International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1690-1702 www.ijprajournal.com ISSN: 2249-7781

Estimation of Total Phenolic Content, Total Flavonoid Content and Assessment of in vitro Antioxidant Activity of Extracts of plant Cleome viscosa Linn.stembark

Dr. Umesh Prabhakar Joshi[1], Dr. Ravindra Banilal Patil[2]
[1, 2]*DCS's A. R. A. College of Pharmacy, Nagaon, Dhule, Maharashtra, India, 424006*

Submitted: 15-05-2023 Accepted: 30-05-2023

ABSTRACT:
The study consists of estimation of Total Flavonoid Content, Total Phenolic Content and Assessment of in vitro Antioxidant Activity of Extracts of stembark of plant Cleome viscosa Linn.by using various in-vitro methods. In this study the Total Flavonoid Content, Total Phenolic Content, Antioxidant activityof aqueous, methanolic and hydroalcoholic extract of stembark of plant Cleome viscosa Linn.was evaluated by Folin–Ciocalteau reagent, Aluminium chloride, 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) radical scavenging, Nitric oxide radical scavenging assay, Hydrogen peroxide and reducing assay methods and compared. The result indicated that methanolic extract of the leaves of plant exhibited potent antioxidant activity as compared to hydroalcoholic and aqueous extract with reference to Ascorbic acid (Vitamin C) as standard. The present estimation and assessment showed that the plant Cleome viscosa Linn. used as potential antioxidant activity with maximum concentration of Total Flavonoid and Total Phenolic Content.

KEYWORDS: Plant Cleome viscosa Linn.Antioxidant, Total Phenolic, Total Flavonoid, DPPH scavenging activity, Nitric oxide scavenging activity, Reducing power, Hydrogen peroxide etc.

I. INTRODUCTION

Free radicals are naturally occurring by-products of body metabolism. Free radicals are electrically charged molecules that attack various cells, tissues, organs, tearing through impermeable cellular membranes to react with the nucleic acids, proteins and enzymes present in the body [1]. Free radicals can cause the lipid peroxidation in foods which leads to their deterioration [2]. Oxidation is called as to be the major cause of foods and materials degradation [3].Lots of research work is clearly showed that free radicals would damage nearby structures including DNA, Proteins and Lipids. Radical scavenging antioxidant substances are particularly important in antioxidative-defence which are protecting the cells from the injury of free-radical [4].

Plants are very good source of biologically active compounds known as phytochemicals. The phytochemicals are found to be act as potent antioxidants by scavenging free radicals and may have important therapeutic potential for free radical associated disorders [5]. It is well known that free radicals which are the major causes of various chronic and degenerative diseases, such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer.

Oxidative stress is the major driving factor responsible for the initiation and progression of cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases among other syndromes[].

Therefore, it is very important to find out antioxidant activity of the plants used in the herbal medicine either to elucidate the mechanism of their pharmacological action or to provide information on antioxidant activity of these herbal plants [7].

Reactive oxygen species (ROS) like as superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are generated as by products of biological reaction or from exogenous factors [8]. In vivo, some of these Reactive oxygen species (ROS) are play an important role in cell metabolisms like as energy production, phagocytosis and intercellular signaling [9]. However, generally these Reactive oxygen species (ROS) are produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic reactions which have a wide variety of pathological effects like as DNA damage, carcinogenesis and various degenerative disorders such as cardiovascular diseases, aging and neuro-degenerative diseases [10-12].A potent broad spectrum scavenger of these species may acts as a possible preventive intervention for free radical mediated cellular damage and diseases [13].

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1690-1702 www.ijprajournal.com ISSN: 2249-7781

Antioxidants are substances which are provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA stand breaking [14]. There are many substances from natural sources have been shown to contain antioxidant and they are under the study. Antioxidant compounds like as Phenolic acids, polyphenolic substances and flavonoids which scavenge the free radicals like as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [15]. Ethnomedical literature has reveals that the large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. There are many plants that have been found to possess strong antioxidant activity [16].Recent studies have shown that number of plant products together with polyphenols, terpenes and various plant extracts exerted asan antioxidant action [17-20].

There is presentlymassive interest in natural antioxidants and their role in human health and nutrition [21].

Considerable amount of data have been generated on antioxidant properties of food plants around the globe [22, 23]. However, traditionally used medicinal plants awaits such screening. On the other hand, the medicinal properties of plants have also been investigated in the light of recent scientific developments through out the world, due to their potent pharmacological activities, low toxicity and economic viability [24].

Cleome viscosa Linn.family:- Capparaceae is an annual, erect, 30-90 cm high plant. Stem of plant is grooved, densely clothed with glandular and simple hairs. Leaves are 3-5 foliolate. Lower leaves petioles are 2.5-5 cm long gradually becoming shorter upwards. Leaflets are elliptical–oblong or obovate. Flowers of plant are yellow in colour, axillary, growing out into a lax raceme. Seeds are brown–black in colour when ripe [25, 26].

The whole plant and its parts like as leaves, seeds and roots) are widely used in traditional and folk system of medicine. Various parts of plant Cleome viscosa are used as traditional folk medicines for the treatment of helmintic infections, joint pain, rubifacient, vesicant. The research work has done on antipyretic, anticonvulsant, hepatoprotective, analgesic, psychopharmacological, antidiarrheal, carminative, febrifuge, and cardiac stimulant[27, 28].

This research paper reports total flavonoid, total phenolic and antioxidant potential of methanolic, hydroalcoholic and aqueous extracts of stembark of plant Cleome viscosa Linn.evaluated by Folin–Ciocalteau reagent, Aluminium chloride, 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) radical scavenging, Nitric oxide radical scavenging assay, Hydrogen peroxide and reducing assay methods. Further an attempt has also been made to find the relationship between phenolic content and antioxidant activity of this plant.

II. MATERIALS AND METHODS

Plant Collection

The stembark of plant Cleome viscosa Linn.were collected from local area of Dhule district, Maharashtra, India. The selected plantwas authenticated by Dr. D. A. Dhale, Asst. Professor, PG & Research Dept. of Botany SSVPS's, L.K.Dr.P.R.Ghogrey Science College, Dhule, Maharashtra. Stembarks were dried at room temperature to avoid loss of chemical constituents and milled with the aid of grinding machine.

