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EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 1
609-616
Year: 2018

PHYTOCHEMICAL ANALYSIS AND *IN VITRO* ANTIOXIDANT ACTIVITIES OF *CLEOME VISCOSA* L.

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Article Received on 15/11/2017 **Article Revised on 06/12/2017** **Article Accepted on 27/12/2017**

ABSTRACT
Cleome viscosa L. (capparidaceae) commonly known as weed is a popular traditional medicine. It is a well-known medicinal plant, which possessed many bioactive compounds of high therapeutic value.The present study; the aerial parts of *C. viscosa* were sequentially extracted with hexane, chloroform, ethyl acetate and methanol. Among the solvent extracts, methanol showed rich in phytochemical profile and antioxidant activity. Phytochemicals such as alkaloids (15.42 mg/g) present in higher amount followed by phenols (15.23 mg/g); fatty acids (5.06 mg/g); flavonoids (14.52 mg/g); tannins (7.38 mg/g) carbohydrates (5.37 mg/g). And then the methanolic extract of *C.viscosa* showed the significant antioxidant activity such as DPPH, $ABTS^{\bullet+}$, ferrous ion chelating and FRAP assays. Based on this substantial activity of methanolic extract of *C. viscosa* could be the best source for the isolation of anticancer molecules.

KEYWORDS: *Cleome viscosa*, Phytochemical screening, Antioxidant, GC-MS.

1. INTRODUCTION

Cleome viscosa L. (Cleomaceae formerly known as Capparidaceae) is a rainy season, self-compatible and sexually reproducing herb. Commonly known as "wild or dog mustard," is an annual, sticky herb found as a common weed all over the plains of India and throughout the tropics of the World (Tripti Joshi *et al.*, 2015). It is an annual uncultivated weed with yellow flowers and strong pungent odour. The leaves of the plant are rubefacient, vesicant and sudorific, the seeds are small, dark brown or black and granular (Wael Abdullah *et al.*, 2016, Ravi Kant Upadhyay, 2015). *C. viscosa* is well documented in Indian and Chinese traditional systems of medicine and as well as like in ayurveda and unani as a folklore medicine for the cure of various ailments of human beings (Ravindra and Mali, 2010). In Indian traditional medicine, found throughout the greater part of India, often in woodlands, fallow lands, roadsides, refuse heaps, waste grounds and agricultural land and waste places (Asolkar *et al.*, 1965-1981; Shveta Saroop and Veenu Kaul, 2014). The leaves of the plant are rubefacient, vesicant and sudorific. The seeds are small, dark brown or black and granular. They are reported to have rubefacient, vesicant and anthelminthic properties. The seeds are occasionally used as a condiment in curries (Parimala Devi *et al*., 2003).In this study, an attempt has been made to screen the qualitative and quantitative phytochemicals analysis of crude extract of aerial parts *C. viscosa* using different organic solvents. To assess their *in vitro* antioxidant DPPH, $ABTS^{\bullet+}$, Fe^{3+} reducing power and ferrous ion chelating assays were carried out.

2. MATERIALS AND METHODS

2.1. Collection of plant material

The disease free and healthy aerial parts of *C. viscosa* were collected from Guindy Campus of University of Madras during the month of August to October. The aerial parts of *C. viscosa* were washed twice with tap water and finally with distilled water to remove soil and dust. The plant parts were shade dried for 10 days, pulverized (100 g) in a mechanical grinder and stored in an air tight container for further use. Taxonomic identification of the plant was made by Prof. K. Murugesan, CAS in Botany, University of Madras. The voucher specimen was deposited in our laboratory for future reference.

2.2.1. Preparation of plant extracts

The shade dried powered aerial parts of *C. viscosa* (100 g) were sequentially extracted with different organic solvents such as hexane, chloroform, ethyl acetate and methanol with increasing polarity by using Soxhlet extractor. The extraction procedure was repeated until the solvent becomes colourless in the thimble, after which it was filtered through Whatman No.1. filter paper and the solvent was evaporated completely by using a rotary evaporator (Lark Rotary Evaporator, Model RE 100-

Pro).The same procedure was followed for other solvent extracts (Hossain and Nagooru, 2011).

