IJPSR (2013), Vol. 4, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 October, 2012; received in revised form, 18 November, 2012; accepted, 26 January, 2013

RADICAL SCAVENGING POTENTIAL OF *CLEOME VISCOSA* L. AND *CLEOME BURMANNI* W. & A. (CLEOMACEAE)

Lakshmi S. Pillai* and Bindu R. Nair

Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram- 695 581, Kerala, India

Keywords:

Cleome viscosa, *Cleome burmanni*, antioxidants, ROS, antioxidant assays, phenols, flavonoids,proanthocyanidins

Correspondence to Author:

Lakshmi S. Pillai

Research Scholar, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram- 695 581, Kerala, India

E-mail: abrus09@yahoo.in

ABSTRACT

The present study evaluates the reactive oxygen species (ROS) scavenging and *in vitro* antioxidant activities of two species of *Cleome*, *C. viscosa* and *C. burmanni*. The antioxidant potential of the methanol extracts of species of *Cleome* was tested using different assays such as FRAP, DPPH, ABTS, hydroxyl, superoxide, nitric oxide, and hydrogen peroxide. The extracts gave positive results for all the assays and the radical scavenging ability was detected to be comparable to those of the corresponding standards. Quantitative estimation of antioxidant phytochemicals, such as phenols, flavonoids and proanthocyanidins were also done in both species of *Cleome*. The results revealed that the phytochemicals content was greater in *C. viscosa* when compared to *C. burmanni.*

INTRODUCTION: Oxidative stress plays an important role in the pathogenesis of various diseases such as atherosclerosis, alcoholic liver cirrhosis and cancer. The reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide formed *in vivo* due to several metabolic activities in cells, initiate oxidative stress. ROS is also known to initiate the lipid peroxidation of phospholipids in cell membranes, thereby propagating a chain reaction and ultimately resulting in cell damage [1, 2].

Antioxidants are substances that counteract oxidative damage from ROS by one or more of the following mechanisms: reducing activity, free-radical scavenging activity, potential complexing of pro-oxidant metals and quenching of singlet oxygen. Antioxidants are produced *in vivo* or may be chemically synthesized. Natural antioxidants are known to have a significant role in curing human ailments [3, 4].

Phytochemicals such as phenols and flavonoids are reported to possess antioxidant activities and inhibit the propagation of free radical reactions [5].

Phenolic compounds act as cellular support material and form an integral part of cell wall structure [6]. Polyphenols can protect the human body from the oxidative stress which causes many diseases, including cancer, cardiovascular problems and ageing [7].

Flavonoids have also been reported to possess anti-inflammatory, oestrogenic, enzyme inhibition, antimicrobial, antiallergic activites, apart from antioxidant and cytotoxic antitumor activity [8].

The present study involves the analysis of radical scavenging potential of two species of genus *Cleome* (Cleomaceae). Many species of *Cleome* found growing as roadside weeds are reportedly used in traditional systems of medicines [9, 10].

Cleome viscosa, the most common species, is reported to possess rubefacient, vesicant, expectorant, astringent, antispasmodic, repellent, antifeedant, nematicidal and anthelmintic properties [11, 12, 13]. *Cleome burmanni* is reported to possess anthelmintic property [13].

However, there are no reports on the antioxidant properties of either of these plants. The present study therefore investigates the *in vitro* antioxidant and free radical scavenging potential of *C. viscosa* and *C. burmanni* and checks for the presence of antioxidant phytochemicals.

MATERIALS AND METHODS: The plant samples, *Cleome viscosa* and *C. burmanni* were collected from Kariavattom, Thiruvananthapuram.

Preparation of Extract: Methanol extracts of *Cleome viscosa* and *C. burmanni* were prepared from shade-dried plant parts. About 20g of the powdered plant material from each sample was subjected to extraction by soxhlet apparatus using 300 ml of methanol. The extracts were then concentrated under reduced pressure and preserved in refrigerator until further use.