Preparation of Plant extract

The leaves of plant were dried at room temperature and transformed to coarse powder. The powder of stembarkswere extracted with three solvents i.e methanol, water and water-ethanol separately by Soxhlet extraction method. Finally, the extract was evaporated and dried under vacuum to obtain thick sticky extract.

Chemicals

2-2 diphenyl-1 picryl hydrazyl (DPPH), Methanol, Sodium nitroprusside, Sulphanilamide, Potassium ferricyanide,Trichloroacetic acid, Ascorbic Acid, Ferric chloride, N-(1- naphthyl) ethylenediamine dihydrochloride), Hydrogen Peroxide solution, Phosphate Buffer and allother reagents were of analytical grade.

Instrument

Shimadzu UV – visible spectrophotometer

Determination of Total Phenolics Content[29]

The Total soluble phenolics in the extracts were determined with Folin–Ciocalteau reagent according to the method reported by Singletion et al., (1999) using gallic acid as a standard phenolic compound. About 500µl (20mg/ml) of plant sample was added to 25mlof distilled water and 1ml of Folin-Ciocalteu reagent (1:10). Thenthis mixture

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1690-1702 www.ijprajournal.com ISSN: 2249-7781

was kept at room temperature for 3 minutes, after then1.5ml of 2% sodium bicarbonate was added, soon after vortextingthe reaction mixture for 1 hour at room temperature, theabsorbance was measured at 760nm. All the tests were performedin triplicates and the results were averaged. The concentration oftotal phenolic compounds in methanolic stembark extracts wasdetermined as microgram of gallic acid equivalent by using anequation that was obtained from the standard gallic acid graph (10-100 µg/ml).

Determination of Total Flavonoid Content[30]

The aluminium chloride colorimetric assay was used fortotal flavonoids determination, as described by Zhishen et al.(1999). 100µl (20mg/ml) of the extract was mixed with 2.5 ml ofdistilled water and 300µl of 5% sodium nitrate. Then, it wasincubated at room temperature for 5 minutes and 300µl of 10%aluminium chloride, 2ml of 1M sodium hydroxide and 1ml ofdistilled water were added. Then, absorbance of the reactionmixture was measured at 512nm, along with the standard,quercetin and blank. The total flavonoids content was determinedas microgram, quercetin equivalent by using the standard,quercetin graph, obtained by comparing the calibration curveprepared from a reference solution containing quercetin (10-100µg/ml).

DPPH radical scavenging assay[31]

The antioxidant activity of the methanolic, aqueous and hydroalcoholic extracts of stembark of plant Cleome viscosa Linn. was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH carriedout by using the method of Molyneux. About 1 ml of 100 µM DPPH solution in methanol, equal volume of theextract in methanol of different concentrations of the extract in methanol was added and incubated in dark for 30min and 1ml of methanol served as control. The change in colour was observed in terms of absorbance using aspectrophotometer at 517 nm. The different concentrations of ascorbic acid were used as reference compound.Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and wascalculated using the formula:

$$\text{Percentage Inhibition} = \frac{\text{Absorbance Control} - \text{Absorbance test}}{\text{Absorbance Control}} \times 100$$

Nitric oxide radical scavenging assay[32]

Nitric oxide radical scavenging activity was measured spectrophotometrically according to the method described by Govindharajan et al. When sodium nitroprusside was mixed with aqueous solution at physiological pH, suddenly it generates nitric oxide, which reacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Nitric oxide scavengers compete with oxygen leading to reduced production of nitrite ions. About 1 ml of Sodium nitroprusside (5 mM) in phosphate buffer (pH 7.4, 0.1 M) was mixed with different concentrations of the methanolic, aqueous and Hydroalcoholicextract (25 - 200 µg/ml) in phosphate buffer (pH 7.4, 0.1 M). The tubes were then incubated at 25°C for 2 h. After incubation 1.5 ml of reaction mixture was removed and diluted with 1.5 ml of Greiss reagent [1% sulphanilamide, 2% O-phosphoric acid and 0.1% of N-(1- naphthyl) ethylenediamine dihydrochloride]. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-(1- naphthyl) ethylenediamine dihydrochloride) was measured spectrophotometrically at 546 nm. Control tube was maintained with all chemicals excluding Cleome viscosa Linn. extract. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula

$$\text{Percentage Inhibition} = \frac{\text{Absorbance Control} - \text{Absorbance test}}{\text{Absorbance Control}} \times 100$$

Hydrogen peroxide radical scavenging activity analysis

Hydrogen peroxide radical scavenging activity was determined by the method of Ruch et al. (1984)[33]. Hydrogen peroxide (H_2O_2) is a biologically important oxidant because of its ability to generate the hydroxyl radical which is extremely potent. The ability of the hydroxyl radical to remove or add hydrogen molecules to unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems. Its very short half-life, however, restricts its diffusion capability and its potency [34, 35]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of the methanolic, aqueous and hydroalcoholic extracts of stembark of plant

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1690-1702 www.ijprajournal.com ISSN: 2249-7781

Cleome viscosa Linn.(25-200 µg/ml) in phosphate buffer were added to a H_2O_2solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. Blank solution was containing phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging of stembark of plant Cleome viscosa Linn. and Ascorbic acid as standard compound was calculated as

H_2O_2 radical scavenging activity (%) = [{Ao – A1/Ao}] ×100.

Where, Ao is the absorbance of the H_2O_2,

A1 is the absorbance of the presence of the extract in H_2O_2 solution [36, 37].

Reducing power assay [38]

The reducing power was determined according to the method of Berker et al. The methanolic, aqueous and hydroalcoholic extracts of stembark of plant Cleome viscosa Linn. (25-200 µg/ml, 2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50ºC for 20 min. After the addition of 2.5 ml of 10% trichloroacetic acid the reaction mixture was centrifuged at 3000 rpm for 10 min. About 5 ml of the upper layer was mixed with 5 ml of deionised water and 1 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power.

Statistical analysis

All the assays were carried out in triplicate and each experiment was independently repeated at least three times,through which means and standard deviations (SD) were generated. The results are presented as the average and standard error of three experiments. The data was analyzed by using Sigma plot 10.0.

Table (1): Total Phenolic Content present in extracts of stembark of plant Cleome viscosa Linn.

