI : https://doi.org/10.61096/ijamscr.v14.iss3.2026.1570.1575

Study of Phytochemical Investigation of the Aerial Parts of *Cleome viscosa L.*

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Published on: 22.09.2026
Published by: Futuristic Publications

Abstract: Cleome viscosa L. (Cleomaceae), commonly known as wild mustard or Hurhur, is a widely distributed annual herb used across Ayurveda, Siddha, Unani and folk medicine for the treatment of wounds, fever, diarrhoea, bronchitis, rheumatism and microbial infections. Despite extensive documentation of whole-plant, seed and root extracts, the aerial parts (leaves and stems) remain comparatively under-characterised. In the present study, the aerial parts of C. viscosa were sequentially extracted with petroleum ether, methanol and water by maceration, and the extracts were subjected to qualitative phytochemical screening and gas chromatography-mass spectrometry (GC-MS) analysis, with the eventual aim of evaluating anticancer activity. Screening confirmed the presence of flavonoids, alkaloids, glycosides, tannins and saponins in both the methanolic and aqueous extracts, while sterols, terpenoids and phenolic compounds were not detected under the conditions used. GC-MS analysis of the ethanolic extract identified eighteen compounds, dominated by fatty acids and their esters (notably tetradecanoic acid, hexadecanoic acid esters and decanedioic acid bis (2-ethylhexyl) ester) together with catechol and minor nitrogen heterocycles. These results provide a chemical rationale for the traditional therapeutic uses of the plant and establish a foundation for bioassay-guided isolation and anticancer screening.

Keywords: Cleome viscosa, Cleomaceae, phytochemical screening, GC-MS, aerial parts, secondary metabolites, anticancer

1. Introduction

Medicinal plants are the therapeutic backbone of much of the world's population; the World Health Organization estimates that nearly 80% of people globally rely on traditional plant-based medicine for primary healthcare. India and China together account for a substantial share of global medicinal-plant diversity, and roughly 90% of India's herbal raw material is still collected from forest and wild sources. Rising interest in natural remedies, coupled with concerns over the adverse effects of synthetic drugs, has renewed scientific attention on traditionally used medicinal herbs.

Cleome viscosa L. (family Cleomaceae), known variously as wild mustard, dog mustard or Hurhur, is an annual, glandular-hairy weed distributed across the plains of India, Africa, Pakistan and South-East Asia. In Indian traditional systems it is known by regional names such as HulHul, Kanputi, Talwani and Pashugandha. Leaves are applied externally for wounds, ulcers, skin infections and inflammation; seed preparations are used for fever, diarrhoea, dysentery and bronchitis; and the roots serve as an anthelmintic and remedy for rheumatic pain. Reported pharmacological activities include antimicrobial, anti-inflammatory, analgesic, antioxidant, antipyretic, hepatoprotective, antidiabetic, wound-healing, anticancer, gastroprotective, immunomodulatory and insecticidal effects, attributed to a broad phytochemical profile of

alkaloids, flavonoids, tannins, glycosides, terpenoids, steroids, phenolics, coumarins, saponins and essential oils.

While the whole plant, seeds and roots of C. viscosa have been reasonably well studied, the aerial parts (leaves and stems) remain comparatively less characterised. This project was undertaken to systematically extract, phytochemically screen and chemically profile the aerial parts, as a foundation for subsequent pharmacological evaluation, particularly anticancer activity.

2. Scope and Aim

The aerial parts of C. viscosa were extracted using solvents of increasing polarity (petroleum ether, methanol and water) to determine the most effective solvent system for recovering bioactive constituents. Specific objectives were: (i) qualitative and quantitative screening for major secondary metabolites such as steroidal saponins, flavonoids (quercetin, kaempferol) and alkaloids; (ii) identification of the optimal extraction solvent; and (iii) pharmacological evaluation of the extracts, with emphasis on anticancer properties.

3. Literature Review

Prior reviews and studies have consistently confirmed C. viscosa as a medicinally important herb rather than a mere weed. Joshi et al. reviewed its use for epilepsy, irritable bowel syndrome and protozoal/worm infections, alongside notable antioxidant and free-radical scavenging properties. Singh et al. documented anthelmintic, carminative, anticonvulsant, antidiarrhoeal, antimicrobial and wound-healing activity, and identified terpenes, flavonoids and phenol carboxylic acids—including coumarinolignoids cleomiscosin A and B—as major bioactive classes.