2.2.2. Percentage yield of different solvent extracts of *C. viscosa*

It is the amount of total extract obtained from the calculated mass of plant material. The extraction process was repeated thrice and total yield of extracts was recorded and tabulated as follows;

$$\text{Yield (\%)} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

2.2.3. Preliminary phytochemical screening of different extracts of *C. viscosa*

Screening for the presence of phytochemicals from aerial parts of *C. viscosa* was carried out using standard methods (Trease and Evans, 1989; Harborne, 1998).

2.2.4. Gas Chromatography-Mass Spectroscopy (GC-MS)

Gas chromatography (GC) is used to separate volatile compounds in a mixture. The separated compounds could be identified and quantified. To achieve the identification of different compounds, three steps can be distinguished in a GC-MS system such as injection, separation and detection. Depending on the sample (gas, liquid or solid), compounds in a mixture need to be volatilized or extracted from the matrix by one of the injection techniques. After injection of a mixture, separation is achieved in the capillary column. Many different detectors can be used for detection of the separated compounds. Mass spectrometer (MS) combines high sensitivity with the unique property of being able to determine the molecular composition. The fragmentation pattern measured is characteristic for each molecule, making identification possible.

GC-MS analysis of the methanolic extract of *C. viscosa* was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 mL/min, and an injection volume of 2 µL was employed (a split ratio of 10:1). The injector temperature was maintained at 250ºC, the ion-source temperature was 200ºC, the oven temperature was programmed from 110ºC (isothermal for 2 min), with an increase of 10ºC/min to 200ºC, then 5ºC/min to 280ºC, ending with a 9 min isothermal at 280ºC. Mass spectra were taken at 70 eV a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC-MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

2.2.5. Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more patterns, the spectrum of the component was compared with the spectrum of the known components stored in the library. The chemical name, molecular formula, molecular weight and structure of the component of the test material were identified.

2.3. Evaluation of *in vitro* antioxidant and radical scavenging activities

2.3.1. DPPH free radical scavenging assay

The antioxidant activity of different extracts of aerial parts of *C. viscosa* was measured on the basis of the radical scavenging activity of stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical by the method Brand-Williams *et al*., 1995. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentration (20-120 µg/mL) of different solvent extract of *C. viscosa*. The mixture was then allowed to stand for 30 min incubation in dark. Ascorbic acid was used as the reference standard. One mL methanol and 1 mL DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517 nm. The percentage of inhibition was calculated using the following formula:

$$\%\ \text{of DPPH}^{\cdot}\ \text{radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

The analysis was performed in triplicates. The sample concentration providing 50% inhibition (IC_{50}) under assay condition was calculated from the graph of inhibition percentage against sample concentration.

2.3.2. ABTS•+ radical cation scavenging assay

The antioxidant capacity was estimated in terms of the $ABTS^{\bullet+}$ radical cation scavenging activity following the procedure described by Delgado-Andrade *et al*., 2005. $ABTS^{\bullet+}$ was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12-16 hrs before use. The ABTS solution (stable for 2 days) was diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance of 0.70±0.02 at 730 nm. After the addition of different solvent extracts of varying concentrations (10-60 µg/mL) to 1 mL of diluted $ABTS^{\bullet+}$ solution, the absorbance was measured after 10 min. The $ABTS^{\bullet+}$ radical-scavenging activity of the samples was expressed as:

$$\%\ \text{of ABTS}^{\cdot+}\ \text{radical cation inhibition} = \frac{\text{Control-Sample}}{\text{Control}} \times 100$$

2.3.3. Reducing power assay

The reducing power of methanol extract of aerial parts of *C. viscosa* was determined by the slightly modified method of Oyaizu, 1986. One mL of plant extract of different concentrations (20-120 µg/mL) was mixed with phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferric cyanide [$K_3Fe(CN)_6$] (1 mL, 1%). The mixtures were then incubated at 50°C for 20 min. One mL of trichloroacetic acid (10%) was added to each mixture. Then to the mixture 1 mL of $FeCl_3$ (0.1%) was added and the absorbance was measured at 700 nm using spectrophotometer. Ascorbic acid was used as the standard reference.