Chemicals: Methanol, Gallic acid, Ascorbic acid, Folin-ciocalteu reagent, Sodium carbonate, Vanillin, Aluminium chloride, Potassium acetate, Phosphate buffer, ferric chloride, acetate buffer, 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ), Butylated hydroxyl toluene (BHT), 1, 1- Diphenyl-2-picryl hydrazyl (DPPH), 2, 2'-azino-bis (3- ethyl- benzthiazoline-6-sulphonic acid(ABTS), Potassium persulphate, Trolox, Myoglobin, Sodium nitroprusside, Hydrogen peroxide, Sulfanilic acid, Glacial acetic acid, Naphthyl ethylenediamine dichloride (NEDD), Potassium metabisulphite, EDTA, DMSO, Ammonium acetate, Acetone, NADH. All chemicals were of analytical grade.

Methods: The radical scavenging potential and *in vitro* antioxidant activity were tested using the following assays:

A. Antioxidant assays:

1. **FRAP (Ferric reducing ability of plasma) -** (Benzie and Strain, 1996) [14]: The FRAP reagent (2.5 ml of 10mM TPTZ solution in 40mM HCl, 2.5ml of 20mM $FeCl_3$ and 25 ml of 0.3 M acetate buffer - pH 3.6) was freshly prepared. An aliquot of various concentrations (20-100µg/ml) of test solution was mixed with 180µl of FRAP reagent. The absorbance was measured at 595nm.

2. **2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay-** (Brand-Williams *et al*, 1999) [15]: One ml of 0.135mM DPPH prepared in methanol was mixed with 1.0 ml of methanol extract ranging from 0.2-0.8 mg/ml. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517nm. The scavenging ability of the plant extract was calculated using the equation:

DPPH scavenging activity (%) =

[(*Abs control-Abs sample)] / (Abs control)] x 100

*Abs control is the absorbance of DPPH + methanol; Abs sample is the absorbance of DPPH radical + sample.

3. **Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) scavenging activity-**(Miller and Evans, 1997) [16]: Trolox standards were prepared for a standard curve of different concentrations. The ABTS substrate working solution was prepared by adding 25µl of 3% Hydrogen Peroxide solution to 10 ml of ABTS substrate solution. The assays were prepared in the 96 well plates. In the wells for the trolox standard curve, 10µl of Trolox standard and 20µl of Myoglobin working solution were added. In the wells for the test samples, 10µl (0.2-0.8 mg/ml concentrations) of methanol extracts and 20µl of Myoglobin working standard were added. Then 150µl of ABTS substrate working solution to each well was added. The solution was incubated for 5 min at room temperature. The stop solution (100µl) was added to each well. The endpoint absorbance was read at 405nm using a plate reader.

4. **Hydroxyl Radical Scavenging Activity** (Klein, 1995) [17]: Various concentrations (20, 40, 60, 80 and 100 µg/ml in methanol) of extracts were taken in different test tubes and evaporated to dryness. One ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of EDTA (0.018%) and 1ml of DMSO (0.85% v/v in 0.1M phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5ml of 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80-90^{o}C for 15 min., the reaction was terminated

by the addition of 1ml of ice-cold TCA (17.5% w/v). Three ml of Nash reagent (75.0 g of ammonium acetate, 3 ml of glacial acetic acid and 2ml of acetyl acetone were mixed and made up to 1L with distilled water) was added to all of the tubes and left at room temperature for 15min for color development. The intensity of the yellow color formed was measured spectrophotometrically at 412nm against the reagent blank.

5. **Nitric oxide Scavenging Activity**-(Garrat, 1964) [18]: Sodium nitoprusside in aqueous solution at physiological pH spontaneously generate nitric oxide, which interacts with oxygen to produce nitrite ions determined by the use of Griess reagent. Two ml of 10mM Sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of plant extract at various concentrations (0.2-0.8 mg/ml). The mixture was incubated at 25^{o}C. After 150 min, 0.5 ml of incubation solution was withdrawn and mixed with 0.5ml of Griess reagent [(1.0ml sulfanilic acid reagent (0.33%) in 20% glacial acetic acid at room temperature for 5 min with 1ml of naphthylethyl enediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min. The absorbance was measured at 540nm. The amount of nitric oxide radical was calculated following this equation:

% of inhibition of NO = [*Ao- A_1] / Ao x 100.

