International Journal of Phytomedicine 3 (2011) 386-391

http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Comparison of the antioxidant activity and total phenolic, flavonoid content of aerial part of *Cleome viscosa* L.

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Abstract

Cleome viscosa L. (Capparidaceae), commonly known as "wild mustard", is an annual, sticky herb found as common weed all over the plains of India and throughout the tropics of the world. In traditional systems of medicine the plant is reported to possess beneficial effects as an anthelmintic, antiseptic, carminative, antiscorbutic, febrifuge, and cardiac stimulant.

The aim of the present study was to evaluate the antioxidant activity of 70% methnolic extract of leaf and stem part of *Cleome viscosa* (CV) by using different *in vitro* model such as β carotene bleaching assay, reducing power and free radical scavenging activity (DPPH and hydroxyl radical scavenging activity). Total phenolic content were estimated by the Folin–Ciocalteu colorimetric method using gallic acid as standard and expressed as mg/g gallic acid equivalent (GAE) and total flavonoid content was estimated by aluminium chloride colourimetric method.

The total phenolic, flavonoid content and antioxidant activity of *Cleome viscosa* leaves were found to be 66.38±0.82mg/g, 0.54±0.04mg/g and 77.30% respectively. *Cleome viscosa* leaves showed high free radical scavenging activity as evidenced by the low IC50 values in both DPPH (1,1-diphenyl-2-picryl hydrazyl) (373.18 µg/ml) and hydroxyl radical (573.55 µg/ml) methods.

Cleome viscosa leaves possess high phenolic, flavonoid content and potential antioxidant activity, reducing power and free radical scavenging activity in comparison to stem.

Keywords: *Cleome viscosa,* total phenolic and flavonoid content, β carotene bleaching assay, reducing power, free radical scavenging activity.

Introduction

Medicinal plants are an important source of antioxidants [1]. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke [2]. The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants

such as leaves, fruits, seeds, stems and bark [3]. There are many synthetic antioxidants in use. It is reported, however, they have several side effects such as risk of liver damage and carcinogenesis in laboratory animals [4, 5]. Therefore there is a need for more effective, less toxic and cost effective antioxidants. Medicinal plants appear to have these desired comparative advantages, hence the growing interest in natural antioxidants from plants.

Cleome viscosa is a weed distributed throughout the tropics of the world and the plains of India [6]. The plant is an annual, sticky herb with a strong penetrating odor. In Ayurvedic system of medicine, this plant is used in fever, inflammations, liver diseases, bronchitis, diarrhoea and infantile convulsions [7]. The seeds used as anthelmintic and the leaves are useful in healing the wounds and ulcers. The juice of the plant diluted with water and given internally in small quantities in fever [8, 9]. *Cleome viscosa* is highly effective in a wide spectrum of diseases and reported to possess analgesic [10], antidiarrheal [11], antipyretic [12], psychopharmacological [13] and antimicrobial [14] properties including in vitro Helicobacter pylori [15].

The objective of the present study was to determine the antioxidant activity of *Cleome viscosa* leaves and stem using standard methods.

Materials and methods

Materials

Plants of *Cleome viscosa* were collected from the rural area around Kanpur, India in the month of August. The plant material was identified and authenticated by Dr. Tariq Husain, taxonomist of National Botanical Research Institute, Lucknow, India and a voucher specimen (No. UIOP/M-121) was deposited at the herbarium section of departmental museum for future reference.

Table 1: Percentage yield of different parts of *Cleome viscosa*

S. No.	Sample	Solvent used for extraction	% Yield (Extract)
1.	Leaves	MeOH : H_2O (7:3)	8.90
2.	Stem	MeOH : H_2O (7:3)	5.70

Method

Preparation of extract

The freshly collected leaves and stem part of *Cleome viscosa* were washed with distilled water and air-dried under the control conditions and powdered. The powdered plant material was percolated with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with of 70% methanol for 3 days. The extract was filtered, concentrated on rotavapour and then freeze-dried under high vacuum (1.33 Pa) and at temperature of - 40± 2 °C, obtain solid residue and their extractive value are given in Table 1.

Estimation of total phenolic content (TPC)

TPC was analysed by the Folin–Ciocalteu colorimetric method for leaf and stem part of *Cleome viscosa* using gallic acid as standard developed by [16] with modification and expressed as mg/g gallic acid equivalent (GAE) on dry weight basis. The 25 mg plant extract was dissolved in 10 ml of 50% MeOH: H2O (1:1), at room temperature and in its 1.0 ml, 1.0 ml of Folin's Reagent (1N) and 2.0 ml of Na_2CO_3 (20 %) were added subsequently. The test mixture was mixed properly on cyclomixer, left at room temperature for 30 min and maintained to 25 ml with water. The absorbance of test mixture was

measured at 725 nm. The reported TPC were expressed as gallic acid equivalent (GAE) mg/g.

Estimation of total flavonoid content (TFC)

TFC was estimated by aluminium chloride colorimetric method for leaf and stem part of *Cleome viscosa* [17]. 0.5ml of stock solution (1g/ml) of the extract, 1.5 ml methanol, 0.1ml potassium acetate (1M) was added to reaction test tubes and volume was made up to 5 ml with distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. Total flavonoid content was calculated by extrapolating the absorbance of reaction mixture on standard curve of rutin. The experiment was repeated thrice and the total flavonoid content was expressed as equivalent to rutin in mg/ g of the extracts.

