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Phytochemical Profiling and Antioxidant Potential of *Cleome viscosa* Leaf Extracts

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Abstract

Cleome viscosa is a native medicinal plant that displays important seasonal biodiversity in the north -western regions of India. Its leaves, seeds and roots have long been working in traditional and folklore systems of medicine for the treatment of various diseases. The present study was undertaken to evaluate the phytochemical composition, antioxidant, and cytotoxic potential of methanol and acetone leaf extracts of *C. viscosa*. Phytochemical screening of extracts revealed the presence of diverse secondary metabolites. Acetone extracts consisted of alkaloids, saponins, flavonoids, terpenoids, glycosides, and phenolic compounds, while methanol extracts additionally performed tannins and steroids. The antioxidant capacity of both extracts was evaluated through hydroxyl radical manual scavenging and nitric oxide scavenging assays. Methanol extracts demonstrated comparatively high scavenging activity, which can be attributed to phytoconstituents, especially the broad spectrum of tannins and steroids. The antioxidants and cytotoxic activities indicate that the bioactive compounds present in *C. viscosa* contribute to its medicinal efficacy. These findings align as an antimicrobial, anticancer and antioxidant remedy with traditional applications of the plant. The study provides scientific evidence supporting the ethno medicinal use of *C. viscosa* and underlines its ability as a natural source to develop medical agents. Further studies that focus on separation, characterization and mechanical evaluation of individual compounds are warranted to detect their clinical relevance.

Keywords

Cleome viscosa, phytochemical screening, antioxidant activity, cytotoxicity

Introduction

Cleome viscosa Linn. commonly known as the Asian Spider flower or tick weed, belongs to the family Cleomaceae. It is a widely distributed annual herb, found predominantly in tropical regions, especially in the plains and moist areas of India. The plant is commonly seen in waste lands and prefers sunny, light soils, thriving under both seasonally dry and perennially moist conditions. It can also grow in sandy or calcareous soils. Morphologically, *C. viscosa* is a sticky herb characterized by a strong penetrating odour, also contains yellow flowers, long slender pods, with dark seeds.

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Traditionally, *C. viscosa* has been valued for its diverse therapeutic properties and ethnomedicinal uses. This herb is used for the treatment of fever, inflammation, liver disorders, bronchitis, and diarrhea (Chatterjee & Wernerfelt, 1991) as a part of Ayurveda medicine. The extract of this plant juice from the seeds used to cure or treat the infantile convulsion and mental disorders in the populations of rural areas in India. The plant liquid crude extract is given orally to the patients who are affected with severe fever and the crushed leaves are applied to the wounds as external medicine (Nadkarni, 1982). *C. viscosa* also used as anti-helminthic activity to treat the worms in the gastrointestinal infections such as flatulence, colic, dyspepsia, and diarrhea. The other medicinal properties include the management of cough, bronchitis, cardiac conditions, and fungal infections like ringworm (Ahmad, 2012). The plant extract contains bio molecules that can cure liver diseases thus it has the hepatoprotective activity.

As a part of Indian knowledge system 80% of the population still depends on this plant extract as the primary healthcare medicine and it was appreciated by the World Health Organization (World Health Organization, 2019). In India most of the herbs contains many medicinal values that have the bioactive compounds that cure and prevent most of the infectious diseases. Most of the plant has the natural antioxidant properties including the *C. viscosa*. In other words, the synthetic antioxidants have adverse effect on human physiology therefore the natural plants were given significant attention. Considering this, the exploration of natural antioxidants from such plants has gained significant attention as potential alternatives to synthetic antioxidants, which may have adverse effects. Therefore, the plant *C. viscosa* is given attention for its phytochemical rich compounds and least explored for the antioxidant activity. The present study is to evaluate the antioxidant property of this plant extract is scientifically valid and has the potential value based on its therapeutic use by biochemical assay.