*Ao is the absorbance before reaction and A_1 is the absorbance after reaction has taken place.

6. **Superoxide anion Scavenging Activity** (Yen and Chen, 1995) [19]: The reaction mixture consisting of 1ml of plant extract (0.2-0.8 mg/ml), 1 ml of PMS (60µM) prepared in phosphate buffer (0.1 M pH7.4) and 1 ml of NADH (phosphate buffer) was incubated at 25^{o}C for 5 min. The absorbance was read at 560nm against blank samples.

7. **Hydrogen peroxide Scavenging Activity** (Ruch *et al.,* 1989) [20]: The plant extracts (4ml) prepared in distilled water at various concentrations (0.2-0.8 mg/ml) were mixed with 0.6 ml of 4mM H_2O_2 solution prepared in phosphate buffer (0.1M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the plant extract without H_2O_2.

B. Quantitative estimation of antioxidant phytochemicals.

1. **Determination of total phenol-** (Spanos, 1990) [21]: 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of Na_2CO_3 (2% w/v) was added to 0.5ml of each sample of plant extract solution (1mg/ml). The resulting mixture was incubated at 45^{o}C with shaking for 15 min. The absorbance of the samples was measured at 765nm using UV/Visible light. Results were expressed relative to Gallic acid (0-0.5 mg/ml) dissolved in distilled water.

2. **Determination of total flavonoids-** (Harborne, 2000) [22]: Aluminium chloride colorimetric method was used for flavonoid determination. One ml of sample (1mg/ml) was mixed with 3ml of methanol, 0.2ml of 10% aluminium chloride, 0.2ml of 1M potassium acetate and 5.6ml of distilled water and allowed to remain at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420nm with UV-visible spectro-photometer. The content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoids was expressed in terms of mg/ml.

3. **Determination of total proanthocyanidins -** (Sun *et al*, 1998) [23]: The mixture of 3ml vanillin-methanol (4% v/v), 1.5ml of hydrochloric acid was added to 0.5 ml (1mg/ml) of methanol extracts and vortexed. The resulting mixture was allowed to stand for 15 min at room temperature followed by measurement of the absorbance at 500nm. Total proanthocyanidin content was expressed as gallic acid equivalent (mg/ml) from the standard curve.

RESULTS: The results of the antioxidant assays are presented in **tables 1-7**. The reducing capacity (FRAP Assay) of the methanol extracts of *C. viscosa* and *C.burmanni* was found to be appreciable.

The results showed that there was an increase in reducing power of the plant extracts as the extract concentration increased (**Table 1**).

In DPPH assay, the methanol extract of *C. viscosa* appeared to be more potent than *C. burmanni* when compared with BHT at the same concentration (**Table 2**).

In ABTS assay also, *C. viscosa* extracts had greater ABTS radical scavenging activity than *C. burmanni* extracts (**Table 3**).

In hydroxyl radical assay, the ability to quench hydroxyl radicals was higher in *C. viscosa* than *C.burmanni* in comparision to Ascorbic acid used as standard (**Table 4**).

In nitric oxide scavenging assay, the methanol extracts of *C. viscosa* showed higher percentage of inhibition values almost nearing BHT (**Table 5**).

Both the plant extracts were found to be potent scavengers of superoxide radical but the value was lesser than the standard (**Table 6**).

Hydrogen peroxide assay showed that the hydrogen peroxide scavenging activity of both the extracts was significant when compared to BHT (**Table 7**).

Quantitative estimation revealed that the content of phenols, flavonoids and proanthocyanidins was greater in *Cleome viscosa* when compared to *C. burmanni* (**Table 8**). Flavonoids formed the major group compared to phenols and proanthocyanidins in that order.