2.3.4. Ferrous ion chelating assay of *C. viscosa*

The ability of plant extracts and bioactive compounds to chelate the ferrous ion (Fe^{2+}) was investigated according to the method of Dinis *et al.*, 1994. Briefly, 50 µL (2 mM), $FeCl_2$ was added to 1mL of different concentration of samples (20-120 µg/mL). The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine solution. After which the reaction mixture was vigorously shaken and left at room temperature (28±2°C) for 10 min. The absorbance was measured at 562 nm; Na_2EDTA was used as a positive control and percentage inhibition of ferrozine (Fe^{2+}) complex formation was calculated:

$$\text{Ferrous ion chelation}\,(\%) = \frac{\text{control} - \text{sample}}{\text{control}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Percentage yield of different solvent extracts of *C. viscosa*

To determine the yield of bioactive principles present in *C. viscosa* using various solvents extraction was the aim of this study. The colour of the extracts was ranged from light yellow to dark green and their consistency was a paste. The maximum yield was observed in the methanolic extract (5.80 ± 0.18%) followed by ethyl acetate (3.91 ± 0.11%), chloroform (2.28 ± 0.25%) and hexane (1.85 ± 0.24%) (Table 1). Higher yield of bioactive metabolites from methanolic extract, might be due to the fact that methanol could easily penetrate into the cellular membrane and solubilize the intracellular ingredients from the plant cells. Moreover the results indicated plant contained more polar substances than others (Nhat Minh Phan *et al.*, 2016). Analysis of secondary metabolites present in the plant extracts is a daunting task, due to bioactive molecules, relatively low abundance and variability even within the same species.

3.2. Preliminary phytochemical screening of aerial parts of *C. viscosa*

Phytochemical screening of aerial parts of *C. viscosa* was carried out with different organic solvents such as hexane, chloroform, ethyl acetate and methanol. The results showed the presence of various bioactive constituents such as alkaloids, steroids, flavonoids, tannins, phenols, saponins, terpenoids and glycosides. Abundant presence of almost all the phytochemicals were observed in the methanolic extract followed by ethyl acetate, chloroform and hexane (Table 2) Taiwo Elufioye and Joel Onoja, 2016 reported that previously phytochemicals present in the methanolic extract of *C. viscosa* which is more relevant to our findings and supported strongly our results. The methanolic extract contains secondary metabolites at the more amount than the other extracts.

3.3. Quantitative phytochemical analysis of *C. viscosa*

Screening for quantitative phytochemicals was carried out all the solvent extracts. Comparatively, methanolic extract contained higher amount of active metabolites such as flavonoids (14.52 mg/g), phenols (15.23 mg/g), tannins (7.38 mg/g), fatty acids (5.06 mg/g), alkaloids content (3.92 mg/g) and followed by other extracts (Table 3).

Mostly, methanol and ethyl acetate were proved to be the good extractors of the maximum phytoconstituents from the plant (Nhat Minh Phana *et al*., 2016; Tan *et al*., 2017). It revealed that the high polar solvents are responsible for the extraction of higher quantity of phytochemicals. Some studies are showed that aqueous extract of *C.viscosa* having alkaloid, tannins, flavonoids, fixed oil, fats, gum and mucilage, sterols (Packialakshmi and Oviya, 2014). The presence of various phytoconstituents in *C. viscosa* used to cure the several disease in human beings.

3.4. Chromatographic studies of methanolic extract of *C. viscosa*

3.4.1. Thin Layer Chromatographic profile of methanolic extract of *C. viscosa*

Thin Layer Chromatographic (TLC) analysis was performed to analyze various components present in the methanolic extract of *C. viscosa*. The optimized solvent system was hexane: toluene: methanol in the ratio of 1.0:0.8:0.2 and showed six distinct spots on the TLC plate when viewed under the UV light at 254 nm. The retention front (R_f) was determined and calculated as 0.31, 0.34, 0.41, 0.58, 0.65 and 0.73 respectively (Fig. 1).

