Antimicrobial and antioxidative properties of *Cleome viscose* leaf extract

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Research Article

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Abstract

The annual, sticky plant *Cleome viscose* Linn. (Capparidaceae), sometimes known as "Jakhya," thrives as a weed across India's plains and throughout the tropics. The entire plant and its components are extensively used in traditional and folklore medicine (seeds, leaves, roots and barks). We evaluated the antimicrobial and antioxidant capacity of *Cleome viscose* leaf extract.

Methods: Methanol was used to extract the leaves. Extract was analysed qualitatively and quantitatively. Antioxidative properties of extract was assayed using DPPH, FRAP, ABTS H_2O_2 scavenging assay. Correlation between antioxidant activity, phenolic content and flavonoid content was estimated. Antimicrobial activity of *Cleome viscose* Linn leaf methanolic extract was estimated against *Staphylococcus aureus, Vibrio cholera, Salmonella Typhimurium*.

Result: The methanolic extract of *Cleome viscose* leaves showed good antioxidant and antimicrobial potential. The extract was having alkaloids, phenolics, flavonoids and tannins as major phytochemicals. The extract showed good ABTS scavenging activity and moderate DPPH scavenging activity. The TPC strongly while TFC moderately correlated with antioxidant activity of the extract.

Conclusion: Methanolic extract of *Cleome viscose* leaves demonstrated significant antioxidant and antibacterial activity. The phytoconstituents found in plant leaves, primarily phenolics, flavonoids, alkaloids, and tannins, are thought to be responsible for the plant's antioxidant and antimicrobial properties, making it a therapeutically important plant with the potential to be used as a drug as an antioxidant or against microbial diseases.

Introduction

Cleome L. is a big genus in the Capparidaceae family with 200 species. This genus is traditionally been used in ethnomedicine for its indigenous medicinal properties, which include anthelmintic [1], carminative [2], anticonvulsant [3], antidiarrheal [4], antibacterial [5] and wound-healing [6] properties. *Cleome viscose* Linn, *Cleome rutidosperma* DC, *Cleome arabica* L, *Cleome enrichment*, and *Cleome gynandra* L have been used in traditional medicine to treat diarrhoea, fever, scabies, inflammation, rheumatic pains, blood problems, uterine complaints, malaria, diabetic hyperglycemia, paralysis, anthelmintic problems, epilepsy, convulsion [7,8]. According to traditional usage, the leaves, roots, and seeds of the *Cleome* genus are employed as stimulants, antiscorbutics, anthelmintics, rubefacients, vesicants, and carminatives [9].

Cleome genus is found to have alkaloids, phenolic compounds, fatty acids, flavonoids, terpernes, minerals, vitamins, coumarino-lignan, gallotannins, saponins, kaempferol, hexacosanol, iridoid and proteins. Various compounds have been isolated from different part of *Cleome viscose*. Seeds of *Cleome viscose* is very nutritive and contain 26% oil consisting of mainly Linoleic acid, palmitic, stearic, oleic, and linolenic [10] five fatty acids, seven amino acids, and sucrose [11].

Cleome viscose (CV) Linn. (Capparidaceae) is a weed found across the world's tropics and Indian plains. The plant is a sticky annual with a pungent odour and glandular and simple hairs on its surface. It is branching and grows to a height of 30–90 cm. The leaves are 3–5 foliated, obovate, and obtuse, and they become shorter as you rise. The yellow blooms are axillary and grow in a loose raceme. When mature, the seeds are somewhat transversely striate, subglobose, and brownish-black. The fruits are crushed capsules that are hairy all around. Wild mustard, dog mustard, and sticky *cleome* are all names for *Cleome viscose*. Hul-Hul, Pashugandha, Pivala tilvana, Kanphuti, Jakhya, and Talwani are some of the local names for the plant in India. According to ethnobotanical studies and ancient medical systems such as Ayurveda and Unani, the plant is a popular cure for a number of diseases [12]. Following folk claims of cures for numerous ailments, the plant's potential as a medicinal agent has been scientifically investigated. The current study offers a complete description of traditional usage as ethnobotanical, phytochemical, and pharmacological research on the plant, which may explain the herb's varied significance.