A. Antioxidant Assays:

1. Reducing Power Assay (FRAP):

TABLE 1: FRAP ASSAY USING METHANOL EXTRACTS OF TWO SPECIES OF *CLEOME*

Concentration of the extract µg/ml	% of inhibition using *C. viscosa* extracts	% of inhibition in *C. burmanni* extracts	% of inhibition by *BHT
20	0.071	0.05	0.085
40	0.145	0.130	0.160
60	0.221	0.205	0.230
80	0.285	0.262	0.301
100	0.360	0.339	0.386

*BHT- Butylated Hydroxyl Toluene

2. DPPH Assay:

TABLE 2: DPPH ASSAY USING METHANOL EXTRACTS OF TWO SPECIES OF C*LEOME*

Concentration of the extract mg/ml	% of inhibition using *C. viscosa* extracts	% of inhibition in *C. burmanni* extracts	% of inhibition by BHT
0.2	29.39	25.92	42.60
0.4	40.00	37.21	52.00
0.6	58.80	55.23	73.01
0.8	77.30	60.55	80.32

3. ABTS Scavenging Assay:

TABLE 3: ABTS SCAVENGING ASSAY USING METHANOL EXTRACTS OF TWO SPECIES OF *CLEOME*

Concentration of the extract mg/ml	% of inhibition using *C. viscosa* extracts	% of inhibition in *C. burmanni* extracts	% of inhibition by Trolox
0.2	40.87	31.76	52.00
0.4	53.40	43.29	63.35
0.6	69.21	55.00	77.00
0.8	73.11	60.95	79.12

4. Hydroxyl Radical Scavenging Assay:

TABLE 4: HYDROXYL RADICAL SCAVENGING ASSAY USING METHANOL EXTRACTS OF TWO SPECIES OF *CLEOME*

Concentration of the extract µg/ml	% of inhibition using *C. viscosa* extracts	% of inhibition in *C. burmanni* extracts	% of inhibition by ascorbic acid
20	24.42	17.71	31.00
40	39.16	24.76	38.24
60	46.11	45.24	60.27
80	58.51	56.17	71.12
100	65.20	61.25	79.22

5. **Nitric Oxide Scavenging Assay**:

TABLE 5: NITRIC OXIDE SCAVENGING ASSAY USING METHANOL EXTRACTS OF TWO SPECIES OF *CLEOME*

Concentration of the extract. mg/ml	% of inhibition using *C. viscosa extracts*	% of inhibition in *C. burmanni extracts*	% of inhibition by BHT(*)
0.2	36.02	26.29	40.21
0.4	51.80	37.02	46.18
0.6	62.95	55.45	60.82
0.8	78.59	62.37	80.11

6. **Superoxide Scavenging Assay**:

TABLE 6: SUPEROXIDE SCAVENGING ASSAY USING METHANOL EXTRACTS OF TWO SPECIES OF *CLEOME*

Concentration of the extract mg/ml	% of inhibition using *C. viscosa extracts*	% of inhibition in *C. burmanni extracts*	% of inhibition by BHT(*)
0.2	60.65	53.17	59.18
0.4	66.23	58.15	72.46
0.6	72.16	62.40	77.11
0.8	76.82	70.21	78.00

7. **Hydrogen Peroxide Scavenging Assay**:

TABLE 7: HYDROGEN PEROXIDE SCAVENGING ASSAY USING METHANOL EXTRACTS OF TWO SPECIES OF CLEOME

Concentration of the extract mg/ml	% of inhibition using *C. viscosa extracts*	% of inhibition in *C. burmanni extracts*	% of inhibition by BHT(*)
0.2	50.13	42.00	68.60
0.4	56.88	49.17	73.22
0.6	62.10	53.26	76.00
0.8	70.85	60.73	79.19