Methodology

Collection and Authentication of Plant Material

Fresh leaves of *Cleome viscosa* L. were collected from the local area of Western Guards of Tamil Nadu, India. The plant was authenticated at the Department of Botany, Tamil Nadu Agricultural University, Coimbatore, and a voucher specimen was deposited in the departmental herbarium for reference. The leaves were separated, shade-dried for seven days, pulverized using a mechanical grinder, and stored in an airtight container until further use.

Preparation of Plant Extracts

Ten grams of the powdered leaf material was extracted separately with 100 ml of methanol and acetone. The mixtures were plugged with cotton wool and placed on a rotary shaker (220 rpm) for 24 h. The extracts were filtered, and the solvents were evaporated to one-fourth of the original volume. The concentrated extracts were stored at 4°C for further analysis.

Qualitative Phytochemical Screening

The methanolic and acetone extracts of *C. viscosa* leaves were subjected to preliminary phytochemical screening using standard methods (Harborne, 1998; Kokate et al., 2008). Presence of secondary metabolites was confirmed based on characteristic colour changes or precipitate formation.

Table 1. Qualitative phytochemical screening of *C. viscosa* leaf extracts

Phytochemical	**Test Performed**	**Colour Observation**	**Inference**
Alkaloids	Mayer's test; Wagner's test	Yellow, brown, red	Presence of alkaloids
Saponins	Foam test	Stable foam persists (>10 min)	Presence of saponins
Flavonoids	Alkaline reagent test	Intense yellow (disappears in HCl)	Presence of flavonoids
Glycosides	Legal's test; Killer Killion's test	Pink, red, yellow	Presence of glycosides
Phenols	Ferric chloride test; Lead acetate test	Bluish black, yellow	Presence of phenols
Tannins	Ferric chloride test; Lead acetate test	Bluish black, yellow, brown	Presence of tannins
Steroids	Salkowski's test	Red colour in chloroform layer	Presence of steroids
Thiols	Sodium nitro preside test	Transient magenta	Presence of thiols
Anthraquinones	Borntrager's test	Pink, red	Presence of anthraquinones

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity was assessed by the deoxyribose degradation method (Madhe et al., 2014). The reaction mixture contained 0.1 ml EDTA, 0.01 ml $FeCl_3$, 0.1 ml H_2O_2, 0.36 ml deoxyribose, 1 ml extract (100–500 µg/ml), 0.33 ml phosphate buffer (50 mM, pH 7.9), and 0.1 ml ascorbic acid. After incubation at 37 °C for 1 h, TBARS were measured by adding 0.5 ml thiobarbituric acid (TBA) and 0.5 ml HCl, heating for 20 min, cooling, and recording absorbance at 532 nm.

Nitric Oxide Scavenging Assay

Nitric oxide scavenging activity was determined using sodium nitroprusside (Madhe et al., 2014). The reaction mixture (6 ml) contained 4 ml sodium nitroprusside, 1 ml phosphate-buffered saline (PBS), and 1 ml extract (100–500 µg/ml in DMSO). After incubation at 25 °C for 15 min, 0.5 ml of the mixture was treated with 1 ml sulphanilic acid reagent (5 min) and 1 ml naphthyl ethylene diamine dihydrochloride (30 min, diffused light). The absorbance of the resulting pink chromospheres was measured at 540 nm. Rutin was used as standard. The scavenging activity was calculated as equation 1. Hydroxyl radical scavenging activity was assessed by deoxyribose degradation inhibition, while nitric oxide scavenging activity was determined by nitrite formation inhibition, as shown in Figure 1.

$$\text{Scavenging activity (\%)} = (A0 - A1) \times 100 / A0 \quad \text{- (Eq. 1)}$$

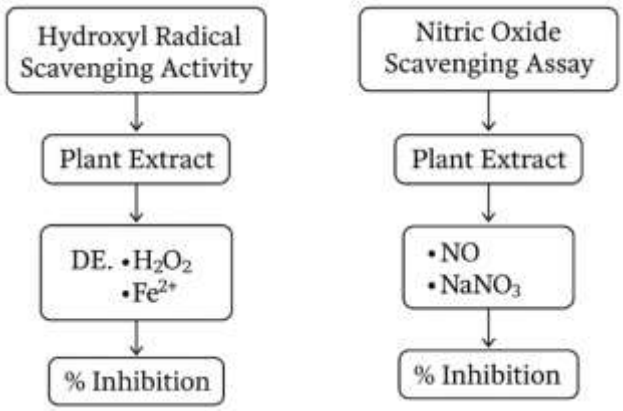


Figure 1. Flowchart representing the in vitro antioxidant assays of *Cleome viscosa* leaf extracts.