Biological activity of *Cleome viscose* extracts:

Cleome viscose has been scientifically evaluated for a variety of pharmacological actions, including anthelmintic, antibacterial, analgesic, antiinflammatory, immunomodulatory, antipyretic, psychopharmacological, antidiarrheal, and hepatoprotective properties. Table summarises the many pharmacological actions documented for the plant.

Antimicrobial activity

Harpreet *et al*., investigated antimicrobial activity of *Cleome viscose*. Various extracts of *Cleome viscose* Linn. seeds were used in the antibacterial investigation. A total of eight microbial species were employed in this study. These eight microbial species were tested using chloroform, patroleum ether, ethyl acetate aqueous and ethanol extracts of *Cleome viscose* Linn. seeds. Ear discomfort sufferers' pus samples were also utilised in the study. All the tested samples exhibited antimicrobial activities. The zones of inhibition were discovered to be

10 mm to 17 mm wide. Extracts' minimum inhibitory concentrations (MIC) against microorganisms were also measured, ranging from 0.1 to 0.45 [13].

Antioxidant activity

Alcoholic extract of the leaf of *Cleome viscose s*howed high antioxidant potentials. At a concentration of 0.1 mg/ml it can scavenge 43.33% of DPPH free radicals [14]. Various research on the antioxidant potential of the *Cleome viscose* leaf extract confirmed their therapeutic importance as antioxidant in various diseases.

Materials And Methods

Collection of plant sample

The leaves of *Cleome viscose* were obtained from the local market in Uttarakhand. The plants were authenticated by Dr. Anamika, Department of Botany, Vardhman college Bijnor, The Department of Botany identified plant material. The leaves were cleaned and dried in the shade for two to four weeks. The powdered dried leaves were kept in an airtight container. In a nutshell, 5 g powder was sequentially Soxhlet extracted for 6-7 hours, with 3-4 cycles each hour with methanol solvent. After roughly every four cycles, the extraction was verified by taking some extract from the extractor syphon tube, spotting it on a TLC plate, and seeing it in an iodine chamber. The absence of a spot signifies that extraction has been completed.

Phytochemical analysis of extract:

Plants were qualitatively tested for phytoconstituents. Alkaloid [15], flavonoid [16], Phenolic compounds [17], tannins [18], cardiac glycosides [19], steroids (Liebermann-Burchard reaction) [20], saponins [21], and carbohydrate tests: molisch, fehling's, and benedict's tests were performed on extracts.

Total Flavonoid content:

The total flavonoid was estimated using the aluminium chloride colorimetric technique [22]. In a nutshell, 0.5 ml of 1.2 % aluminium chloride and 0.5 ml 1M potassium acetate were added to 0.1 ml of leaf extract. Methanol was used to dilute the reaction mixture to 3 ml, and it was incubated at room temperature for 30 minutes. The absorbance was measured at 415 nm. Blank was set by adding all reagents except the extracts. Rutin standards ranging from 0.1 mg/ml to 1 mg/ml were used to create a standard curve.

Total Phenolic content:

TPC was estimated by modified protocol of Singleton *et al*., 1999 [23]. Gallic acid (GA) was used as the standard, while pure water was used as the blank sample. Briefly 0.5 mL distilled water, 2.5 mL Folin-Ciocalteu reagent, and 0.05 mL extract were mixed together. For 5 minutes, the solution was allowed to react. After that, 2 mL of a 7 % Na_2CO_3 solution was added, the mixture was stirred, and the volumetric flask was filled to the necessary capacity with distilled water. After 90 minutes of incubation in the dark and at room temperature (23 °C), the solution absorbance was measured at 765 nm with a spectrophotometer. Gallic acid standards ranging from 0.01 mg/ml to 0.05 mg/ml were used to create a standard curve.

