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Phytochemical Screening, Antioxidant, and Antimicrobial Activities of *Cleome viscosa* Leaves

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ABSTRACT

Cleome viscosa is a widely distributed plant specie, that has been used in traditional medicine to treat various illnesses. This study aimed to investigate the phytochemical composition, antioxidant potential, and antimicrobial activity of *Cleome viscosa* leaves. Phytochemical screen was done via standard methods. The antioxidant activity was evaluated using DPPH radical scavenging. The antimicrobial potential of the extracts was assessed against a panel of clinically relevant bacterial strains, including both Gram-positive and Gram-negative bacteria. Qualitative phytochemical screening revealed the presence of bioactive compounds such as resin, flavonoids, oxalates, quinones, essential oil, tannins, and phenols in the leaf extracts. The results demonstrated the potent antioxidant properties of the *Cleome viscosa* leaf extracts, which may be attributed to the presence of phenolic compounds and other antioxidant phytochemicals. The findings showed the effectiveness of the *Cleome viscosa* leaf extracts in inhibiting the growth of these pathogenic bacteria. The phytochemical screening, antioxidant evaluation, and antimicrobial assessment provide valuable insights into the medicinal potential of *Cleome viscosa* leaves and suggest their possible applications in the development of natural products and pharmaceuticals.

Keywords: *Cleome viscosa*, phytochemicals, antioxidant activity, antimicrobial activity.

INTRODUCTION

Plants are principal sources of herbal remedies and anti-infective agents [1-2]. All over the world, particularly in developing countries, people count on plants for primary health care and curing infectious and serious diseases [3]. Screening plants for phytochemicals and biological activities has become a research stream of international concern [4]. A leading impetus behind this interest is the emergence of infections and intoxifications driven by microbial pathogens with acquired resistance related to the improper use of synthetic drugs [5-6]. This microbial property provides a need to develop novel therapeutic formulations with resistance-combating capacity [2]. Plants represent a major reservoir of diverse secondary or natural products that are of medicinal value and that can be exploited for new medications [7].

Cleome viscosa, commonly known as Asian spider flower or wild mustard, is a widely distributed plant species that has garnered attention due to its potential therapeutic properties [8]. This plant belongs to the Cleomaceae family and is native to various regions, including Asia, Africa, and parts of the Americas. The leaves of *Cleome viscosa* have been used in traditional medicine to treat ailments, including skin infections, gastrointestinal disorders, and inflammatory conditions [9].

In recent years, there has been interest in exploring the phytochemical composition and biological activities of *Cleome viscosa,* particularly its antimicrobial properties [10]. Phytochemical screening of the plant's leaves revealed the presence of various bioactive compounds which possess pharmacological activities [11]. The antioxidant potential of *Cleome viscosa* extracts has been investigated using *in vitro* assays, such as DPPH radical scavenging, ferric reducing antioxidant power (FRAP), and total phenolic content determination [12].

Several studies have reported the effectiveness of *Cleome viscosa* extracts in scavenging free radicals and exhibiting potent antioxidant activity, which may be attributed to the presence of phenolic compounds and other antioxidant phytochemicals [13]. The antimicrobial potential of *Cleome viscosa* plants has been studied against pathogenic bacteria, including both Gram-positive and Gram-negative strains. Based on literature search, there is little, or no research conducted on the leaves of *Cleome viscose*.

The present study conducted a phytochemical screening of *Cleome viscosa* leaves, evaluated their antioxidant activity, and assessed their antimicrobial potential against a relevant bacterial strain. The findings of this study will contribute to the understanding of the medicinal potential of this plant and provide valuable insights into its possible therapeutic applications.

MATERIAL AND METHODS

Collection and processing of plant materials

The leaves of *Cleome viscose* were collected at Yobe State University, Damaturu, Nigeria and was identified by Mr. Abdullahi Salihu of the Herbarium unit, Department of Biological Science, Yobe State University, Damaturu. After collection, the leaves were washed under running tap water and spread on mesh trays for shade drying. Then, the leaves were ground to powder and stored in airtight glass containers until required for analysis.

Preliminary phytochemical analysis

The crude n-hexane, ethyl acetate and methanol extracts of leaves of *Cleome viscosa.* were tested for the presence of phytochemicals using the following qualitative procedures [2, 14].