Results and Discussion

Phytochemical Analysis

The phytochemical analysis of *C. viscosa* leaf extract compounds was presented in the Table 2. This preliminary analysis was done by methanol revealed the presence of all common biochemical such as alkaloids, flavonoids, steroids, saponins, phenolic compounds, glycosides, terpenoids, and tannins. Interestingly, tannins, steroids, and anthraquinones were absent in the acetone extract but were detected in the methanol extract, suggesting that methanol was more efficient in extracting a broader range of phytoconstituents. These differences highlight the solvent-dependent variation in phytochemical composition, with methanol demonstrating a higher affinity for polar compounds such as tannins and steroids.

Table. 2 Preliminary phytochemical screening of Methanol and Acetone extract of *C. viscosa.*

Sample	Acetone Extract	Methanol extract
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	+
Glycosides	+	+
Phenolic Compounds	+	+
Tannins	-	+
Steroids	-	+
Thiols	+	+
Anthraquinones	-	+

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity of the methanolic extract of *C. viscosa* leaves was evaluated and compared with the standard antioxidant, vitamin C (Table 3). The results showed a clear dose-dependent increase in scavenging potential for both the extract and the standard. At the lowest concentration (100 µg/ml), the methanol extract exhibited 10.80 ± 0.20% inhibition, which was slightly lower than vitamin C (12.30 ± 0.20%). With increasing concentrations, the activity improved progressively, reaching 54.55 ± 0.40% inhibition at 500 µg/ml, whereas vitamin C recorded 60.50 ± 0.50% at the same concentration. Although the methanolic extract displayed lower activity than the standard, its comparable scavenging

potential highlights the presence of potent hydrogen-donating phytochemicals capable of neutralizing hydroxyl radicals is shown in Figure 2.

Table 3. Hydroxyl radical scavenging activity of methanolic extract of *C. viscosa* leaves compared with standard vitamin C. Values are expressed as mean ± SD (n = 3). Methanol extract showed a dose-dependent increase in hydroxyl radical scavenging activity, though slightly lower than standard vitamin C.

Concentration (µg/ml)	% Inhibition (Vitamin C)	% Inhibition (Methanol extract)
100	12.30 ± 0.20	10.80 ± 0.20
200	25.32 ± 0.30	19.22 ± 0.20
300	36.25 ± 0.93	30.22 ± 0.50
400	51.34 ± 0.58	43.25 ± 0.30
500	60.50 ± 0.50	54.55 ± 0.40