B. Quantitative estimation of antioxidant phytochemicals:

TABLE 8: PHYTOCHEMICAL COMPONENTS IN METHANOL EXTRACTS OF *CLEOME VISCOSA* AND *CLEOME BURMANNI.*

Plant name	Phytochemicals (extract equivalent of gallic acid (mg/g)		
	Phenols	Flavonoids	Proanthocyanidins
Cleome viscose	3.4	4.6	2.2
Cleome burmanni	2.1	4.0	1.1

DISCUSSION: Oxidative stress is the prime cause for all permanent modifications to the genetic material and represents the first step in almost all the detrimental processes such as mutagenesis, carcinogenesis and ageing leading to cell damage and death. The modulation of cell signaling pathway by antioxidants could help prevent cell damage in the following ways:

1) Preserving normal cell cycle regulation;
2) Inhibiting proliferation and inducing apoptosis;
3) Inhibiting tumor invasion and angiogenesis;
4) Suppressing inflammation and other such effects.

Due to these healing effects, antioxidant constituents especially from plant materials have raised interest among scientists, food manufacturers and consumers for their role in the maintainence of human health [24, 25].

The antioxidant potential of compounds is usually being tested by various antioxidant assays. The assays conducted in the present study and their utility is discussed below;

The principle of FRAP method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous colored form in the presence of antioxidants.

A compound exhibiting a positive result in the FRAP assay is an electron donor which terminates the oxidation chain reaction by reducing the oxidized intermediates into the stable form. The reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process, so they can act as primary and secondary antioxidants [19]. Both the plant extracts showed potent reducing ability when compared to BHT (Table 1).

DPPH is a stable free radical having a characteristic absorption at 517nm. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and DPPH solution is decolourised as the colour changes from deep violet to light yellow [15]. The degree of reduction in absorbance measurement is indicative of radical scavenging power of the plants. The results of DPPH (stable free radical) scavenging activity in this study indicated that *C.viscosa* was potentially active. The plant extracts possibly contain compounds capable of donating hydrogen to a free radical in order to remove the odd electron, responsible for radical's reactivity (Table 2).

The principle of ABTS assay is formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which oxidizes the ABTS to produce a radical cation, $ABTS^+$, a soluble chromogen that is green. $ABTS^+$ is a blue coloured chromophore which is reduced to ABTS on a concentration dependant manner upon addition of plant extracts. The scavenging activity of $ABTS^+$ radical by the *C.viscosa* extract was found to be appreciable (Table 3). This implies that the plant extract may be useful for treating radical related pathological damage especially at higher concentrations [26].

Hydroxyl radical is an extremely reactive species formed in biological systems and has been implicated as highly damaging in free radical pathology, capable of damaging almost every molecule found in living cells [27]. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contribute to carcinogenesis, mutagenesis and cytotoxicity.

This species is considered to be one of the quick initiators of lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids [28]. The results of the study indicated that the extracts of *C.viscosa* were strong hydroxyl radical scavengers when compared to *C.burmanni* (Table 4).

Nitric oxide radicals are produced by macrophages at the time of inflammatory response. They play an important role in various types of inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis. These radicals interfere in DNA processes because of its mutagenic nature [29].

The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite anion that is well restrained by the extracts. The results indicated that the extracts of *C. viscosa* possess potent NO scavenging activity (Table 5).

Superoxide anion is one of the strongest reactive oxygen species among the free radicals generated [18]. The scavenging activity of this radical by the extracts of species of *Cleome* compared favourably with the standard BHT suggesting that the plants were potent scavengers of superoxide radical (Table 6).

Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell [30]. Scavenging of H_2O_2 by the extracts of species of *Cleome* may be attributed to their phenolics which donate electron to H_2O_2, thus reducing it to water. The plant extracts were capable of scavenging H_2O_2 in a concentration dependant manner (Table 7).

On the whole, it appears that *C. viscosa* has a greater radical scavenging ability than *C. burmanni* and may be utilized effectively.