Plant Extracts	Absorbance mean ± SEM (n=3)	Total Phenolic content (mg gallic acid equivalent/g of dried extract)
Aqueous	0.152±0.004	50.67±0.72
Methanolic	0.285±0.003	95±1.02
Hydroalcoholic	0.188±0.002	62.67±0.90

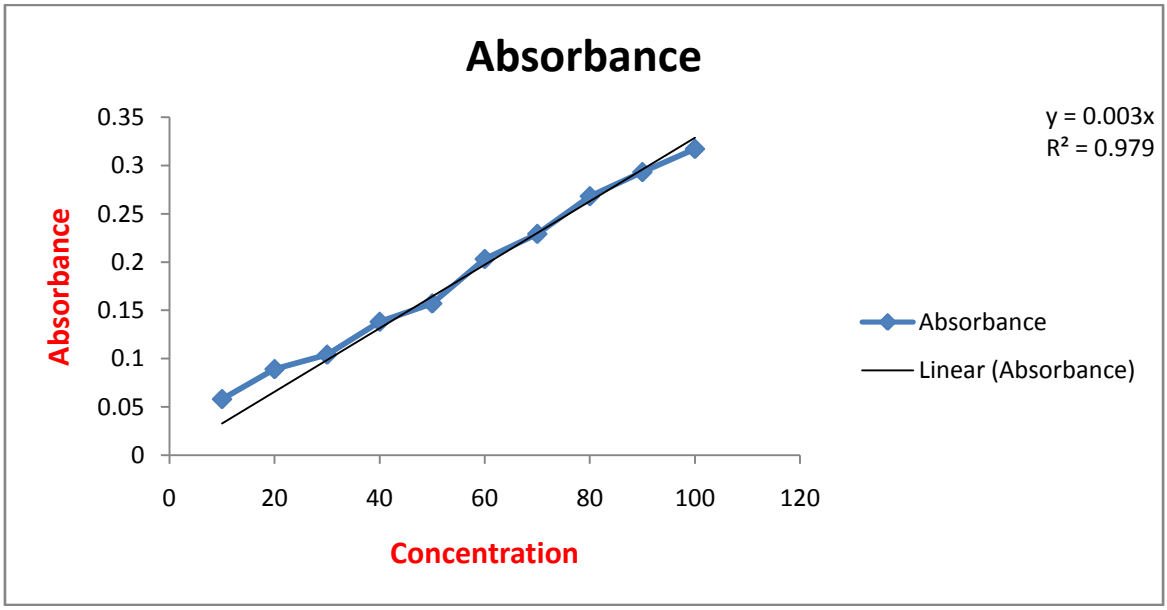

Figure (1): Calibration curve of Gallic acid

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1690-1702 www.ijprajournal.com ISSN: 2249-7781

Table (2): Total Flavonoid Content present in extracts of stembark of plant Cleome viscosa Linn.

Plant Extracts	Absorbance mean ± SEM (n=3)	Total flavonoid (mg querecetin equivalent/g of dried extract)
Aqueous	0.217±0.002	54.25±0.53
Methanolic	0.321±0.003	80.25±0.81
Hydroalcoholic	0.252±0.002	63.00±0.59

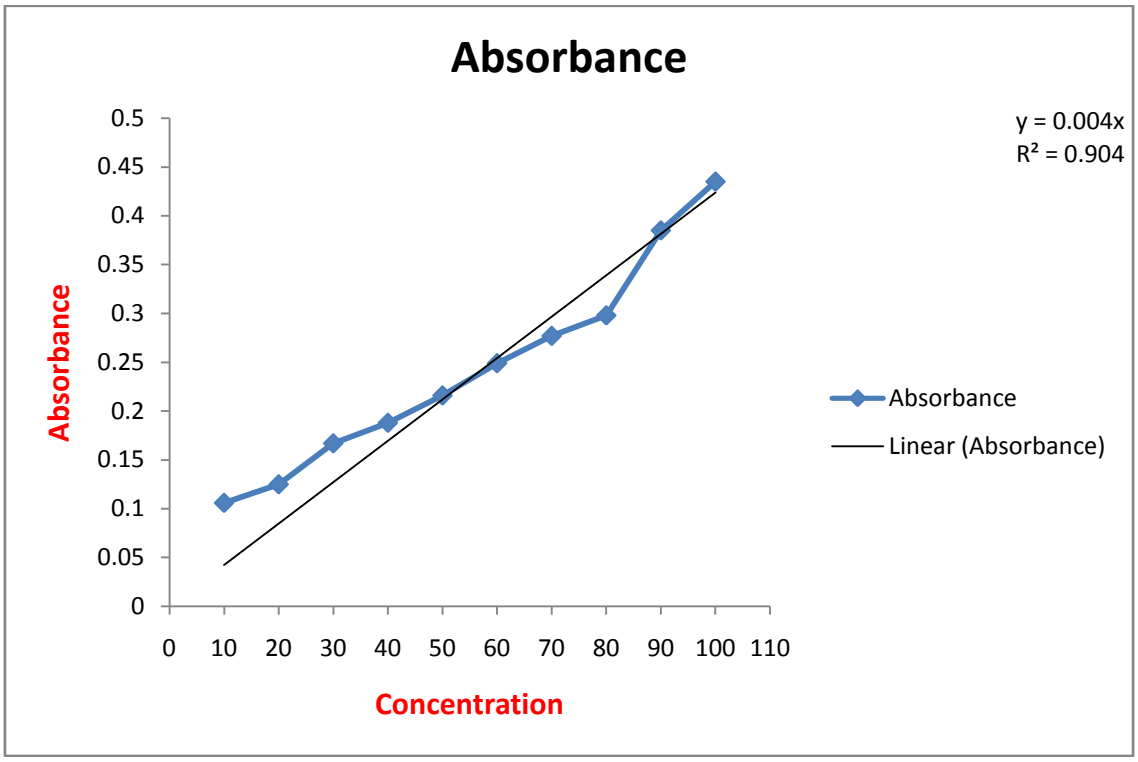

Figure (2): Calibration curve of Querecetin

Table (3): DPPH free radical scavenging activity of extracts of stembark of plant Cleome viscosa Linn.

