WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 6, 973-988. Research Article ISSN 2277 – 7105

QUANTITATIVE ANALYSIS AND *IN VITRO* FREE RADICAL SCAVENGING ACTIVITY OF *CAYRATIA TRIFOLIA* (L.)

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Article Received on
16 June 2014,

Revised on 11 July 2014,
Accepted on 06 August 2014

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ABSTRACT

Cellular damage caused by reactive oxygen species has been implicated in several diseases and hence antioxidants have significant importance in human health. The present study was to evaluate the quantitative analysis and *in vitro* free radical scavenging activity of stem ethanolic extract of *Cayratia trifolia*. The content of total phenols, tannins, saponins, flavonoids, alkaloids analyzed. Free radical activity was assayed using 1,1-diphenyl-2-picryl hydroxyl (DPPH), 2,2' azinobis-3 ethylbenzothiozoline-6 sulfonic acid (ABTS+) cation decolourization test, hydroxyl radical (OH.), hydrogen peroxide assay (H_2O_2), nitric oxide radical inhibition activity (NO), superoxide radical, metal chelating assay, ferric reducing antioxidant power assay (FRAP), reducing power activity using established assay procedure. The ethanolic extract of *Cayratia trifolia* exhibited major content of secondary metabolites present in the stem extract. The antioxidant activity of the extract was compared with standard ascorbic acid. In conclusion, the results presented in the stem of *Cayratia trifolia* have a strong antioxidant property against free radicals and it may serve as a good pharmacological property.

KEY WORDS: *Cayratia trifolia*, Ethanolic extract, Quantitative analysis, Free radical scavenging activity.

INTRODUCTION

Free radicals are incessantly produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function [1]. These free radicals are usually produced through aerobic respiration. Although the human body produces antioxidant enzymes to neutralize the free radicals [2]. When the generation of ROS overtakes the antioxidant defense of the cells the free radicals start attacking cellular proteins, lipids and carbohydrates leading to the pathogenesis of many disorders including arthritis and connective tissue disorders, liver disorders, neurodegenerative disorders, cardiovascular disorders, diabetes, chronic inflammation, mutagenesis, carcinogenesis and in the process of ageing [3]. Antioxidants provide protection for living organisms from damage caused by uncontrolled production of reactive oxygen species (ROS) and the concomitant lipid peroxidation, protein damage and DNA strand breaking [4].Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases. This activity is due to the ability of antioxidants to reduce oxidative stress by neutralizing or scavenging of reactive species by hydrogen donation [5].Recent studies have confirmed that free radicals would damage nearby structures including DNA, proteins or lipids. Radical scavenging antioxidants are mainly significant in protecting cells from the injury of free radical [6]. Thus, antioxidants with free radical scavenging activities may have enormous significance in the prevention and therapeutics of diseases [7].

Natural products derived from food and medicinal plants are the potential sources of antioxidant molecules [8]. Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [9]. These phytochemicals have been found to act as antioxidants by scavenging free radicals and many have therapeutic potential for free radical associated disorders [10]. *Cayratia trifolia* Linn. Domin syn (Vitaceae) is native of India, Asia and Australia. It is a weak herbaceous climber having trifoliated leaves with (2-3 cm), long petioles and ovate to oblong-ovate leaflets. Flowers are small greenish white brown in color [11]. The ethanolic extract of *Cayratia trifolia* possesses a good free radical scavenging activity which may due to the presence of alkaloids and flavonoids [12]. The whole plant is used as diuretic, in tumors, neuralgia and splenopathy, leucorrhea. The paste of tubers is applied on the affected part in the treatment of snake bite. It is reported to possess antiviral, antibacterial, antiprotozoal, hypoglycaemic, anticancer and

diuretic activity etc [13]. This plant also contains kaempferol, myricetin, quercetin, triterpenes and epifriedelanol [14]. The aim of the study is to investigate quantitative analysis and *in vitro* free radical scavenging activity of stem ethanolic extract of *Cayratia trifolia*.