3.5. Gas Chromatography-Mass Spectral (GC-MS) analysis of methanolic extract of *C. viscosa*

GC-MS is one of the best techniques to identify the constituents based on the peak area, retention time and molecular formulae. It was commonly used to identify volatile compounds, long chain hydrocarbons, branched chain hydrocarbons, alcohols, acids and esters. The present study, GC-MS analysis of methanolic extract of *C. viscosa* showed the presence of six major chemical constituents such as Flavones (12.1), E,E,6,8 Tridecadien-2-ol, acetate (14.17), 11 Hexadecynoic, mether ester (15.95), pentadecanoic acid, 14 methyl–methyl ester (17.13), Phytol (19.02) and Hexadecanoic acid 3,7,11,15 tetramethyl; methyl ester (21.65) the methanolic extract from aerial part of *C. viscosa*. The results revealed that peak area (%), and retention time

(RT) is representing in Table 4 and Fig. 2. This showed the presence of six bioactive phytochemical compounds in the methanolic extract of *C. viscosa*. Mass spectra of the compounds were matched with that of NIST/NBS spectral database. To supportively the same genus of *Cleome gynandra* ethanolic extract was identified the major compounds such as (E)-9-Octadecenoic acid, ethyl ester (21.81%) and Hexadecanoic acid, ethyl ester (11.23) and the six minor compounds such as methyl (2)-5,11,14,17-eicosatetraenoate (10.28%) Diethyl phthalate (7.48%) phenol 1, 3-pentadecyl (5.63%) 1-Hexadecanol, 2-methyl (2.68%) at the GC-MS analysis (Ahmed John and Annadurai, 2015). These compounds are act as highly responsible for their antioxidant and anticancer activities (Joseph *et al*., 2014).

3.6. *In vitro* antioxidant properties of different organic solvent extracts of *C. viscosa*

Plants are the major source of natural antioxidants because the presence of secondary metabolites like polyphenols, flavanoids etc. The antioxidant activity was determined by using antioxidant assays such as radical scavenging, reducing power, hydrogen donors and metal chelators.

3.6.1. DPPH radical scavenging activity of different solvent extracts of *C. viscosa*

The DPPH radical scavenging assay is used for preliminary screening of the antioxidant activity by the mechanism of the proton radical scavenging activity is known to be an important of antioxidants. The *C. viscosa* extracts of hexane, chloroform, ethyl acetate and methanol was evaluated by DPPH radical scavenging activity. Antioxidant activity was observed during the reduction of DPPH radical, while the purple colour change into yellow colour due to proton donating ability of different solvent extracts of C. viscosa and the absorbance was calculated at 517 nm. Comparatively, the maximum radical scavenging activity was found to be methanolic extract with an IC_{50} concentration at 101.25 µg/mL followed by ethyl acetate 105.363 µg/mL; chloroform 115.464 µg/mL and hexane 118.512 µg/mL concentrations (Fig. 3a). But the previous reports found that antioxidant activity of *C. viscosa* methanolic extract showed DPPH radical scavenging capacity at the IC_{50} values of 910 µg/mL (Elufioye and Onoja, 2016). Comparatively our plant exhibited significant activity due to the reason of ecological factors vary from place to place. Moreover, the methanolic extract was found to be the higher activity than the other solvents. It could be suggested that very strong radical scavenging activity and the activity was dose dependent through scavenging ability of free radicals. The ability of compounds present in the extract to donate a hydrogen atom which result is inhibiting the oxidatation (Prakash Chandra Gupta *et al*., 2011).

3.6.2. $ABTS^{\bullet+}$ radical cation scavenging ability of different extract of *C. viscosa*

Various solvent extracts of *C. viscosa* was determined $ABTS^{\bullet+}$ radical scavenging activity and compared with ascorbic acid as standard. The highest $ABTS^{\bullet+}$ radical scavenging activity was found in methanol extract with an IC_{50} value of 51.13 µg/mL followed by ethyl acetate 52.63 µg/mL and chloroform 53.68 µg/mL (Fig. 3b). Whereas, hexane extract is the lowest ability to scavenge $ABTS^{\bullet+}$ cation radical with an IC_{50} value of 60.87 µg/mL and also it can be attributed due to the presence of flavonoids and polyphenols. These differences in correlation between phytochemicals and antioxidant assays could be attributed to the different mechanism of the free radical antioxidant reaction. The extent of reduction or decolourization of reaction mixture is directly proportional to increased concentration. This results that radical scavenging activity of the extracts might be attributed to their strong proton-donating ability.