Concentration (ug/ml)	Percentage Inhibition (Mean ± SEM) (n=3)			
	Standard ASC	MET	HAL	AQE
25	17.70±0.25	12.21±0.23	6.62±0.18	3.71±0.17
50	28.10±0.27	18.32±0.11	10.35±0.14	5.49±0.19
75	42.34±0.26	30.25±0.19	21.95±0.16	14.88±0.11
100	57.55±0.24	48.21±0.21	34.96±0.26	27.47±0.18
125	73.82±0.45	57.42±0.22	48.22±0.19	41.57±0.19
150	83.24±0.27	63.45±0.13	58.75±0.28	58.93±0.26
175	85.34±0.14	79.54±0.25	78.29±0.12	67.13±0.25
200	93.95±0.48	87.53±0.27	84.48±0.23	76.89±0.17
IC50	96.34	113.12	124.38	140.44

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1690-1702 www.ijprajournal.com ISSN: 2249-7781

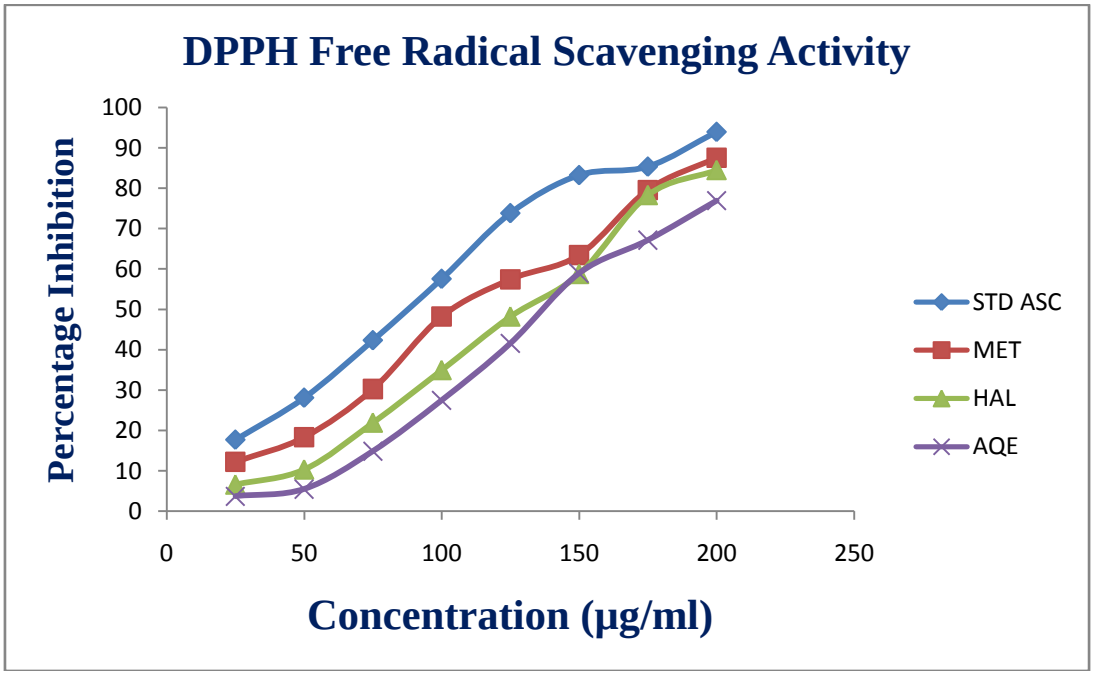

Figure (3): DPPH free radical scavenging activity of extracts of stembark of plant Cleome viscosa Linn.

Table (4): Nitric oxide free radical scavenging activity of extracts of stembark of plant Cleome viscosa Linn.

Concentration (ug/ml)	Percentage Inhibition (Mean ± SEM) (n=3)			
	Standard Ascorbic Acid	MET	HAL	AQE
25	16.22±0.08	14.58±0.21	7.37±0.28	4.45±0.11
50	33.37±0.12	23.66±0.11	16.12±0.11	11.23±0.11
75	47.8±0.18	37.72±0.16	31.95±0.26	23.72±0.11
100	63.45±0.12	47.22±0.17	37.06±0.11	35.18±0.19
125	75.42±0.14	55.53±0.18	47.56±0.11	45.88±0.18
150	81.80±0.13	69.41±0.22	57.07±0.07	53.58±0.22
175	88.22±0.07	79.77±0.15	71.17±0.12	66.78±0.11
200	93.45±0.19	88.90±0.37	79.51±0.13	74.22±0.08
IC50	93.98	109.65	127.88	138.12

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1690-1702 www.ijprajournal.com ISSN: 2249-7781

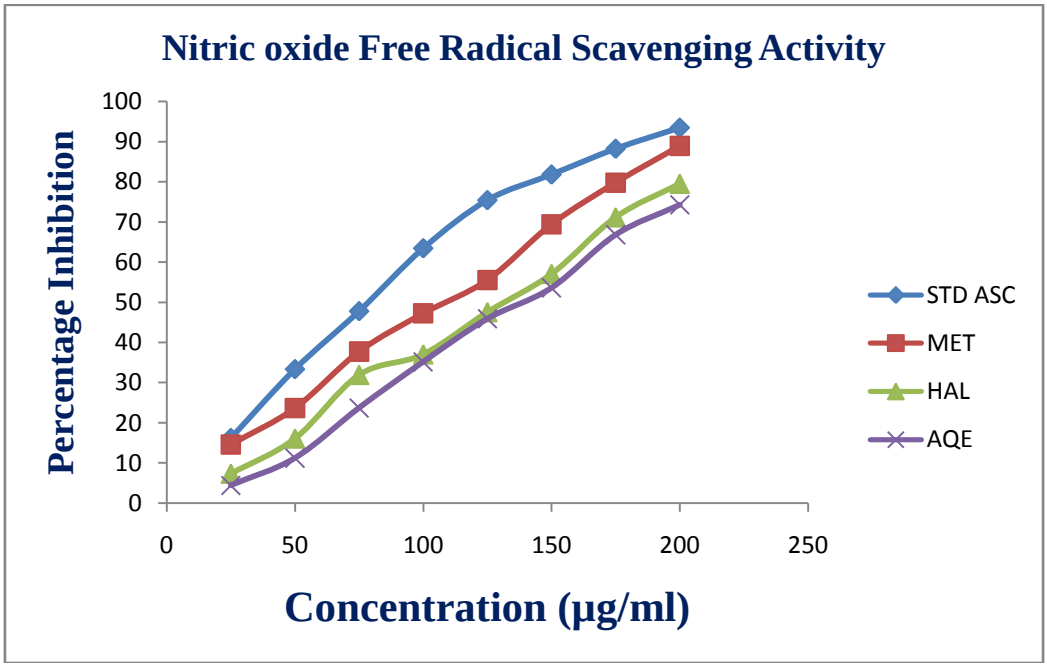

Figure (4): Nitric oxide free radical scavenging activity of extracts of stembark of plant Cleome viscosa Linn.