Test for saponins

About 5 ml of the extract was shaken with 10 ml of water in a test tube. Frothing which persists was taken as preliminary evidence for the presence of saponin.

Test for tannins

To 2 ml of the extract, 10 ml of water was added and a drop of ferric chloride. Green precipitate indicates the presences of tannins.

Tests for flavonoids

To 5 ml of the extract, 0.5 g of magnesium chips was added and a few drops of concentrated H_2SO_4 down the side of the test-tube. Reddish coloration indicates the presence of flavonoids.

Test for essential oils

About 10 ml of the extract were dissolved in 90% alcohol and 3 drops of ferric chloride was added. Green coloration indicates the presences of essential oils.

Test for glycoside

About 5 ml of the extract was boiled with 25 ml of dilute tetraoxosulphate (VI) acid ($2.5cm^3$) for 15 minutes. The resulting solution was cooled and neutralized with 10% potassium hydroxide and of Fehling's solution A and B were added. Formation of a brick red precipitate indicates the presence of glycosides.

Test for phenols

Equal volume of the extract was added to equal volume of ferric chloride. A deep blue bluish solution indicates the presence of phenols.

Test for resin

To 2 ml of the extract, an equal volume of acetic anhydride solution and few drops of concentrated H_2SO_4 were added. Violet coloration will be taken as an indication for the presence of resins.

Test for quinones

Few drops of concentrated, HCl were added to a small portion of extract, formation of yellow precipitate coloration indicates the presence of quinines.

Test for oxalate

About two drops of glacial acetic acid were added to a small portion of the extract, a greenish black color confirm the presence of oxalate.

Antimicrobial activity

About four different concentrations of the extracts were tested for antimicrobial activity using disc diffusion assay according to the method employed by Blažević et al [15]. The test microorganisms used in this study were *Staphylococcus aureus* and *Escherichia coli.*

Disc diffusion assay

The strains of microorganism obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37 ºC for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia), poured on petri-dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimetre were impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37 ºC. The antimicrobial activity was recorded by measuring the width of the clear inhibition zone around the discs using zone reader (mm). Amoxicillin and ketoconazol (100 µg/disc) were used as standards. Dimethylsulfoxide (DMSO) was used as negative control.

Determination of Minimum Inhibition Concentration

The minimum inhibitory concentration was determined by serial dilution method. Serial dilution of the extract was prepared in the test tubes containing peptone water as diluents. About 50 mg of the extract was dissolved in one ml of DMSO which is further subjected for two fold dilution. Totally 10 test tubes were maintained. The final concentration of the extract was now one half of the original concentration in each test tube. Each bacterial isolate was inoculated at 37 °C for 24 h. Then, the tubes were examined for the presence of growth considering turbidity as criterion. The highest dilution in each series that did not show turbidity and thus no growth was considered to be the MIC of the organism.

Determination of antioxidant activity

The antioxidant activity of the aqueous crude extract of cleome leaves was determined using DPPH radical scavenging method. A fresh DPPH methanol solution (0.002%) was prepared. A series of diluted solutions (0.00, 10.0, 20.0, 30.0, 40.0, 50.0 ppm) of ascorbic acid in methanol were prepared. Each 1.5 mL of the ascorbic acid solution was mixed with a 3.0 mL DPPH solution and allowed to stand in dark for 15 min. The control was prepared by taking

3.0 mL of DPPH in 1.5 mL methanol and its absorbance at 517 nm was recorded. The same procedure was applied to prepare different concentrations (0.00, 10.0, 20.0, 30.0, 40.0, 50.0 ppm) from the leaf extract of cleome. The percentage inhibition of the extracts in the presence of DPPH was calculated using Eq. given below.

$$\%Inhibition = \frac{Ac - Ao}{Ac} X\ 100$$

RESULTS AND DISCUSSION

The percentage yield of the employed leaf extract is illustrated in Table 1. The extraction of 200 g of dried *Cleome viscose* leaves produced a total phytochemical yield of 19.6 g. Out of this, ethyl acetate showed the highest extract yield of 7.7 g. This was followed by the n-hexane extract with an extract yield of 7.3 g. The methanol extract yield was 4.6 g, which is the lowest extraction yield. This indicates that the leaves of Cleome viscose contain a high amount of phytochemicals.