3.6.3. Reducing power assay of different solvent extracts of *C. viscosa*

The various solvent extracts of *C. viscosa* was determined by the reducing power antioxidant assay. Methanolic extract was found to be maximum reducing potential and all the extracts monitored up to the concentrations of 120 µg/mL. The reduction power of methanolic extract with the maximum absorbance of 0.437 followed by the ethyl acetate with the maximum absorbance 0.425, chloroform with the maximum absorbance 0.403 and hexane with the maximum absorbance 0.396 shown in the Fig. 3c.

3.6.4. Ferrous ion chelating radical scavenging activity of different extracts of *C. viscosa*

The various organic solvent extracts were estimated by measuring the iron-ferrozine complex. The result showed the methanolic extract was maximum chelating activity with an IC_{50} value at 76.10 µg/mL followed by ethyl acetate 92.31 µg/mL, chloroform 107.22 µg/mL. But the minimal ferrous ion chelating activity was observed on hexane extract at 117.34 µg/mL (Fig. 3d).

Table 1. The percentage yield of different solvent extracts of *C. viscosa*

S. No	Solvents	Dried powder (g)	Color and consistency	Yield of extracts (%)
1	Hexane	100	light yellow-powder	1.85±0.24
2	Chloroform	100	dark yellow-paste	2.28±0.25
3	Ethyl acetate	100	light yellow-paste	3.93±0.11
4	Methanol	100	dark green-paste	5.80±0.18

Values are expressed as mean ± standard deviation (n=3)

Table 2. Qualitative phytochemicals screening for aerial parts of *C. viscosa*

Phyto chemicals	Inference				
	Test	Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	Dragondroffs	-	-	-	+
Steroids	Salkowski	+	-	+	++
Flavonoids	Alkaline reagent	-	+++	+++	+++
Tannins	Ferric chloride	-	-	++	+
Phenols	Ferric chloride	+	+	+	+++
Saponins	Froth	-	+	+	++
Terpenoids	Salkowski	++	++	++	+++
Glycosides	Borntrager's	-	++	+	++

+++: Abundant presence ++: Moderate presence
+: Little presence - : Absent

Table 3. Quantitative estimation of phytochemicals from aerial parts of *C. viscosa*

Secondary metabolites	Hexane	Chloroform	Ethyl acetate	Methanol
	(mg/g)			
Alkaloids	---	---	1.58	3.92
Anthroquinones	1.35	0.62	0.04	3.82
Carbohydrates	0.27	2.15	3.86	5.37
Flavonoids	---	1.94	12.36	14.52
Fatty acids	0.25	0.38	0.21	5.06
Glycosides	---	2.32	1.54	3.65
Phenols	1.95	4.61	3.24	15.23
Proteins	0.12	---	7.32	1.35
Saponins	---	1.28	3.21	4.72
Steriods	4.32	0.87	8.25	10.65
Tannins	---	0.14	3.42	7.38
Triterpenoids	1.35	4.65	4.35	5.28

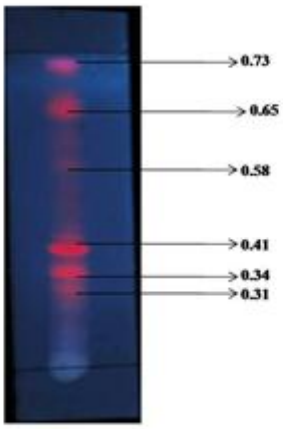

Fig. 1 Thin layer chromatographic profile of methanolic crude extract of *C. viscosa* Solvent system in the ratio of hexane: toluene: methanol (1.0: 0.8:0.2)