Table (5): Hydrogen peroxide scavenging activity of extracts of stembark of plant Cleome viscosa Linn.

Concentration (ug/ml)	Percentage Inhibition (Mean ± SEM) (n=3)			
	Standard Ascorbic Acid	MET	HAL	AQE
25	13.59±0.22	8.43±0.17	5.3±0.02	2.75±0.19
50	31.1±0.14	17.38±0.19	13.28±0.14	09.21±0.15
75	40.48±0.13	26.37±0.21	22.68±0.24	14.68±0.17
100	54.58±0.32	36.28±0.32	34.28±0.16	24.11±0.17
125	63.2±0.18	48.52±0.19	45.52±0.30	35.42±0.25
150	76.52±0.32	64.15±0.14	57.54±0.19	48.52±0.28
175	84.96±0.09	76.92±0.53	73.48±0.16	59.42±0.20
200	93.12±0.13	83.56±0.24	78.56±0.23	70.49±0.15
IC50	100.81	121.36	130.89	158.73

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1690-1702 www.ijprajournal.com ISSN: 2249-7781

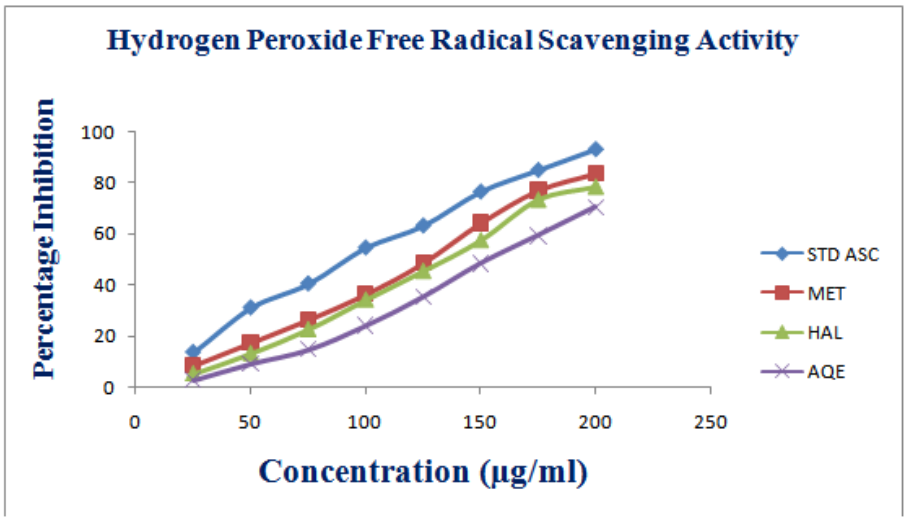

Figure (5): Hydrogen peroxide free radical scavenging activity of extracts of stembark of plant Cleome viscosa Linn.

Table (6): Reducing Power Assay activity of extracts of stembark of plant Cleome viscosa Linn.

Concentration (ug/ml)	Absorbance (Mean ± SEM) (n=3)			
	Standard ASC	MET	AQE	HAL
25	0.052±0.0023	0.035±0.0013	0.015±0.0019	0.008±0.0006
50	0.075±0.0015	0.058±0.0019	0.028±0.0006	0.04±0.0009
75	0.087±0.0023	0.072±0.0013	0.039±0.0013	0.038±0.0016
100	0.104±0.0019	0.083±0.0007	0.072±0.0019	0.053±0.004
125	0.131±0.0022	0.097±0.0019	0.084±0.0019	0.074±0.0016
150	0.146±0.0017	0.106±0.0013	0.095±0.0016	0.086±0.0019
175	0.163±0.0008	0.118±0.0004	0.13±0.0006	0.097±0.0013
200	0.177±0.0018	0.133±0.0013	0.125±0.0018	0.118±0.0017

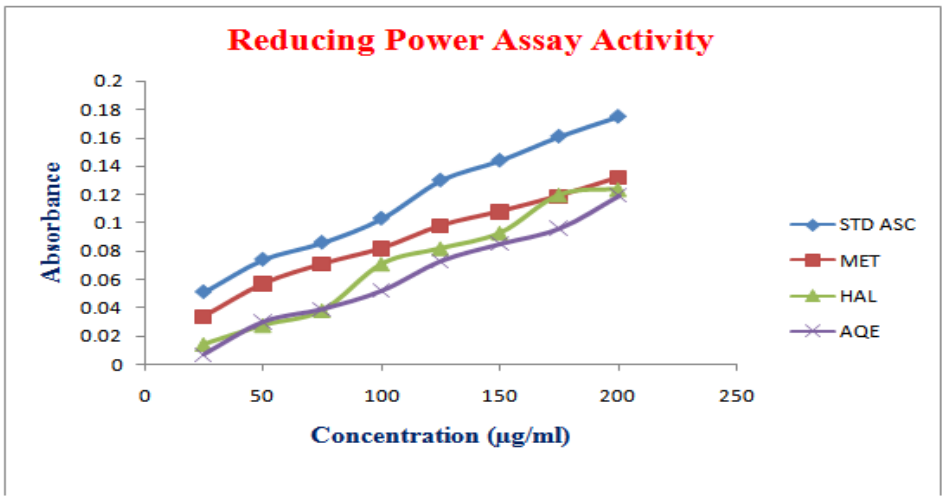

Figure (6): Reducing Power Assay activity of extracts of stembark of plant Cleome viscosa Linn.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1690-1702 www.ijprajournal.com ISSN: 2249-7781

III. RESULT

Total Phenolics Content

The Folin-Ciocalteu method is sensitive to reducing compounds, polyphenols there by producing blue colored complex. The quantative phenolics estimation was performed at 760 nm by change in intensity of Folin-phenolic compounds complex. In methanolic, hydroalcoholic and aqueous extracts of stembark of plant Cleome viscosa Linn., the total phenolic content was found to be 95µg/ml, 62.67µg/ml and 50.67 µg/ml respectively in terms of gallic acid equivalent (Table No.1 and Figure No. 1). In addition it has been determined that the highest extraction yield was found in stembark extract.