MATERIALS AND METHODS

Plant collection

Cayratia trifolia was collected from in and around area of Poonthottam, Thanjavur district, Tamil Nadu, India. The plant was authenticated by Dr. P. Sathyanarayanan, Botanical Survey India, TNAU Campus, Coimbatore. The voucher number is BSI/SRC/5/23/2010-2011/Tech.1527. Fresh leaf, stem and fruit plant materials were washed under running tap water, air dried and powdered.

Preparation of ethanolic extract

50 g of powdered plant material was weighed and extracted with 250 ml of ethanol for 72 hours using occasional shaker. The supernatant was collected and concentrated at 40°C. It was stored at 4°C in air tight bottles for further studies.

Quantitative analysis of secondary metabolites

Estimation of Total Phenol

Total phenolic content was carried out by the method of Singleton and Rossi [15]. The sample extract (0.1 ml) was mixed with distilled water (3 ml) and 0.5 ml of Folin-Ciocalteu reagent was added. After 3 minutes 2 ml of 20% sodium carbonate was added and mixed thoroughly. The tubes were incubated in a boiling water bath for exactly one minute. It was then cooled and the absorbance was measured at 650 nm using spectrophotometer against the reagent blank. Standard curve of gallic acid solution (10, 20, 40, 60, 80 and 100 ppm) was prepared using the similar procedure and the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract.

Estimation of Total Tannin

Total tannin content was determined in the method of Schendrel, [16]. 0.2-1.0 ml of standard tannic acid solution was pipetted out in to a series of test tubes. To another test tube 0.5 ml of extract solution was taken. The volumes of all the tubes were made up to 3.0 ml with distilled water. 3.0 ml of distilled water was taken as blank. To all the tubes added 2.0 ml of 20% Na_2CO_3 followed by the addition of 0.5 ml of Folin-Ciocalteu reagent and incubated at room

temperature for 30 minutes. The absorbance was read against reagent blank at 700 nm. From the standard graph, the amount of tannin present in the sample was calculated.

Determination of Saponins

20 g of plant powder was placed into a conical flask and 100 ml of 20 % aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The combined extract was reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and saponin content was calculated as percentage [17].

Estimation of Total Flavonoid

Total flavonoid content was determined using the method of Ordon *et al*., [18]. 0.5 ml of 2% $AlCl_3$ in ethanol solution was added to 0.5 ml of sample solution. After one hour incubation at room temperature, yellow colour was developed. This was measured at 420 nm with UV-Visible spectrophotometer. A standard graph was prepared using the quercetin and the total flavonoid content was expressed as quercetin equivalent (mg/g).

Determination of total alkaloid

The alkaloid content of sample was determined as described by Harborne [19]. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. The mixture was filtered through Whatman no 1 filter paper and the filtrate concentrated to ¼ of its original volume on a water bath maintained at 90°C. Alkaloid was precipitated from each sample, using a concentrated ammonium hydroxide solution (NH_4OH) and then allowed to sediment. The whole solution was allowed to settle and the precipitated was collected and washed with concentrated NH_4OH and then dried in a hot air oven.The residue is alkaloid and is calculated thus: (%) Alkaloid = W2-W1 / W x 100, Where, W1 = Initial weight before drying, W2 = Final weight after drying, W = weight of sample

***In vitro* free radical scavenging activity**

DPPH radical scavenging assay

The scavenging activity for DPPH free radicals was measured according to the procedure described by Blios [20]. Methanol solution of the sample extract at various concentrations (100, 200, 300, 500 and 500 µg/mL) was added separately to each 5 mL of 0.1 mM methanolic solution of DPPH and allowed to stand for 20 min at 27ºC. After incubation, the absorbance of each solution was determined at 517 nm using spectrophotometer. Ascorbic acid was used as standard. The corresponding blank reading was also taken and DPPH radical scavenging activity was calculated by using the following formula: % Radical scavenging activity = [Control OD – Sample OD)/ Control OD] × 100. The percentage inhibition versus concentration was plotted and the concentration required for 50% inhibition of radicals was expressed as IC_{50} value.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the ethanolic extracts of *Cayratia trifolia* was measured with a slight modification of Elizabeth and Rao [21]. All the solutions were freshly prepared. The 1 mL reaction mixture contained, 2-deoxy-2-ribose (2.8 mM); KH_2PO4-KOH buffer (20 mM, pH 7.4); $FeCl_3$ (100 µM); EDTA (100 µM); H_2O_2 (1.0 mM); ascorbic acid (100 µM) and various concentrations (40-200 µg/mL) of the test sample. After incubation for 1 h at 37ºC , 0.5 mL of the reaction mixture was added to 1 mL of 2.8% TCA, then 1 mL of 1% aqueous TBA was added and the mixture was incubated at 90ºC for 15 minutes to develop the color. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All tests were performed six times. Ascorbic acid was used as a positive control. Percentage of inhibition was evaluated by comparing the test and blank solutions.