Antioxidant assay of crude extract (ABTS):

The ABTS test was carried out in accordance with Pellegrini *et al*., 1958 [24]. In brief, 7mM ABTS in water was combined with 2.45mM potassium persulphate (1:1) and incubated in the dark for 12-16 hours. After that, the mixture is diluted to achieve an absorbance of 0.7 at 734 nm. Extract was diluted in different concentrations i.e. 0.1875, 0.3125, 0.4375 and 0.5625 mg/ml and 3 ml of reagent were mixed together and incubated in the dark for 10 minutes. The absorbance was measured at 734 nm. The control was made by measuring the absorbance of ABTS and methanol. Ascorbic acid standard curve was constructed concurrently with standards dilution ranges of 0.1875, 0.3125, 0.4375 and 0.5625 mg/ml. The $ABTS^+$ scavenging effect (percent) was estimated using the equation below.

$$ABTS\ ions\ scavenging\ effect(\%) = \left(\frac{Ab - Aa}{Ab}\right)100$$

Where:

Ab=Ab. Of ABTS+ Methanol

Aa= ABTS+ sample/standard

CEAC (Vitamin C Equivalents Antioxidant Capacity) or ascorbic acid content of all the extract was estimated by standard curve of ABTS+ scavenging effect (%) linear curve equation.

Antioxidant assay of crude extract (DPPH):

The DPPH free radical scavenging activity of the crude extract, based on the scavenging of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical was determined [25]. Samples were diluted to different concentration i.e. 0.1875, 0.3125, 0.4375 and 0.5625 mg/ml. Reaction mixture were set by adding 1.0 ml of 0.1 mM of DPPH in each dilution. The mixture was incubated in the dark for 30 min at room temperature. Degree of inhibition of DPPH by monitoring the decrease in absorbance measured at 517 nm. Ascorbic acid was used as positive control. Standard curve of ascorbic acid was prepared by diluting the ascorbic acid in same concentrations as that of samples. Radical scavenging activity was expressed as inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\%Inhibition = \left(\frac{Ao - At}{Ao}\right) 100$$

where Ao was the absorbance of control (blank without sample) and at was the absorbance in presence of sample. All the tests were performed in triplicate and graph was plotted with mean values.

Antioxidant assay of crude extract (FRAP assay):

The FRAP test was carried out basically as previously described [26]. The samples were diluted to a concentration of 1 mg/ml. An aliquot (100 µl) of the correctly diluted extract was added to 3 ml of the standard reaction solution, and absorbance at 593 nm was measured at 0 time and after 6 minutes of standing at room temperature. The measurement was made three times. The standard curve in the 200-1000 M range was created using $FeSO_4$. The FRAP values for both the standard (ascorbic acid 1 mg/ml)) and the samples were computed and represented as µM Fe [II]/gm dry wt.

Antioxidant assay of crude extract (H_2O_2 assay):

H_2O_2 scavenging activity was determined as previously described [27]. A 0.6 ml aliquot of 40 mM H_2O_2 solution was combined with 0.1 ml of diluted crude extract. 2.4 ml of phosphate buffer (0.1 M, pH 7.4) was added to the mixture, which was rapidly agitated and incubated at room temperature for 10 minutes. The absorbance of the reaction mixture was then measured at 230 nm. Positive control was ascorbic acid. The scavenging activity of H_2O_2 was computed as follows:

$$H2O2\ \%\ inhibition = \left(\frac{A1 - A2}{A1}\right) * 100$$

Where A1 is the ascorbic acid absorbance and A2 is the sample absorbance.

Antimicrobial activity of Crude extract:

The bacteria *Salmonella Typhi* Chi–603 (MTCC 3224), *Vibrio cholera* 569B (Classical 01) (MTCC 3904) were procured from MTCC, IMTECH, chandigarh while *Staphylococcus aureus* (MCC 2408) was purchased from NCCS, NCMR, Pune. The antibacterial activity assay was performed using disc diffusion techniques (CLSI 2012 standard).

Disk-diffusion method:

Plant extracts were loaded on filter paper discs of 6 mm diameter with different dilutions (200 mg/ml, 100 mg/ml, 75 mg/ml, and 50 mg/ml) and placed on plates with MHA for bacterial strains with $2x10^8$ microorganisms per plate. Gentamycin was diluted at 2, 5, 7, and 10 µg/ml concentrations and loaded in the same manner as the sample. Bacterial incubation periods at 37°C and 32°C ranged from 16 to 24 hours. The zone of inhibitions (zones surrounding the discs) was measured to determine antimicrobial activity and minimum inhibitory concentration (MIC). Gentamycin served as the positive control, while dimethyl sulfoxide served as the negative control [28].

Statistical analysis:

Data were presented as mean SD for at least three separate measurements in triplicate for each experimental point. The IC50 values in antioxidant experiments were calculated using the GraphPad Prism version 8.0 software.