Table 1: The percentage yield of the leaves of *Cleome viscose*

S/N	Solvent used	Weight of sample used (g)	Weight of extract (g)	Percentage yield (%)
1.	n-hexane	200	7.3	3.05
2.	Ethyl acetate	200	7.7	3.90
3.	Methanol	200	4.6	2.0

Phytochemical screening

The results of the phytochemical screening for the presence of n-hexane, methanol, and ethyl acetate extracts of *Cleome viscosa* leaves are presented in Table 2. The n-hexane extract contains resin, flavonoids, essential oil, and saponins. In contrast, the methanol extract includes resin, flavonoids, oxalates, essential oil, tannins, phenols, and saponins, while the ethyl acetate extract comprises resin, flavonoids, oxalates, essential oil, tannins, and saponins. Quinones were not detected in any of the extracts. The n-hexane extract yielded four compounds, while the methanol and ethyl acetate extracts yielded seven and five phytochemicals, respectively. This variation may be attributed to the higher solubility of these compounds in polar protic solvents like methanol compared to nonpolar solvents such as n-hexane and ethyl acetate, highlighting the solvent-dependent nature of extracting phytochemical compounds from plants. The presence of these bioactive secondary metabolites is linked to antibacterial properties [16]. These phytochemicals are recognized for

their medicinal and physiological effects [17]. Flavonoids, a group of polyphenolic metabolites, exhibit antioxidant properties and play a role in protecting against allergies, inflammation, platelet aggregation, microbial infections, and ulcers. Tannins, traditionally used to treat inflamed oral surfaces, catarrh, wounds, hemorrhoids, and diarrhea, are also known for their antimicrobial properties, making them valuable in pharmaceutical applications [18-19].

Table 2: The result of phytochemical screening of n-hexane, methanol and ethyl acetate.

S/N	Phytochemicals	n-hexane	Methanol	Ethyl acetate
1.	Rasin	+	+	+
2.	Flavonoids	+	+	+
3.	Oxalates	-	+	+
4.	Quinones	-	-	-
5.	Essential oil	+	+	+
6.	Tannins	-	+	+
7.	Phenols	-	+	-
8.	Saponins	+	+	-

Keys (+) = Present and (-) = Absent of phytochemicals

Antioxidant studies

Antioxidants are primarily sourced from food and medicinal plants like fruits, vegetables, flowers, spices, and traditional herbs [21]. Plant-derived natural antioxidants are predominantly polyphenols, encompassing phenolic acids, flavonoids, tannins, anthocyanins, lignans, and stilbenes. Carotenoids (xanthophylls and carotenes), along with vitamins C and E, are also common natural antioxidants [22]. These natural antioxidants, particularly polyphenols and carotenoids, exhibit various beneficial effects such as anticancer, antibacterial, anti-inflammatory, antiviral, and anti-aging properties [23]. In this study, the antioxidant activity was assessed using n-hexane, ethyl acetate, and methanol extracts of *Cleome viscosa* leaves. The evaluation involved measuring the free radical scavenging activity and ferric reducing antioxidant power of the extracts, with ascorbic acid serving as the standard. The IC50 values, representing the 50 % inhibition concentration, were determined by comparing the absorbance of different concentrations of each leaf extract with that of standard ascorbic acid (concentrations ranging from 10 to 50 µg/mL) at 700 nm. The results are presented in the Table 3.

Table 3: Absorbance at 700 nm against concentration of standard ascorbic acid, n-hexane, ethyl acetate, methanol and ethyl acetate extract of *Cleome viscosa* leaves.

S/N	Concentration (10 glml)	Ascorbic acid	n-hexane	Ethyl acetate	Methanol
1.	0	0.685	0.685	0.685	0.685
2.	10	0.277	0.637	0.542	0.643
3.	20	0.177	0.479	0.358	0.482
4.	30	0.158	0.347	0.308	0.351
5.	40	0.132	0.297	0.208	0.213
6.	50	0.129	0.243	0.137	0.178

The data from Table 3 were transformed into a percentage inhibition table for standard ascorbic acid, n-hexane, ethyl acetate, and methanol extracts of *Cleome viscosa* leaves using Microsoft Excel. A graph illustrating the absorbance at 700 nm versus the concentration of standard ascorbic acid, n-hexane, ethyl acetate, and methanol extracts of *Cleome viscosa* leaves was generated. Additionally, a graph depicting the percentage inhibition of standard ascorbic acid relative to concentration was created based on the Table 3 data.