Total Flavonoids Content

The aluminium chloride forms acid stable complexes with the C-4 keto group and either with C-3 or C-5 hydroxyl group of flavones and flavonols. Aluminium chloride also forms acid labile complexes with the ortho - dihydroxyl groups in the A- or B-ring of flavonoids. In methanolic, hydroalcoholic and aqueous extracts of stembark of plant Cleome viscosa Linn., the total flavonid content was found to be 80.25 µg/ml, 63 µg/ml and 54.25 µg/ml respectively in terms of quercetin equivalent (Table No. 2 and Figure No. 2).

DPPH radical scavenging activity

The DPPH assay is purely based on the assumption that an antioxidant serve as a hydrogen donor and thus reduces the DPPH free radicals (the color turns from purple to yellow). This assay is known as a basic and quick tool to carry out evaluation of antioxidant activity of plant extracts. The antioxidant potency of a compound is relative to loss of DPPH free radicals that can be quantified through a decrease in the maximum absorption of DPPH at 517 nm. In this study, results showed that all plant extracts had significant levels of radical scavenging activity in a dose dependent manner (Table No. 3 and Figure No. 3). The DPPH-derived IC_{50} values of plant extracts are also illustrated in Table No.3 The methanolic, hydroalcoholic and aqueous exract of plant inhibited DPPH upto 87.53%, 84.48% and 76.89% at concentration 200ug/ml. Amongst the plant extracts of Cleome viscosa Linn., methanolic extract was found to be the most potent DPPH scavengers, as they could inhibit DPPH free radicals up to 87.53% at 200ug/ml concentration compared to the rest aqueous and hydroalcoholic extract whereas standard Ascorbic acid were shows 93.95% of DPPH scavenging activity.

Nitric oxide radical scavenging assay

Nitric oxide is an unstable free radical which involved in many biological processes and associated with several diseases. It react with oxygen to produce stable product nitrate and nitrite through intermediates and high concentration of nitric oxide can be act as toxic and inhibition of over production is an important goal. In this study, results showed that all plant extracts had significant levels of radical scavenging activity in a dose dependent manner (Table No. 4 and Figure No. 4). The Nitric oxide-derived IC_{50} values of plant extracts are also illustrated in Table 4. The methanolic, hydroalcoholic and aqueous extract of plant inhibited Nitric oxide upto 88.90%, 79.51% and 74.22%, at concentration 200ug/ml.. Amongst the plant extracts of Cleome viscosa Linn., methanolic extract was found to be the most potent Nitric oxide scavengers, as they could inhibit Nitric oxide free radicals up to 88.90% at 200 ug/ml concentration compared to the rest aqueous and hydroalcoholic extract whereas standard Ascorbic acid were shows 93.45% of Nitric oxide scavenging activity.

Hydrogen peroxide scavenging activity

Scavenging of H_2O_2 by extracts may be attributed to their polyphenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The ability of plant extracts to effectively scavenge hydrogen peroxide, determined according to the method of Ruch, where they are compared with that of tocopherol as standard (Ruch et al., 1984). The plant extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner (Table No. 5 and Figure No. 5). Although hydrogen peroxide itself is not very reactive, but sometimes it can cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removal of H_2O_2 is very important living systems. The Hydrogen peroxide-derived IC_{50} values of plant extracts are also illustrated in Table 5. The methanolic, hydroalcoholic and aqueous exract of plant inhibited Nitric oxide upto 83.56%, 78.56% and 70.49% at concentration 200ug/ml.. Amongst the plant extracts ofCleome viscosa Linn., methanolic extract was found to be the most potent Hydrogen peroxide scavengers, as they could inhibit Hydrogen peroxide free radicals up to 83.56% at 200ug/ml concentration compared to the

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1690-1702 www.ijprajournal.com ISSN: 2249-7781

rest aqueous and hydroalcoholic extract whereas standard Ascorbic acid were shows 93.12% of Hydrogen peroxide scavenging activity.

Reducing Power Assay

Reducing capability of an antioxidant substance can be assessed using its ability to convert Fe^{3+} to Fe^{2+}. Intensity of Perl's Prussian blue color caused by this reduction is measured at 700 nm. Higher absorbance indicates higher reducing power. The reducing power of the compound can be contributed to its antioxidant potency. The reducing power assay of the plant extracts of Cleome viscosa Linn. were tested in this study illustrated in Table No. 6 Figure No. 6. The findings revealed that the values of reducing power of the plant extracts of Cleome viscosa Linn. were functions of their concentrations. In this study, results showed that all plant extracts had significant levels of Reducing Power activity in a dose dependent manner. At concentration 200ug/ml, methanolic, aqueous and hydroalcoholic extract had reducing power values of 0.133, 0.1325 0.118 as compare to standard Ascorbic acid 0.177. At this concentration, methanolic extractshowed a remarkable reducing power that was significantly greater than those of the hydroalcoholic and aqueous extract as compared to standard ascorbic acid.

IV. DISCUSSION

Polyphenolic compounds are known as powerful chainbreaking antioxidant[39]. They are important constituentsin plant because of their scavengingability, which is because of their hydroxyl groups[40].

Flavonoids are called as polyphenolic compounds,which exhibit several biological effects such as antioxidant, antiulcer, anticancer, anti-inflammatory, hepatoprotectiveactivities[41]. Flavonoids are capable ofscavenging the reactive O_2 species because of theirphenolic hydroxyl groups and therefore called as potent antioxidants[42].

In the present study several biochemical constituents and free radical scavenging activities of extracts ofplant Cleome viscosa Linn. were evaluated. Free radicals are involved in several disorders like inflammation, cancer, neurodegenerative diseases and AIDS. Antioxidant activity due to their scavenging activity and they areuseful for the control and management of these diseases. DPPH is a stable free radical which is a sensitive way to determine the antioxidant property of plant extracts[43, 44].

The DPPH method as antioxidant activity was evidently introduced nearly 50 years ago by Blois and is used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant potency. The parameter IC_{50} is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color)[45].