Results

The presence of eight phytochemicals termed Alkaloids, Flavonoid, tannins, saponin, cardiac glycosides, phenolic, steroids, and carbohydrates was determined in a methanolic extract of *Cleome viscose* L. It was discovered that the extract lacks saponins and steroids while containing a significant quantity of flavonoid and alkaloid (as determined by Mayer's test) and a moderate quantity of phenols, glycosides, and tannins (Table 1). Secondary metabolites were more abundant in *Cleome viscose.*

Table 1. Qualitative phytochemical screening of *Cleome viscose* leave extract.

Sample	Alkaloid			Flavonoid	Phenol	Glycosides	Tannins	Carbohydrate			Saponins	steroids
	Mayer's test	Dragendorff's test	Wagner test					Molisch	Fehling's	Benedicts		
CVM	++	-	+	++	+	+	+	-	+	-	-	-

CVM refer to *Cleome viscose* methanolic extract. The symbols: ++, + and – refer to appreciable amounts, trace and absent amounts, respectively.

Total phenolic content (TPC) was calculated using linear curve equation Y=12.207x-0.0739, R^2=0.9865 and was obtained by plotting graph between absorbances at 734 nm and concentrations of gallic acid. The total phenolic content of *Cleome viscose* leaf methanolic extract is 4.6 gGAE/100 gm (Table 2) of *Cleome viscose* leaf, which is lower than Gupta *et al*., 2011[29], but much higher than Govindan *et al*., 2018 [30]. According to Gupta *et al*., the phenolic content of leaf extract is 6.63 gGAE/100 gm of sample. It might be due to a variation in the solvent used to prepare the crude extract. They used 70% methanol instead of pure solvent that have isolated water-soluble phenolic compounds also. Govindan assessed the total phenolic content to be 1.523 gGAE/100 gm (Table 2) of sample using pure methanol. Total flavonoid content (TFC) was calculated using the equation Y=1.7222x-0.1846, R^2=0.9885 obtained after plotting graph between concentration of rutin and their absorbances at 415nm. Use of 70% methanol or pure methanol do not yield high total flavonoid content [Gupta, Govindan]. The total flavonoid content of leaf methanolic extract of *Cleome viscose* is found to be 4.25 g RE/100 gm of leaf sample. Much higher than the previous findings [29, 30].

Table 2: Quantitative analysis (TPC and TFC) of *Cleome viscose* leaf extract

Plant	Part used/solvent	Total phenolic content (g GAE/100 gm)	Total Flavonoid content (g rutin/100 gm)
Cleome viscose	Leaves/methanol	4.60±0.156	4.25±0.305

values are means of three replicates of each parameter ± standard deviation.

Antioxidative activity of Clemoe viscosa leaf extract:

Secondary metabolites especially phenolic compounds and flavonoids stabilizes lipid oxidation and are directly or indirectly correlated with the antioxidant activity. Antioxidant activities of these metabolites including Flavonoids and phenolic compounds are due to their capacity to reduce or scavenge free radicals [31]. *Cleome viscose* leaf extract can scavenge ABTS and DPPH free radical by 98.6 and 57.3 % respectively at a concentration of 562 µg/ml (Table3). Data obtained are according to findings of Pillai & Bindu, 2013 [32]. *Cleome viscose* leaf extract is more efficient toward scavenging ABTS than DPPH. Minimum Inhibitory concentration at which the extract is inhibiting the 50% of free radical i.e., IC50 is found to be 260 µg/ml and 424 µg/ml for ABTS and DPPH respectively and was compared to the IC50 for ascorbic acid which is 147 and 129 µg/ml for ABTS and DPPH respectively (Table 4). IC50 concentration for DPPH is very close to IC50 value (483 µg/ml) calculated from Pillai & Bindu, 2013 data [32]. IC50 was calculated using the plot against % inhibition and concentrations of extract and using the standard curve equation Y=121.84x+32.089, R^2=0.9916; Y=178.4x+3.5678, R^2=0.9299 respectively for ABTS and DPPH. Lower the IC50 concentration, lower will be its concentration to scavenge 50% of the free radicals and will be more efficient. IC50 concentration of the extract is higher than that of the ascorbic acid and is not effective than the ascorbic acid. Antioxidant activity of purified and isolated compound will

have better efficiency. Ascorbic acid equivalent antioxidant capacity (AEAC) was found to be very high against ABTS than DPPH. The stronger the antioxidant activity, the higher the AEAC value.