Antioxidant activity in plants is mostly due to the presence of phytochemicals such as phenols, flavonoids and proanthocyanidins. The results indicated that the plant extracts contain significant amount of phenols and flavonoids. Flavonoid content was higher compared to phenols and proanthocyanidins (Table 8). These compounds have good antioxidant potential and their effects on human nutrition and health are considerable.

Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane bound enzymes such as ATPase and phospholipase A2 [31]. The mechanism of action of flavonoids is through scavenging or chelating process [32]. Phenols are important plant constituents because of their scavenging ability due to their hydroxyl and carboxyl groups which bind, particularly to copper and iron and play a major role in plant development [33].

Proanthocyanidins have anticancer and antitumour activity. They suppress the production of a protein endothelin-1 that constricts the blood vessels. They have antioxidant activity and play a role in the stabilization of collagen and maintainence of elastin [34]. Thus the results indicate that the antioxidant activities exhibited may be due to the presence of phenols and flavonoids in the extracts of *Cleome*.

CONCLUSION: The results from various free radical scavenging systems revealed that *C. viscosa* and *C. burmanni* methanol extracts are strong antioxidants, with scavenging activities for the different ROS at different magnitudes of potency. The study affirms the *in vitro* antioxidant potential of the crude extracts of both plants, with results comparable to those of standards such as Gallic acid, BHT, Trolox and Ascorbic acid.

Presence of flavonoids, phenols and proanthocyanidins in significant quantities in extracts is a possible indication of their role in antioxidant activity. Further studies are needed to clarify the *in vivo* potential of these plants in the management of human diseases resulting from oxidative stress.

ACKNOWLEDGEMENTS: The authors are thankful to Dr. Ashalatha S. Nair, Professor and Head, Department of Botany, University of Kerala, Kariavattom, for providing the required facilities for the conduct of this research work.