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons and other cells. It is involved in the regulation of various physiological processes. Excess concentration of Nitric oxide is always associated with several diseases. Nitric oxide is generated in biological tissues by specific nitric oxide synthesis (NOSs), which metabolizes arginine to citralline with the formation of Nitric oxide via a five electron oxidative reaction. These compounds are responsible for altering the structural and functional behavior of many cellular components[46].

The degree of inhibition of the Nitric oxide free radicals was found to be increased in increasing concentration of the Cleome viscosaextracts, this indicates that the extract may contain compounds capable of inhibiting the generation of nitric oxide and offers scientific evidence for the use of Cleome viscosain the treatment of various diseases. The antioxidant principles present in the extract, which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. It is to be noted that Cleome viscosagreater inhibition comparative to other plant extracts but less than ascorbic acid which has shown good inhibition of Nitric oxide.

Hydrogen peroxide is a weak oxidizing agent and because of its ability to penetrate biological membranes, once inside the cell it can probably react with Fe^{2+} and Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects [47].

All the extracts of Cleome viscosawhen added to the reaction mixture scavenge hydroxyl radicals in a concentration dependent manner. The scavenging activity of the hydroxyl radicals may be due to the presence of polyphenolic compounds in the extracts which can donate electrons to H_2O_2, thus neutralizing it to water.

The reducing capacity of a extract may act as a significant indicator of its potential antioxidant activity. However, the activities of antioxidants

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1690-1702 www.ijprajournal.com ISSN: 2249-7781

have been attributed to various mechanisms such as prevention of chain initiation, degradation of peroxides, reducing capacity and radical scavenging[48].

V. CONCLUSION

In the present study, we focused on total phenolic contents, total flavonoid contents and antioxidant property of crude extracts of Cleome viscosa. Generally, the results of this study showed that plant Cleome viscosa have antioxidant property with their radical scavenging and reducing power. In this study, high to moderate positive correlations were observed between the total phenolic contents, total flavonoid contents and antioxidant properties in different in vitro antioxidant assays. As the plant Cleome viscosa promising source of natural antioxidant agent, further studies on isolation and characterization of the active antioxidant phenolic and flavonoid compound should be carried out.

The results would help to determine the potency of the methanolic, hydroalcoholic and aqueous extract from plant Cleome viscosa as a potential basis of natural antioxidants.Further studies are suggested for the isolation and characterization of antioxidant compounds from plant Cleome viscosa. It will also leads to in vivo studies are needed for understanding their mechanism of action as antioxidants.

VI. ACKNOWLEDGEMENT

The Corresponding author is very grateful to Principal Dr. Rajendra D. Wagh, DCS's A.R.A. College of Pharmacy, Nagaon, Dhule, for providing necessary facilities, support and proper guidance throughout the antioxidant activity.

CONFLICTS OF INTEREST

The authors have no conflicts of interest regarding this research.

REFERENCES

[1]. Aher Vaibhav D., Wahi Arunkumar, Pawdey Abhijit M. & Sonawane A. (2011). Antioxidants as Immunomodulator: an expanding research avenue, International journal of current pharmaceutical research. 3(1):8-10.

[2]. Naphade S. S., Khadabadi S.S., Deore S.L., Jagtap N.S. & Hadke S.P. (2009). Antioxidant Activity of different extracts of plant Tricholepsi Glaberrima DC (Asteraceae), International Journal of Pharm Tech Research. 1(3):502-505.

[3]. Mohammad hassanzadeh-khayyat, Mehdi mojarab & Seyyed ahmad emami. (2009). Antioxidant activity of methanolic extracts of different species of Artemisia from Iran, Pharmacology online, 2:797-807.

[4]. Youwei Zeng, Zhao Jinlian & Peng Yonghong. (2008). A comparative study on the free radical scavenging activities of some fresh flowers in Southern China. LWT-Food Sci. Technol. 41:1586-1591.

[5]. Hausladen A. and Stamer JS. (1999). Nirosative stress. Method in Enzymology. 300:389-395.

[6]. W. Arika, C. M. Kibiti, J. M. Njagi, and M. P. Ngugi, "In vitro antioxidant properties of dichloromethanolic leaf extract of Gnidia glauca (Fresen) as a promising antiobesity drug," Journal of Evidence-Based Integrative Medicine, vol. 24, 2019.

[7]. Scalbert A., Manach C., Morand C., Rémésy C.&Jiménez L. (2005). Dietary polyphenols and the prevention of diseases critical. Reviews in Food Sciences and Nutrition. 45: 287-306.

[8]. Kikuzaki H. & Nakatani N. (1993). Antioxidant effects of some ginger constituents. Journal of Food Science. 58(6) :1407-1410.

[9]. Ottolenghi A. (1959). Interaction of ascorbic acid and mitochondria lipids. Arch. Biochem. Biophy. 79:355-363.

[10]. Gyamfi M. A., Yonamine M.&Aniya Y. (2002). Free radical scavenging action of medicinal herbs from Ghana Thonningia sanguine on experimentally induced liver injuries. Gen. Pharmacol. 32: 661-667.

[11]. Osawa T. Postharvest biochemistry. In:Uritani I,, Garcia VV, Mendoza, E. M. (1994). Novel neutral antioxidant for utilization in food and biological systems. Japan: Japan Scientific Societies Press. 241-251.

[12]. Noda Y., Anzai K., Mori A., Kohno M., Shinmei M.&Packer L. (1997). Hydroxyl and superoxide anion radical scavenging activities of natural source antioxidants using the computerized JES-FR30 ESR spectromoter system. Biochem. Mol. Biol. Inter. 42:35-44.

[13]. Ahmad I, Mehmood Z & Mohammad F. (1998). Screening of some Indian

medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 62:183-193.

[14]. Srinivasan R., Chandrasekar M. J. N, Nanjan M. J. & Suresh B. (2007). Antioxidant Activity of Caesalpinia Digyna Root. Journal of Ethnopharmacology, 113:284-291.

[15]. Kumaraswamy M. V. & Satish S. (2008). Antioxidant and Anti-Lipoxygenase activity of Thespesialampas Dalz & Gibs, Advan. Biol. Res., 2(3-4):56-59.

[16]. Badami S., Gupta M. K. & Suresh B. (2003). Antioxidant activity of ethanolic extract of Striga orobanchioides, J. Ethnopharmacol., 85:227-230.