Table 3: Antioxidant assays: ABTS and DPPH assay of *Cleome viscose* leaf extract

Sample	Concentration (mg/ml)	ABTS scavenging activity (%)	DPPH scavenging activity (%)
Cleome viscose Leaf methanolic extract	0.1875	29.53±0.129	30.65±0.154
	0.3125	68.95±0.169	37.39±0.256
	0.4375	84.8±0.129	46.42±0.673
	0.5625	98.58±0.007	57.31±0.934

values are means of three replicates of each parameter ± standard deviation.

Table 4: Ascorbic acid equivalent and Half-maximal inhibitory concentration (IC50) of extract

Samples		Ascorbic acid equivalent antioxidant capacity (AEAC)	IC50 (mg/ml)
Cleome viscose Leaf methanolic extract	ABTS assay	56481.41	0.26
	DPPH assay	27399.4	0.474
Ascorbic acid (1 mg/ml)	ABTS assay	-	0.147
	DPPH assay	-	0.129

Ferric reducing antioxidant power or FRAP is based on Ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex reduction to Ferrous- tripyridyltriazine (Fe^{2+}-TPTZ), blue coloured compound at low pH. Extract exhibiting positive FRAP assay is an electron donor that stops the oxidation reactions by reducing Ferric to ferrous and making the complex stable. The reducing power of *Cleome viscose* leaf extract is 800 µM Fe II/ g of sample (Table 5) and was calculated from linear curve equation, Y=0.0007x, R^2=0.9805 from the plot between $FeSO_4$ Concentrations (µM) and absorbances at 593 nm. The FRAP value of leaf extract is near about the FRAP value (978 µM Fe II/g of sample) of ascorbic acid at a same concentration which make it a potent antioxidant. H_2O_2 % scavenging activity of the extract is found to be 64 at a concentration of 1 mg/ml which is also close to scavenging activity of ascorbic acid at same concentration.

Table 5: Ferric Reducing Antioxidant Power (FRAP) Assay and H_2O_2 scavenging activity of extract

	FRAP value (µM Fe (II)/g dry wt.)	H_2O_2 % scavenging activity
Cleome viscose Leaf methanolic extract (1 mg/ml)	800	64.44±0.002
Ascorbic acid (1 mg/ml)	978	70.32±0.005

values are means of three replicates of each parameter ± standard deviation.

Correlation between Antioxidant capacity, Total phenolic content and Total flavonoid content

Secondary metabolites especially phenol and flavonoids are directly correlated with the antioxidant potentials of the extract [33]. TPC and antioxidant activity were significantly correlated, with R^2=0.7779 and R^2=0.9382 for ABTS and DPPH assays, respectively (Fig 1). While flavonoid is not significantly connected with antioxidant assay, as seen by R^2=0.4492 and R^2=0.7377 for ABTS and DPPH assays, respectively (Fig 1). These results predicted the direct and positive effect of phenolic content of extract on their antioxidant activity.

Antimicrobial activity of Extract:

Methanolic extract of *Cleome viscose* leaf showed significant antimicrobial activity against three chosen microbes namely, *Staphylococcus aureus*, *Salmonella Typhi* and *Vibrio cholerae* (Table 6) with the zone of inhibition from 7 to 14.6 mm. MIC value for *Cleome viscose* leaf extract against *Staphylococcus aureus*, *Salmonella Typhi* and *Vibrio cholera* is found to be 60.2, 43.4 and 48.2 mg/ml respectively (Fig 2) and was compared with gentamycin whose MIC was found to be 1.0, 1.9 and 2.4 µg/ml against *Staphylococcus aureus*, *Salmonella Typhi* and *Vibrio cholera* respectively.