In vitro antioxidant activity of Cleome viscosa

Antioxidant activity (AOA)

β carotene bleaching assay was carried out according to the method developed by [18]. 2.0 mg of β carotene was dissolved in 20 ml of chloroform and its 3 ml was added to 20 µl of linoleic acid and 200 µl tween 40. After removing under reduced pressure and 100 ml of oxygenated water was added and mixed properly to obtain a stable emulsion. 3 ml aliquot of emulsion were added mixed with 40 µl of sample and incubated for 1 hr at 50°C. The absorbance was recorded at 0 and after 60 min of incubation at 470nm.

Estimation of reducing power (RP)

Reducing power was determined using ferric reducing-antioxidant power assay and quercetin as reference standard [19]. Different aliquots of sample maintained to 1ml followed by the addition of 2.5ml phosphate buffer pH (6.6) and 2.5ml of 1 % w/v potassium ferricyanide in each reaction mixture thus obtained were incubated at 50 ºC for 20 minutes. After incubation, reaction was terminated by additions of 2.5ml of 10% w/v tri chloro acetic acid solution; 2.5ml of above solution from each reaction was diluted with equal amount of distilled water. Aliquot of 0.5ml $FeCl_3$ (0.1%) was added in each and absorbance was recorded after 10 minutes at 700 nm. RP expressed as ascorbic acid equivalent (1mM = 1 ASE). The ASE/ml value is inversely proportional to reducing power.

DPPH radical scavenging activity

The free radical scavenging activity of the leaf and stem part of *Cleome viscosa* was assayed using a stable free radical 1, 1 diphenyl-2-picryl hydrazyl (DPPH). The DPPH scavenging assay employed in the present study was a method proposed by [20]. Different aliquot was added to 2.9ml of freshly prepared solution of DPPH (6×10^{-5} M in MeOH). The Absorbance was recorded at 517 nm at 0 times and after 1 hour of incubation and inhibitory concentration (IC_{50}) was calculated as described by [21]. IC_{50} value is defined as the concentration of sample required to scavenge 50% of free radical.

Hydroxyl radical scavenging activity

The deoxy ribose method was used for determining the scavenging effect on hydroxyl radicals as describe by [22]. The reaction mixture contained ascorbic acid (50µM), $FeCl_3$ (20µM), EDTA (2µM), H_2O_2 (1.42mM), deoxy ribose (2.8mM), with different concentration of the leaf and stem extract of *Cleome viscosa* in a final volume of 1ml in potassium phosphate buffer (10mM, pH 7.4). It was incubated at 37 ºC for 1 h and then 1ml of 2.8 % TCA and 1ml of 1 % TBA were added. The mixture was heated in a boiling water bath for 15 min. It was cooled and absorbance was taken at 532 nm.

Results and Discussion

Phenolic compounds have an important role in stabilising lipid oxidation and are associated with antioxidant activity. Typical phenolics that possess antioxidant activity are known to be mainly phenolic acid and flavonoids. Phenolic compounds exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydro peroxides into free

Table 2: Antioxidant activity, reducing power, free radical scavenging activity (DPPH and hydroxylradical) and total phenolic & flavonoid content of different parts of *Cleome viscosa*

S. No.	Sample	β Carotene bleaching (AOA; %)	Reducing Power (ASE/ml)	DPPH (IC50 µg/ml)	•OH (IC50 µg/ml)	TPC (mg/g GAE)	TFC
1.	Leaves	77.30±1.23	0.78±0.07	373.18±7.32	573.55±5.27	66.38±0.82	0.54±0.04
2.	Stem	59.18±2.46	1.05±0.12	511.10±8.16	698.14±8.12	58.46±0.68	0.48±0.03
3.	BHT	56.34±1.10	-----	-----	-----	-----	-----
4.	Quercetin	-----	0.53±0.06	0.024±0.005	0.075±0.07	-----	-----

All presented values were calculated from three experiment repetitions at least and reported with ±95% confidence limits

radicals. The antioxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals [23, 24]. The result obtained in the present study revealed that the level of polyphenol in *Cleome viscoa* leaves was 66.38±0.82mg/g which was higher when compared to the stem of *Cleome viscosa*. *Cleome viscosa* leaves had a flavonoid compound content of 0.54±0.04 mg/g. For TPC, TFC, AOA, reducing power and free radical scavenging activity the results are presented in Table 2. The flavonoid content of *Cleome viscosa* stem was lower than the leaves. In antioxidant activity estimation betacarotene undergoes rapid decolouration in the absence of an antioxidant because of the coupled oxidation of betacarotene and linoleic acid. Leaves of *Cleome viscosa* showed highest antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [25]. During reducing power assay, the presence of reductant in extracts would result in reducing ferric to ferrous. Leaves of *Cleome viscosa* showed high reducing power as indicated by low ASE/ml value. The percentage inhibition/discoloration of free radicals by different extracts was investigated against DPPH. Leaves of *Cleome viscosa* were found to have remarkable high DPPH radical and hydroxyl radical scavenging activity as evident by low IC_{50} in comparison to stem part.

Conclusion

The present investigation revealed that leaves of *Cleome viscosa* have high phenolic and flavonoid content, inhibition of β carotene bleaching (antioxidant activity), reducing power and free radical scavenging activity in comparison to

stem. Thus the present investigation provides a comparative profile of the antioxidant activity of extracts of leaf and stem parts of an important medicinal plant, *Cleome viscosa*, with respect to its phenols and flavonoids content. Further intention of this study is to correlate relationship of these secondary metabolites to possible biological activities and evaluate *Cleome viscosa* as a potential source of natural bioactive chemicals

Acknowledgement

The authors would like to acknowledge Head, Institute of Pharmacy for providing necessary support.

Abbreviations

AOA- Antioxidant activity; ASE-ascorbic acid equivalent; TBA-Thiobarbituric acid; TCA- tetra chloro acetic acid; TPC-total phenolic content; TFC- total flavonoid content

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