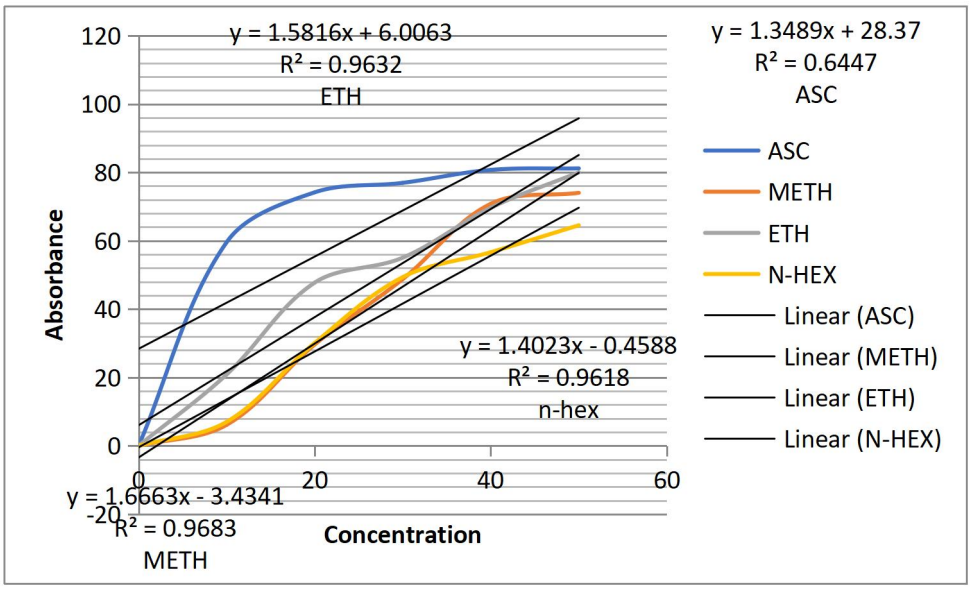

Figure 1: Graph of absorbance at 700nm against concentration of standard ascorbic acid, n-hexane, ethyl acetate, methanol and ethyl acetate extract of *Cleome viscosa* leaves.

The IC50 value of the standard ascorbic acid was calculated from the graph presented (Fig. 1). The graph showed a linear relationship, which was represented by the equation y = 1.34x + 28.37, where y is 50 (the 50% inhibition value) and x represents the IC50 values. Using this

equation, the IC50 value for the standard ascorbic acid was determined. Similarly, the IC50 values for the other test extracts, namely n-hexane, ethyl acetate, and methanol extracts of Cleome viscosa leaves, were calculated in a similar manner and the values are presented in a Table 4.

Table 4: Percentage inhibition of standard ascorbic acid, n-hexane, ethyl acetate, methanol and ethyl acetate extract of *Cleome viscosa* leaves

S/N	Concentration (Ngml)	Standard Ascorbic acid	n-hexane	Ethyl acetate	Methanol
1.	0	0	0	0	0
2.	10	59.562	7.007	20.875	6.131
3.	20	74.161	30.0731	47.737	29.635
4.	30	76.934	49.343	55.036	48.759
5.	40	80.729	56.642	49.635	70.803
6.	50	81.168	64.526	80	74.015

The relationship between the inhibition concentration and the percentage inhibition was plotted using a linear equation, represented by the formula y = 1.4023 x - 0.9618, on a graph. To determine the IC50 value of the n-hexane extract, the value of x was calculated when y was equal to 50, which is the 50% inhibition point. The calculated IC50 value for the n-hexane extract was 52.5 µg/mL. Similarly, the IC50 values for the other test extracts were calculated in a comparable manner, using the linear equation derived from the graph of percentage inhibition plotted using Microsoft Excel. This approach allowed for the determination of the IC50 values for the various extracts tested.

Table 5: The IC50 value of cleome viscose (leaves) extract as compared to standard ascorbic acid.