Table 6: Zone of inhibition of *Cleome viscose* leaf extract at different concentrations against *Staphylococcus aureus, Salmonella Typhi* and *Vibrio cholera*

Cleome viscose extract (mg/ml)	*Staphylococcus aureus*	*Salmonella Typhi*	*Vibrio cholera*
200	10.8±0.23	14.6±0.21	14.42±0.17
100	8±0.1	12±0.28	11±0.16
75	7±0.8	11±0.18	10±0.23
50	7±0.5	9±0.13	9±0.21
Gentamycin (µg/ml)	***Staphylococcus aureus***	***Salmonella Typhi***	***Vibrio cholera***
10	23±0.3	24±0.34	10±0.07
7	22±0.26	19±0.25	9±0.11
5	17±0.67	16±0.12	7±0.09
2	14±0.54	12±0.32	7±0.03

Data are represented as Mean±SD

MIC of gentamycin is found to be1.0 µg/ml against *Staphylococcus aureus* and is in the range of 0.25-2.5 µg/ml reported by Sorensen & Sorensen, 1993 against different strains of *Staphylococcus aureus* including the control ATCC strain and strains isolated from wound [34]. MIC of gentamycin against *Salmonella Typhi* and *Vibrio cholera* is 1.9 and 2.4 µg/ml respectively which is near to 2 µg/ml [35] and within the range of 0.029-2.5 µg/ml [36] for *Salmonella Typhi* and within the range of 1-16 µg/ml [37]. Lower the MIC more efficient the extract is against the microbes. *Cleome viscose* leaf extract is found to have potent antimicrobial activity against the all the selected microorganism. The leaf extract of *Cleome viscose* showed the greatest inhibitory effects against *Salmonella Typhi* (MIC 43.3 mg/ml) and the least against *Staphylococcus aureus* (MIC 60.24 mg/ml) (Fig 2), however its activity in bacteria was significantly lower than gentamycin. That antimicrobial activity is due to the secondary metabolites mainly tannins and alkaloid [38]. If the bioactive is purified, the MIC of the extract can be increased even more.

Conclusion

The study found that the methanolic extract of *Cleome viscose* leaves had strong antioxidant capacity as well as substantial antibacterial activity. The phytoconstituents present in the plant leaves, primarily phenolics, flavonoids, alkaloids, and tannins, may be responsible for their antioxidant and antimicrobial activities, making it a therapeutically important plant with the potential to be used as a drug as an antioxidant or against microbial diseases. To gain a better knowledge of the method of action, bioactive substances can be isolated, purified, and characterised. The study used just three pathogenic bacteria, but it may be expanded by testing the purified compounds against a broader spectrum of Gram positive, Gram negative, pathogenic, and pathogenic microorganisms. The antifungal efficacy of the leaf extract, as well as isolated components, may be investigated further against a variety of fungus species.

Declarations

Conflict of interest

The authors state that they have no financial or other conflicts of interest.