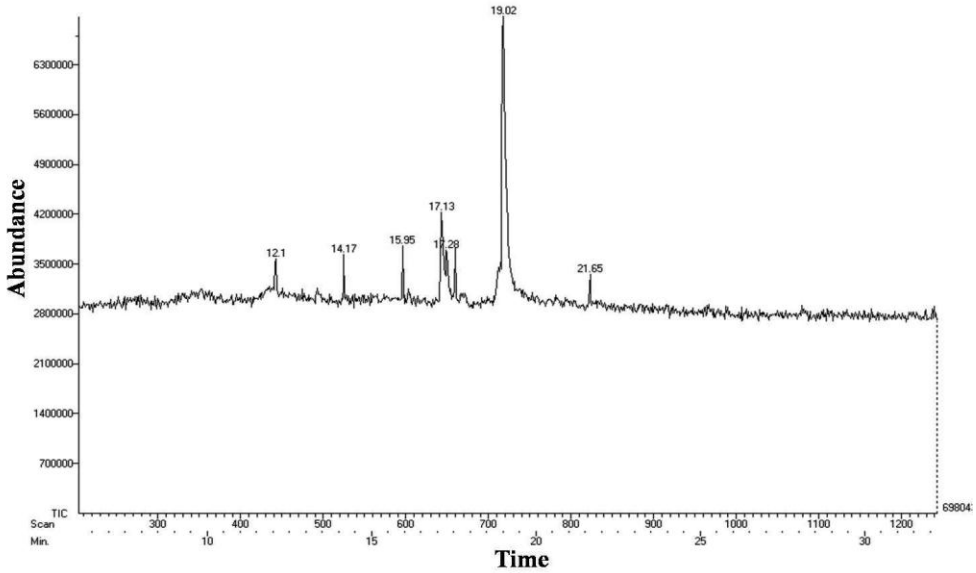

Fig. 2: GC-MS chromatogram of methanolic extract of *C. viscosa*.

Table 4. GC-MS analysis of methanolic crude extract of *C. viscosa*

Peak	Retention Time	area (%)	Name of the compound	Molecular formula	Molecular weight (g/mol)
1	12.1	7.4	Flavone	$C_{15}H_{10}O_2$	222.24
2	14.17	13.2	E,E,6,8 Tridecadien-2-ol , acetate	$C_{16}H_{28}O_2$	252.39
3	15.95	23.8	11 Hexadecynoic , mether ester	$C_{17}H_{32}O_2$	268.435
4	17.13	30	pentadecanoic acid, 14 methyl – methyl ester	$C_{17}H_{34}O_2$	270.4507
5	19.02	54.9	Phytol	$C_{20}H_{40}O$	296.5310
6	21.65	6.7	Hexadecanoic acid 3,7,11,15 tetramethyl ; methyl ester	$C_{21}H_{42}O_3$	342.564

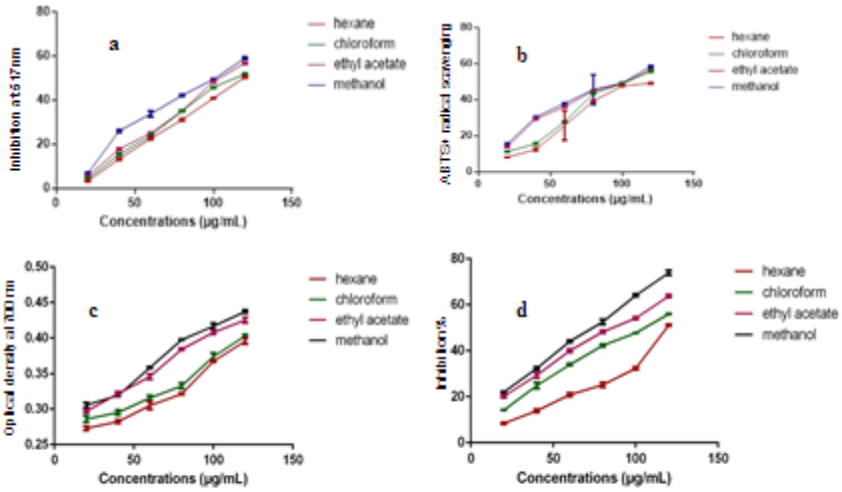

Fig. 3. Antioxidant activity for solvents extract of *C.viscosa.*
a. DPPH radical scavenging, b. $ABTS^{\bullet+}$ radical cation scavenging, c. Reducing potential,
d. Ferrous ion chelating activity.

4. CONCLUSION

C. viscosa contains rich source of secondary metabolites such as alkaloids, steroids, terpenoids, tannins, glycosides, carbohydrates, flavonoids, fatty actids, phenols and saponins. Methanolic extract exhibit the significant antioxidant activity and the active volatile and semi volatile compounds were ascertained by GC-MS analysis. These secondary metabolites used for human beings for the treatment of various diseases and its leads to the opening of biomolecules isolation.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENTS

The authors grateful to The Director, CAS in Botany for providing the laboratory facilities to carry out this work, the author (G. L) thankful to UGC-UPE Phase II for providing financial support and also thankful to SAIF – IIT Madras for helping GC-MS analysis.

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