S/N	Test Sample	IC50 (Nglml)
1.	Ascorbic acid	16.05
2.	n-hexane	52.5
3.	Ethyl acetate	27.8
4.	Methanol	32.07

The results of the IC50 values indicated that the standard ascorbic acid exhibited higher antioxidant activities with a value of 16.05 µg/ml. This was followed by the ethyl acetate,

methanol, and n-hexane extracts with 27.8, 32.07, and 52.5 µg/ml, respectively. The results indicate that all the extracts show antioxidant activities, with the ethyl acetate extract exhibiting the highest antioxidant activity among the Cleome viscose leaf extracts. Generally, the plant can be regarded as a good source of antioxidants. The results have shown that *Cleome viscosa* extracts exhibit potent antioxidant activity, which may be attributed to the presence of phenolic compounds and other antioxidant phytochemicals.

Antibacterial activities

In this study, the antibacterial effects of standard drugs and the methanol, ethyl acetate, and n-hexane extracts of Cleome viscosa were investigated against isolates of one Gram-positive bacterium (*Staphylococcus aureus)* and one Gram-negative bacteria (Escherichia coli) [23]. The results of the antibacterial activity of the standard drug (Erythromycin) and the methanol, ethyl acetate, and n-hexane extracts of the leaves are presented in the Table 6. The results of the *in vitro* antibiotic sensitivity test showed that the isolates of *Salmonella typhi* and *Staphylococcus aureus* were generally susceptible to the test compounds. The studies have demonstrated the effectiveness of *Cleome viscosa* extracts in inhibiting the growth of bacteria, such as Salmonella typhi and Staphylococcus aureus. The findings suggest that *Cleome viscosa* could be a promising source of natural antibacterial agents with potential applications in the treatment of bacterial infections.

Table 6: Zones of inhibition diameter of cleome viscose extracts.

Sample	Organism	Concentration of extract in (mglml)	Zone of inhibition (mm/dm)
Ethyl Acetate	E.coli	200	32
		100	15
		50	10
		25	0
		12.5	0
	S.aureus	200	43
		100	37
		50	25
		25	15
		12.5	0
N-Hexane	E. Coli	200	0

		100	0
		50	0
		25	0
		12.5	0
	S. Aureus	200	12
		100	9
		50	0
		25	0
		12.5	0
Methanol	E. Coli	200	55
		100	40
		50	32
		25	23
		12.5	17
	S. Aureaus	200	47
		100	32
		50	19
		25	11
		12.5	8

CONCLUSIONS

The phytochemical screening, antioxidant, and antibacterial activity of *Cleome viscosa* leaves provided valuable insights into the medicinal potential of this plant. The results have shown that *Cleome viscosa* leaves contain a variety of bioactive compounds, which are known to possess diverse pharmacological activities. The antioxidant activity evaluation revealed that *Cleome viscosa* extracts exhibit potent antioxidant activity, which may be attributed to the presence of phenolic compounds and other antioxidant phytochemicals. This suggests that *Cleome viscosa* could be a promising source of natural antioxidants with potential applications in the treatment of oxidative stress-related diseases. The antibacterial activity evaluation demonstrated that *Cleome viscosa* extracts are effective against a wide range of pathogenic bacteria, including both Gram-positive and Gram-negative strains. This indicates that *Cleome viscosa* could be a potential source of natural antibacterial agents with applications in the treatment of bacterial infections.

The phytochemical screening in this study was limited to the detection of major classes of compounds. However, other bioactive compounds might have been overlooked. The phytochemical composition of *cleome viscosa* can vary significantly depending on geographical location, climate and soil conditions. This study only used sample from specific region which may not represent the plant's global phytochemical profile. The study also focused on demonstrating the presence and general activity of phytochemicals, antimicrobial and antioxidant properties. However, the underlying mechanisms of actionfor these bioactivities were not explored.

Conducting comparative studies on *Cleome viscosa* samples from different geographical regions and environmental conditions can help in understanding the variability in phytochemical content and bioactivity. This would also assist in identifying the optimal growing conditions for maximum therapeutic benefit. Research should aim to elucidate the molecular mechanisms underlying the antimicrobial and antioxidant activities of *Cleome viscosa.* This can involve studying the interaction of the plant's bioactive compounds with microbial cells and oxidative stress pathways.

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