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Figures

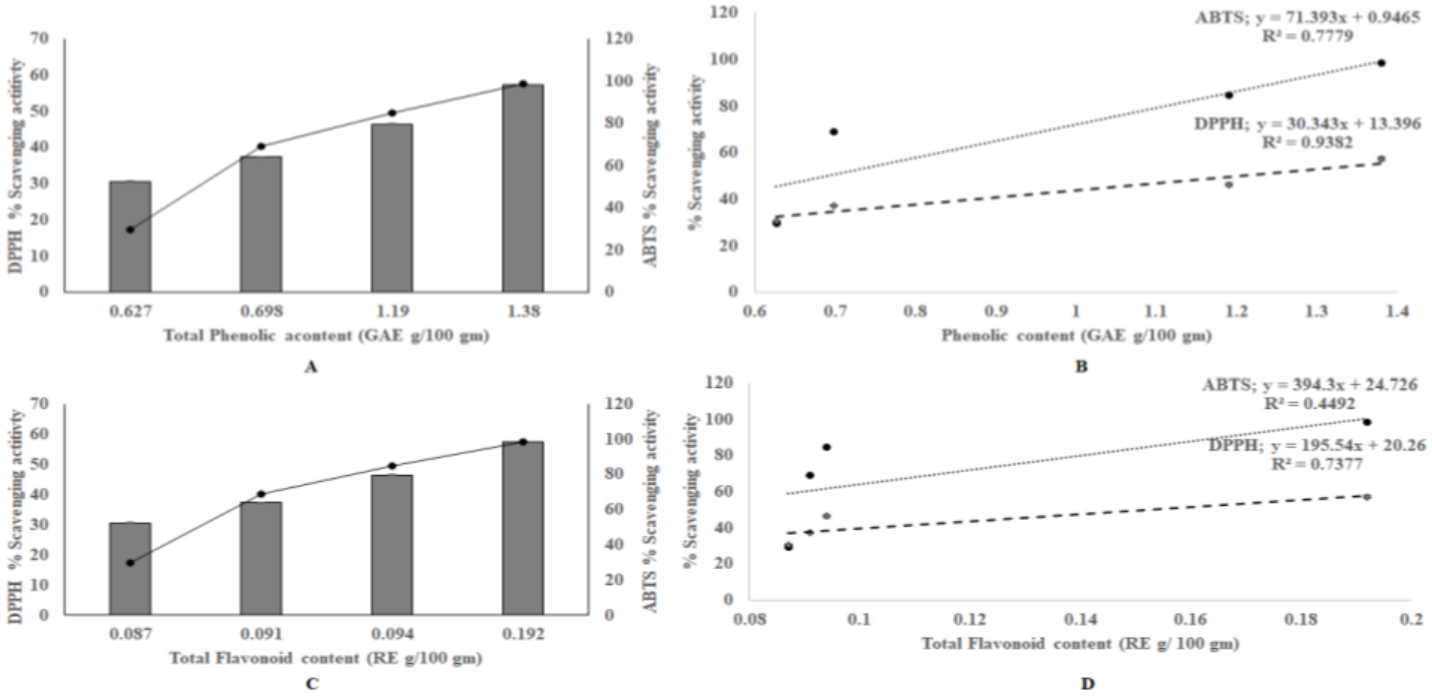

Figure 1

Correlation between Antioxidant assay, TPC and TFC of leaf extract, A: Between TPC, DPPH and ABTS % scavenging activity; B: Standard curve between TPC, DPPH and ABTS % scavenging activity; C: Between TFC, DPPH and ABTS % scavenging activity; D: Standard curve between TFC, DPPH and ABTS % scavenging activity.

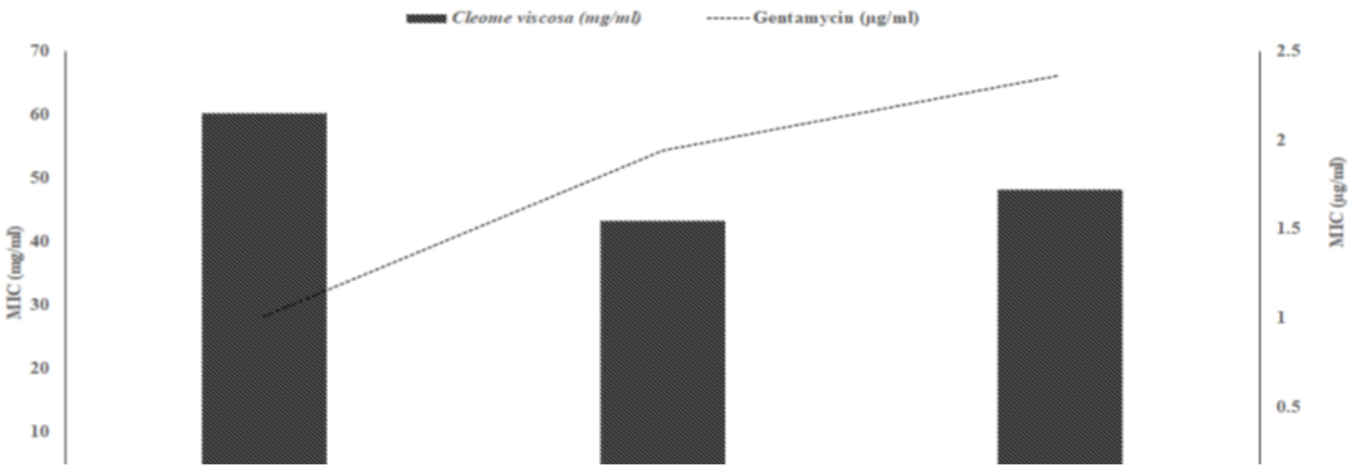

Figure 2

Minimum inhibitory concentrations of *Cleome viscose* leaf methanolic extract (mg/ml) and positive control Gentamycin (µg/ml)

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