Deventhiran et al. compared GC-MS profiles of wild and micropropagated plants, finding differences in ethanol-extract compound counts (eight versus six) despite similar morphology. Yarrappagaari et al. demonstrated antiradical and antidiabetic activity of methanolic whole-plant fractions, with the most active fraction showing high phenolic/flavonoid content and dual alpha-glucosidase/alpha-amylase inhibition; HPLC identified protocatechuic acid hexoside and rutin. Usha et al. detected 78 phytochemicals by GC-MS and used in-silico docking against PARP-1, EGFR and HPV-related cancer targets to shortlist candidate anticancer phytomolecules.

Gupta et al. established significant, dose-dependent gastroprotective activity of C. viscosa extract via reduced lipid peroxidation and modulated H+/K+-ATPase activity, with HPTLC confirming quercetin and gallic acid. Namphonsaen et al. reported strong anti-inflammatory activity (NO and TNF-alpha inhibition) for a 95% ethanolic extract in Thai traditional use. Senthamilselvi et al. isolated quercetin-3-O-(2-acetyl)-glucoside with confirmed anti-inflammatory and antimicrobial activity against S. aureus and E. coli. Collectively, this body of work supports the pharmacological plausibility of the phytochemical classes identified in the present study.

4. Plant Profile

C. viscosa is distributed throughout tropical India, South-East Asia, Malaysia, tropical Australia, tropical Africa and Southern Arabia, thriving in crop fields, wastelands and wet grassy areas during and after the rainy season, with particularly high seasonal biodiversity in north-western India, including Uttarakhand. In India it is known by regional names such as HulHul, Kanputi, Talwani, Pivala Tilwana and Pashugandha, and locally as Hurhur (Hindi), Nayikkadugu (Tamil) and Hurhuria (Bengali).

Table 1a. Taxonomic classification of Cleome viscosa L.

Rank	Taxon
Kingdom	Plantae
Division	Angiosperms
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Brassicales
Family	Cleomaceae
Genus	Cleome

Species	C. viscosa L.

Botanically, the plant is a yellowish-green, viscid, glandular-hairy herb 30–160 cm tall, branched, with petioles 1.5–4.5(–8) cm and 3–5 foliate leaves bearing ovate-to-oblanceolate leaflets 0.6–6 × 0.5–3.5 cm, with entire, glandular-ciliate margins. Racemes are 5–10 cm (10–15 cm in fruit), with deciduous, trifoliate bracts. Flowers are bright yellow (occasionally purple-based), tetramerous, radially arranged at anthesis, with dimorphic stamens (4–10 shorter adaxial ones) and a densely glandular ovary. Capsules are cylindrical, 3–10 cm long, viscid and minutely beaked, dehiscing partway from apex to base. Seeds are numerous, light-to-dark brown, finely ridged transversely, about 1.2–1.8 × 1–1.2 mm (2n = 20).

5. Materials and Methods

5.1 Extraction

Materials used included beakers, aluminium foil, filter paper, distilled water, petroleum ether and methanol. Aerial parts (leaves and stems) were collected fresh, shade-dried away from direct sunlight to minimise degradation of thermolabile constituents such as flavonoids and glycosides, and then powdered to a coarse consistency suitable for maceration.

The powdered material was successively macerated with solvents of increasing polarity to achieve selective, sequential recovery of constituents. It was first extracted with petroleum ether to remove non-polar impurities such as waxes, fats and chlorophyll pigments, yielding a dark-green extract. The resulting marc was then extracted with methanol to recover moderately polar constituents (flavonoids, alkaloids, glycosides), again giving a dark-green extract. Finally, the marc was extracted with water to recover the most polar constituents (tannins, saponins, some glycosides), giving a yellowish-green aqueous extract. Each extract was filtered, concentrated by solvent removal, and stored separately in labelled containers for phytochemical and instrumental analysis (Table 1b, main results).

Table 1b. Extract colour by solvent

Solvent	Extract Colour
Petroleum ether	Dark green
Methanol	Dark green
Aqueous	Yellowish green

5.2 Preliminary Phytochemical Screening

The dried powder (coarse, light-green, aromatic, producing frothing with water) was subjected to standard qualitative tests: Liebermann-Burchard and Salkowski tests for steroids; Shinoda and lead-acetate tests for flavonoids; Molisch's and Fehling's tests for carbohydrates; Mayer's, Wagner's, Dragendorff's and Hager's reagents for alkaloids; Legal's test for steroidal glycosides; ferric chloride, gelatin and lead-acetate tests for tannins; Biuret and Ninhydrin tests for proteins/amino acids; and ferric chloride test for phenolic compounds.