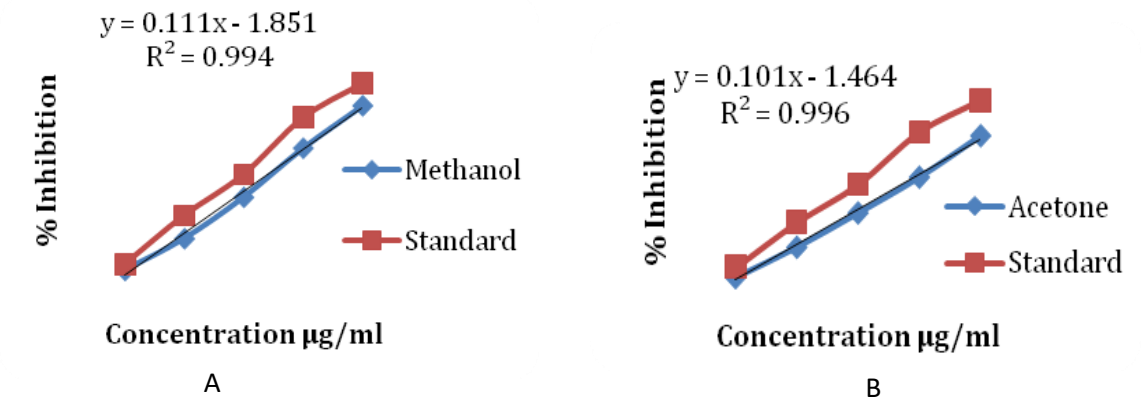


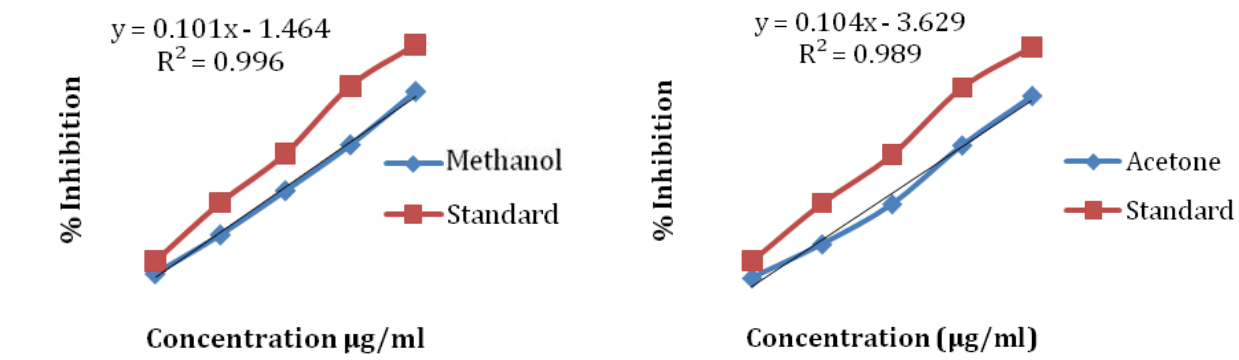
Figure 3. Hydroxyl radical scavenging activity of *C. viscosa* leaf methanol (A) and acetone (B) extracts compared with standard antioxidant.

Nitric Oxide Scavenging Activity

The hydroxyl radical scavenging potential of the acetone extract of *C. viscosa* leaves was also evaluated and compared with vitamin C as the standard antioxidant (Table 4). Similar to the methanol extract, the acetone extract exhibited a concentration-dependent increase in radical scavenging activity. At 100 µg/ml, the extract showed 9.58 ± 0.20% inhibition, which progressively increased to 50.08 ± 0.40% at 500 µg/ml. Although the acetone extract demonstrated notable activity, its scavenging ability remained consistently lower than that of vitamin C (60.50 ± 0.50% at 500 µg/ml). When compared to the methanol extract, the acetone extract showed slightly reduced activity across all concentrations, indicating that methanol is a more effective solvent for extracting hydroxyl radical–scavenging phytochemical from *C. viscosa* leaves is shown in Figure 4A, B.

Table 4. Hydroxyl radical scavenging activity of acetone extract of *C. viscosa* leaves compared with standard vitamin C. Values are expressed as mean ± SD (n = 3). Acetone extract showed dose-dependent hydroxyl radical scavenging activity, though consistently lower than standard vitamin C.

Concentration (µg/ml)	% Inhibition (vitamin C)	% Inhibition (Acetone extract)
100	12.30 ± 0.20	9.58 ± 0.20
200	25.32 ± 0.30	18.24 ± 0.30
300	36.25 ± 0.93	28.04 ± 0.30
400	51.34 ± 0.58	38.24 ± 0.20
500	60.50 ± 0.50	50.08 ± 0.40



A B

Figure 4. Nitric oxide scavenging activity of methanol extract (A) and acetone extract (B) of *C. viscosa* leaves

The overall findings from the phytochemical profiling and antioxidant assays highlight that *C. viscosa* leaves are a rich source of diverse secondary metabolites, including alkaloids, flavonoids, glycosides, phenolic compounds, tannins, steroids, thiols, and anthraquinones, many of which are well known for their antioxidant potential. Both methanol and acetone extracts demonstrated hydroxyl radical scavenging activity in a dose-dependent manner, indicating that *C. viscosa* possesses free radical neutralizing capacity. However, the methanol extract consistently outperformed the acetone extract, suggesting that methanol is a more efficient solvent for extracting antioxidant phytochemical from the leaves. These results provide strong evidence that *C. viscosa* could serve as a natural source of bioactive compounds with therapeutic applications, particularly in managing oxidative stress–related disorders.