REFERENCES

1. Braca A, Sortino C, Politi M, Morelli I and Mendez J: Antioxidant activity of flavonoids from *Licania licaniaeflora*. J. Ethnopharmacol 2002; 79:379-381.
2. Maxwell SR: Prospects for the use of antioxidant therapies. Drugs 1995; 49:345-361.
3. Pulido R, Bravo L and Saura-Calixto F: Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry 2000; 48(8): 3396-3402.
4. Sumino M, Sekine T, Ruangrungsi N, Igarashi K and Ikegami F: Ardisiphenols and other antioxidant principles from the fruits of *Ardisia colorata*. Chemical and Pharmaceutical Bulletin 2002; 50(7): 1484-1487.
5. Terao J and Piskula MK: Flavonoids as inhibitors of lipid peroxidation in membranes. In C. A. Rice-Evans & L. Packer (Eds.), Flavonoids in health and disease 1997; pp. 277-295.
6. Gupta VK, Singh GD, Singh S and Kaul A: Medicinal plants. Phytochemistry, Pharmacology and Therapeutics, Daya publishing house, Delhi, 2010.
7. Robards K, Prernzler PD, Tucker G, Swatsitang P and Glover W: Phenolic compounds and their role in oxidative processes in fruits. Food Chem 1999; 66:401-36.
8. Havsteen B: Flavonoids- a class of natural products for antimicrobial agents. Eur. J. Clin. Microbiol. Infect. Dis. 1990; 9:455-61.
9. Jansen PC: *Cleome rutidosperma* L. and *C. viscosa* L. In: Grubben, G.J.H. and Denton, O. A. (Editors). Plant resources of Tropical Africa. Prota Foundation, Backhury's Publishers, Leiden Netherlands/ CTA Wageningen, 2004.
10. Mnzava NA and Ngwerume FC: *Cleome gynandra* L. In: Grubben, G.J.H. and Denton, O. A. (Editors). Plant resources of Tropical Africa. Prota Foundation, Backhury's Publishers, Leiden Netherlands/ CTA Wageningen, 2004; pp. 191-195.
11. Fletchers R: *Cleome gynandra* (Cat's whiskers). The Australian New Crops Newsletter 1999; Vol. 11.
12. Lazzeri L, Leoni O and Manici LM: Biocidal plant dried pellets for biofumigation. Industrial Crops and Products 2004; 20:59-65.
13. Pillai LS and Nair BR: A comparative study on the anthelmintic potential of *Cleome viscosa* L. and *C. burmanni* W. &A. Indian Journal of Pharmaceutical Sciences 2011; 73 (1): 98-100.
14. Benzie IF and Strain JJ: "The ferric reducing ability of plasma as a measure of antioxidant power: the FRAP assay". Analytical Biochemistry 1996; 239: 70-76.
15. Brand-Williams W, Cuvelier M and Berset C: Use of a free radical method to evaluate antioxidant activity, Lebensmitted Wissenschaft and Technologic 1999; 28: 25-30.
16. Miller NJ and Rice-Evans CA: Factors influencing the antioxidant activity determined by the ABTS+ radical cation assay. Free Radic. Res. 1997; 26: 195-199.
17. Klein SM, Cohen G and Cederbum AI: Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical generating system. Biochemistry 1991; 20:6006-6012.
18. Garrat DC: The quantitative analysis of Drugs. 3rd Edition, Chapman and Hall, Japan, 1964.
19. Yen G. and Chen HJ: Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem. 1995; 43:7-32.
20. Ruch RJ, Cheng SJ and Klaunig JE: Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogens 1989; 10:1003-1008.
21. Spanos GA and Wrolstad WE: Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. J. Agric. Food Chem. 1990; 38:1565-1571.
22. Harborne JB and Williams CA: Advances in flavonoids research since 1992. Phytochemistry 2000; 55: 481-504.
23. Sun JS, Tsuang YW, Chen JJ, Huang WC, Hang YS and Lu FJ: An ultraweek chemiluminescence study on oxidative stress in rabbits following acute thermal injury. Burns 1998; 24:225-231.
24. Milner A: Functional foods and health promotion. J. Nutr. 1999; 129: 1395-1397.
25. Valko M, Rhodes CJ, Moncol J, Izakovic M and Mazur M: Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. 2006; 160: 1-40.
26. Wang M, Li J, Rangarajan M, Shao Y, La Voie EJ, Huang T and Ho C: Antioxidative phenolic compounds from Sage. J Agric Food Chem. 1999; 46:4869-4873.
27. Halliwell B: Reactive oxygen species in living systems: Source, biochemistry and role in human disease. Am. J. Med. 1991; 91:S14-S22.

28. Kappus H: Lipid peroxidation-mechanism and biological relevance. Free radicals and food additives, London, UK, 1991; 59-75.
29. Miller MJ, Sadowska-Krowicka H, Chotinaruemol S, Kakkis JL and Clark DA: Amelioration of chronic ileitis by nitric oxide synthase inhibition. The J Pharmac and Experi Thera 1993; 264:11-16.
30. Gulcin I, Okay M, Kirecci E, Kuifrevioglu OI: Screening of antioxidants and antimicrobial activities of Pimpinella anisum L. seed extracts. Food Chem 2003; 83:371-382.
31. Li H, Wang Z and Liu Y: Review in the studies on tannins activity of cancer prevention and anticancer. Zhang-Yao-Cai 2003; 26(6):444-448.
32. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF and Bilaloglu V: Comparison of antioxidant and antimicrobial activities of Tilia, Sage and Black Tea extracts. J Agri Food Chem 2000; 48: 5030-5034.
33. Cook NC and Samman S: Flavonoids-chemistry, metabolism, cardioprotective effects and dietary sources. Nutr Biochem 1999; 7:66-76.
34. Corder R, Mullen W, Khan WQ, Marks SC, Wood EG, Carrier MJ, Crozier A: Oenology- Redwine procyanidins and vascular health. Nature 2006; 444(7119): 566.

How to cite this article:
Pillai LS and Nair BR: Radical Scavenging potential of *Cleome viscosa* L. and *Cleome burmanni* W. & A. (Cleomaceae). *Int J Pharm Sci Res*. 2013; 4(2); 698-705.