5.3 GC-MS Analysis

The ethanolic extract was analysed on a Scion 436-GC (Bruker) with a TQ Quadrupole Mass Spectrometer, using a BR-5MS column (30 m × 0.25 mm ID, 0.25 µm film), helium carrier gas (1 mL/min, split 10:1), an oven programme of 110°C (3.5 min hold) ramped to 200°C at 10°C/min and then to 280°C at 5°C/min (12 min hold), injector temperature 280°C, and a total run time of 40.5 min. MS parameters used the NIST-11 library, inlet-line temperature 290°C, source temperature 250°C, electron energy 70 eV, and mass scan range m/z 50–500.

6. Results and Discussion

6.1 Phytochemical Screening

Table 1 summarises the qualitative screening results. Flavonoids, alkaloids, glycosides, tannins and saponins were present in both the methanolic and aqueous extracts, while sterols, terpenoids and phenolic compounds were not detected under the conditions used. The frothing observed on shaking the powder

with water is consistent with saponin content. This profile aligns with prior reports of flavonoid-, alkaloid- and saponin-rich extracts from other parts of C. viscosa and supports its documented antioxidant, anti-inflammatory and antimicrobial activities.

Table 1. Phytochemical screening of aerial-part extracts

Constituent	MeOH	Aqueous
Sterols	-	-
Terpenoids	-	-
Flavonoids	+	+
Alkaloids	+	+
Glycosides	+	+
Tannins	+	+
Saponins	+	+
Phenolics	-	-

(+) present, (-) absent

6.2 GC-MS Profile

GC-MS analysis of the ethanolic extract resolved eighteen compounds (Table 2). The profile is dominated by fatty acids and their esters — tetradecanoic acid (1.17%), hexadecanoic acid methyl ester (0.39%) and ethyl ester (0.24%), dodecanoic acid (0.38%), and decanedioic acid bis(2-ethylhexyl) ester (1.03%) — together with catechol (0.61%), a well-documented phenolic antioxidant, and minor nitrogen heterocycles such as cycloserine, semioxamazide and 1,3,5-triazine-2,4,6-triamine. Several of these fatty-acid derivatives and phenolics have reported antioxidant, antimicrobial and anti-inflammatory activity elsewhere in the literature, reinforcing the plausibility of the traditional and pharmacologically reported uses of C. viscosa.

Table 2. GC-MS profile of the ethanolic extract (aerial parts)

Compound	Formula	MW	%
Cycloserine	C3H6N2O2	102	0.25
Semioxamazide	C2H5N3O2	103	0.06
1,3,5-Triazine-2,4,6-triamine	C3H6N6	126	0.08
Catechol	C6H6O2	110	0.61
2-Octenoic acid, 4,5,7-trihydroxy	C8H14O5	190	0.05
n-Pentadecanol	C15H32O	228	0.02
Homovanillyl alcohol	C9H12O3	168	0.15
Dodecanoic acid	C12H24O2	200	0.38
3,8-Dimethylundecane	C13H28	184	0.21
Propanoic acid, 3-chloro-, methyl ester	C4H7ClO2	122	0.98
Tetradecanoic acid	C14H28O2	228	1.17
Hexadecanoic acid, methyl ester	C17H34O2	270	0.39
Hexadecanoic acid, ethyl ester	C18H36O2	284	0.24
Heptadecanoic acid	C17H34O2	270	0.02
Dodecanoic acid, 3-hydroxy	C12H24O3	216	0.02
11,14,17-Eicosatrienoic acid, methyl ester	C21H36O2	320	0.19
11-Methyltricosane	C24H50	338	0.10
Decanedioic acid, bis(2-ethylhexyl) ester	C26H50O4	426	1.03

6.3 Significance for Anticancer Evaluation

The co-occurrence of flavonoids, alkaloids and phenolic fatty-acid derivatives is notable given prior in-silico docking work linking C. viscosa phytomolecules to cancer-associated targets such as PARP-1, EGFR

and HPV proteins. The present phytochemical and GC-MS profile of the aerial parts therefore provides a rational basis for prioritising the methanolic and ethanolic extracts for subsequent in vitro anticancer screening.