Cleome viscosa is a traditionally used medicinal plant with diverse ethno pharmacological applications, including treatment of inflammation, fever, skin diseases, and microbial infections (Gupta & Dixit, 2009; Kumar et al., 2013). Its therapeutic value has been attributed to its rich phytochemical repertoire, particularly phenolics, flavonoids, alkaloids, and terpenoids, which are often associated with antioxidant, antimicrobial, and anticancer properties. Investigating the phytochemical and antioxidant potential of *C. viscosa* leaves is therefore justified, as oxidative stress plays a central role in the onset of several chronic and degenerative diseases, and plant-derived antioxidants remain a promising source for safer therapeutic alternatives.

Phytochemical investigations on *C. viscosa* have demonstrated variability depending on plant part and solvent system. Adanlemegbe et al. (2020) reported that dichloromethane extracts of fruits, leaves, stems, and roots contained alkaloids and tannins, with flavonoids absent in fruits. Ethanol extracts indicated flavonoids and tannins in all plant parts, whereas alkaloids and saponins were restricted to leaves. Similarly, Yadav et al. (2010) showed that methanol, aqueous, ethyl acetate, and n-hexane extracts of the whole plant revealed a wide range of phytochemicals, with methanol yielding the richest profile. Methanol extract of aerial parts contained high levels of flavonoids, phenols, tannins, fatty acids, and alkaloids compared to other solvents.

Methanol extract as superior in phytochemical yield, agree with earlier studies. These phytoconstituents are pharmacologically important, as flavonoids and phenols are well-known for their antioxidant and anti-inflammatory properties, while alkaloids and glycosides contribute to antimicrobial and anticancer activities (Kumar et al., 2013; Harborne, 1998). Hydroxyl radical scavenging is a crucial indicator of hydrogen-donating and chain-breaking antioxidant capacity. The superior activity of methanol extract suggests the presence of abundant phenolic and flavonoid constituents. Gupta & Dixit (2009) reported similar findings, where *C. viscosa* methanol extract showed strong hydroxyl radical scavenging activity with an IC_{50} of 573.55 μg/ml. Yadav et al. (2010) also demonstrated that methanol extracts possess significant antioxidant potential across DPPH, $ABTS^{+}$, FRAP, and metal chelation assays.

Nitric oxide is a free radical involved in inflammatory processes and oxidative stress. Excessive nitric oxide production is implicated in neurodegenerative and cardiovascular disorders. The methanol extract of *C. viscosa* demonstrated stronger nitric oxide scavenging capacity than the acetone extract, corroborating earlier reports. Devi et al. (2002) reported that ethanolic root extracts of *C. viscosa* exhibited significant nitric oxide and DPPH scavenging activities. Similarly, Devi et al. (2013) highlighted the presence of non-enzymatic antioxidants such as ascorbic acid, α-tocopherol, glutathione, and flavonoids in different parts of *C. viscosa*, contributing to its free radical scavenging potential.

The strong activity of the methanolic extract is attributed to its high content of phenolic compounds and flavonoids, which are known to effectively neutralize nitric oxide and hydroxyl radicals. These findings suggest that *C. viscosa* leaves, particularly the methanolic extract, may serve as a natural source of antioxidants with potential therapeutic applications in oxidative stress-related disorders.

Conclusion

Cleome viscosa leaves are a rich source of diverse bioactive phytochemical with significant antioxidant potential. Among the tested extracts, methanol proved most effective in extracting compounds capable of scavenging free radicals. These findings suggest that *C. viscosa* could serve as a natural source of therapeutic agents for managing oxidative stress–related disorders.

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