[17]. Zhou Y. C. & Zheng R. L. (1991). Phenolic compounds and an analog as superoxide anion scavengers and antioxidants. Bioch. Pharm. 2:1177-1179.

[18]. Quinn L. A. & Tang H. H. (1996). Antioxidant properties of phenolic compounds in macadamia nuts. J. Am. Oil Chem. Soc. 73:1585-1588.

[19]. Seymour T. A., Li S. J. & Morrissey M. T. (1996). Characterization of a Natural Antioxidant from Shrimp Shell Waste. J. Agri. Food. Chem. 44:682-685.

[20]. Prasad K., Laxdal V. A., Yu M. & Raney B. L. (1996). Evaluation of hydroxyl radical scavenging property of garlic. Mol. C. Bioch. 154:55-63.

[21]. Aruoma O. I. (1994). Nutrition and health aspects of free radicals and antioxidants. Food Chem. Toxic. 32:671-683.

[22]. Cao G., Srfie E. & Prior R. L. (1996). Antioxidant capacity of tea and common vegetables. Journal of Agricultural and Food Chemistry. 44:3425-3431.

[23]. Kaur C. & Kapoor H. C. (2002). Antioxidant activity and phenolic content of some Asian vegetables. International Journal of Food Science and Technology. 37:153-161.

[24]. Auddy B., Ferreira M., Blasina F., Lafon L., Arredondo F. & Dajas F. (2003). Screening of antioxidant activity of three Indian medicinal plants traditionally used for the management of neurodegenerative diseases. Journal of Ethnopharmacology. 84:131-138.

[25]. Kirtikar A. R. & Basu B. D. (2005). Indian Medicinal Plants. 2nd ed. Dehradoon: India; 510-512.

[26]. Harpreet Singh, Amrita Mishra & Arun Kumar Mishra. (2015). Cleome viscosa Linn. (Capparaceae): A Review, Pharmacognosy Journal. 7(6):325-329.

[27]. Ravindra G. Mali. (2010). Cleome viscosa (wild mustard): A review on ethnobotany, phytochemistry, and pharmacology, Pharmaceutical Biology. 48(1):105–112.

[28]. Lenkalapally Matsyagiri, Kommu Sudhakar, Takkadpelliwar Santoshi, Masna Nagarjuna & N. L. Gowrishankar. (2012). A Review on Medicinal Uses and Pharmacological Effects of Cleome viscose, Research J. Pharmacognosy and Phytochemistry. 4(1):44-48.

[29]. Singleton V. L., Orthofer R. & Lamuela-Raventós R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent. Methods Enzymol. 299:152-178.

[30]. Zhishen J., Mengcheng T. & Jianming W. (1999). Research on antioxidant activity of flavonoids from natural materials. Food Chem. 64:555-559.

[31]. Molyneux P. (2004). The Use of Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity. J Sci Technol. 26:211-19.

[32]. R. Govindarajan, S Rastogi, &M Vijayakumar. (2003). Studies on the antioxidant activities of Desmodium gangeticum. Biol Pharm Bull. 26:1424-1427.

[33]. Pal R. & Girhepunje K. (2012). Antioxidant and free radical scavenging activity of ethanolic extract of the root of Morinda citrifolia. African journal of pharmacy and pharmacology. 6(5):278-282.

[34]. Priscilla M. Clarkson & Heather S. Thompson. (2000). Antioxidant: what role do they play in physical activity and health, The American journal of clinical nutrition. 72(2):637S-646S.

[35]. Fereidoon Shahidi. (2005). Antioxidant: Science, technology, and Applications, Bailey"s industrial oil and fat products, 6th edition; 6:431-489.

[36]. Chaulya N. C., Haldar P. K. & Mukherjee A. (2010). In Vitro Free Radical Scavenging Activity Of Methanolic Extract Of Rhizome Of Cyperus Tegetum Roxb.(Cyperaceae).International Journal

of Current Pharmaceutical Research. 2(3):39-43.

[37]. Buricova L., Andjelkovic M., Cermakova A. (2011). Antioxidant capacity and antioxidants of strawberry, blackberry and raspberry leaves. Czech J. food sci. 29(2):181-189.

[38]. Berker K. I., Güçlü K., İ Tor, B. Demirata, &R Apak. (2010). Total antioxidant capacity assay using optimized ferricyanide/Prussian blue method, Food Anal Methods. 3:154-68.

[39]. Shahidi F. & Wanasundara PKJPD. (1992). Phenolic antioxidants. Food Sci. Nut.32(1):67-103.

[40]. Hatano T., Edamatsu R., Mori A., Fujita & Yasuhara E. (1989). Effect of interaction of tannins and related poyphenols on superoxide anion radical and on DPPH radical. Chem Pharm Bull. 37:2016-2021.

[41]. Umamaheswari & Chatterjee T. K. (2008). In vitroantioxidant activities of the fractions of Coccinia grandis L. leaf extract. Afr. J. Traditional, complementary and Alternative Medicines. 5(1):61-73.

[42]. Cao G., Sofic E., Prior R. L. (1997). Antioxidant and pro-oxidative behavior of flavonoids: Structure activity relationships. Free Radical.Biol. Med. 22:749-760.

[43]. Koleva I. I., Van Beek T. A.& Linssen J. P. H. (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal. 13:8-17.

[44]. Kumar P. S., Sucheta S., Deepa V. S.& Selvamani P. (2008). Antioxidant activity in some selected Indian medicinal plants. Afr. J. Biotechnol. 7:826-1828.

[45]. Blois M. S. (1958). Antioxidant determinations by the use of a stable free radical. Nature. 181:1199–1200.

[46]. Parul R., Kundu S. K. & Saha P. (2013). In Vitro Nitric Oxide Scavenging Activity Of Methanol Extracts Of Three Bangladeshi Medicinal Plants. The Pharma Innovation – Journal. 1(12):83-88.

[47]. Halliwell B. & Gutteridge J.M.C. (2007). Free radicals in biology and medicine. 4th Edition, Oxford University Press, New York:10-15.

[48]. Yıldırım A., Mavi A., Oktay M. & Kara A. A. (2000). Comparision of antioxidant and antimicrobial activities of Tilia (Tilia argentea Desf Ex DC), Sage (Saviatriloba L.) and Black tea (Camellia sinensis) extracts. J Agric Food Chem. 48(10): 5030-5034.