7. Work Plan and Quality Control

The project was executed in six sequential stages: plant collection, botanical authentication, sequential solvent extraction, preliminary phytochemical analysis, GC-MS profiling, and planned pharmacological evaluation with anticancer activity as the primary endpoint. This staged design ensures that chemical characterisation precedes and informs the choice of extracts and fractions carried forward into bioassay work, so that fractions richest in flavonoids, alkaloids and saponins are prioritised.

Instrumental reliability for GC-MS was maintained through daily autotune verification using perfluorotributylamine (PFTBA) as a tuning standard, with the three characteristic tuning masses (69, 219 and 502 amu) required to fall within specified abundance and peak-width tolerances. Monthly performance checks against petroleum ether and reference hydrocarbon blanks, together with periodic septum and liner maintenance, safeguard the reliability of retention times and NIST-11 library matches used for compound identification.

8. Extended Discussion

The phytoconstituent pattern recovered from the aerial parts is broadly, though not entirely, consistent with prior surveys of C. viscosa conducted on whole-plant, root, stem and seed material. Several earlier studies report steroids and phenolic compounds in other plant parts, whereas these were not detected here in the methanolic and aqueous extracts of the aerial parts under the specific reagent tests used. Such discrepancies commonly reflect differences in the plant organ sampled, seasonal and geographic variation in secondary-metabolite biosynthesis, extraction-solvent polarity, and the relative sensitivity of different qualitative tests — reinforcing the value of part-specific, solvent-specific phytochemical profiling rather than extrapolating whole-plant data to a single organ.

The GC-MS profile obtained for the ethanolic aerial-part extract also differs from whole-plant surveys reported elsewhere, which have identified substantially larger compound counts (over 70 in some methanolic whole-plant extracts) dominated by terpenes and phenol carboxylic acids rather than fatty acids. This divergence likely reflects both the choice of extraction solvent and the specific organs sampled, and points to the value of a systematic solvent-comparative GC-MS study (petroleum ether, methanol, ethanol, aqueous) of the aerial parts specifically, as originally envisaged in the study objectives.

Mechanistically, the co-occurrence of flavonoids, alkaloids, tannins and saponins alongside a fatty-acid- and catechol-rich volatile fraction is plausible as a chemical basis for several bioactivities reported for C. viscosa, including antioxidant free-radical scavenging (flavonoids, catechol), membrane-disruptive cytotoxicity relevant to anticancer and antimicrobial effects (saponins), and anti-inflammatory or analgesic activity (flavonoid glycosides such as quercetin derivatives previously isolated from this species). These considerations directly support prioritising the methanolic and ethanolic aerial-part extracts for anticancer bioassay screening.

9. Limitations

This investigation is preliminary and qualitative. Quantitative estimation of total phenolic and flavonoid content, percentage extraction yield per solvent, and confirmatory isolation of individual marker compounds by chromatographic and spectroscopic methods were beyond the present scope and are recommended as follow-up work. GC-MS identification relies on spectral library matching and should ideally be confirmed against authentic reference standards for compounds selected as candidates for further pharmacological study, particularly the major fatty-acid esters and catechol.

10. Future Scope

The next phase of this project will focus on in vitro anticancer screening of the methanolic and ethanolic aerial-part extracts against suitable cell lines using standard cytotoxicity assays (e.g., MTT assay), together with bioassay-guided fractionation to isolate and identify the constituents responsible for observed activity. In-silico molecular docking of the GC-MS-identified compounds against cancer-relevant protein targets — as previously applied to whole-plant C. viscosa constituents against PARP-1, EGFR and HPV-related proteins — offers a complementary strategy to prioritise compounds for isolation and testing.

11. Conclusion

The aerial parts of Cleome viscosa contain a diverse array of pharmacologically relevant phytoconstituents — flavonoids, alkaloids, glycosides, tannins and saponins — together with fatty-acid derivatives and phenolic compounds identified by GC-MS. These findings substantiate the traditional therapeutic uses of the plant and justify further bioassay-guided fractionation and in vitro anticancer evaluation of the methanolic and ethanolic extracts.

Acknowledgements

The authors thank the Department of Pharmaceutical Chemistry for laboratory facilities, instrumentation access and guidance throughout this project.

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