Superoxide radical scavenging activity

The superoxide scavenging activity of the ethanolic extracts of *Cayratia trifolia* was measured by reduction of nitroblue tetrazolium (NBT) method of Fontana *et al*., [22]. Briefly, Tris HCl buffer (3 mL, 16 mM, pH 8.0) was mixed with 1 ml NBT (50 µM) solution, 1 ml NADH (78 µM) solution and the plant extract (40-200 µg/mL). The reaction was initiated by the addition of 1 mL of phenazine methosulfate (PMS) solution (10 µM) to the mixture. The reaction mixture was incubated at 25ºC for 5 min and the absorbance was read at 560 nm

against the subsequent blank sample. All tests were performed three times. Ascorbic acid was used as a control.

Nitric oxide radical scavenging activity

The Nitric oxide was generated by sodium nitroprusside and measured by the Griess Illosvoy reaction by the method of Garratt [23]. The reaction mixture contained 10 mM SNP, phosphate buffered saline (pH 7.4) and various doses (40-200 µg/mL) of the test solution in a final volume of 3 ml. After incubation for 150 min at 25ºC, 1 mL sulfanilamide (0.33% in 20% glacial acetic acid) was added to 0.5 mL of the incubated solution and allowed to stand for 5 min. Then 1 ml of napthylethylenediamine dihydrochloride (NED) (0.1% w/v) was added and the mixture was incubated for 30 min at 25ºC. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with NED was measured spectrophotometrically at 540 nm against a blank sample. All tests were performed three times. Ascorbic acid was used as a standard reference.

Hydrogen peroxide radical scavenging activity

The ability of the *Cayratia trifolia* extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*., [24]. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both *Cayratia trifolia* extracts and standard compounds were calculated: % Scavenged [H_2O_2] = [(A_C – A_S)/A_C] x 100 Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of *Cayratia trifolia* extract or standards.

Metal chelating activity

The chelating of ferrous ions by various extracts of *Cayratia trifolia* was estimated by the method described by Dinis *et al*., [25]. Various concentrations of the extracts viz., 100, 200, 300, 400, 500 µg/mL of *Cayratia trifolia* were added with 1 mL of 2mM $FeCl_2$ separately. The reaction was initiated by the addition of 5mM ferrozine (1mL). Absorbance was measured at 562nm after 10min. Ascorbic acid was used as standard. Chelating activity (%) = [Control OD – Sample OD)/ Control OD] × 100.

ABTS radical cation scavenging activity

The ABTS radical cation scavenging activity was performed with slight modifications described by Re *et al.*, [26]. The $ABTS^+$ cation radicals were produced by the reaction between 7mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. prior to use, the solution was diluted with ethanol to get an absorbance of 0.700 ± 0.025 at 734 nm. Free radical scavenging activity was assessed by mixing 10 µl of test sample with 1.0 ml of ABTS working standard in a microcuvette. The decrease in absorbance was measured exactly after 6 min. the percentage inhibition was calculated according to the formula: $[(A_0-A_1)/A_0]\times100$, where A0 was the absorbance of the control, and A1 was the absorbance of the sample.

Reducing power assay

The reducing power capacity of the plant was assessed by the modified method of Oyaizu [27]. Various concentrations (40-200 µg/mL) of the extract (0.5 mL) were mixed with 0.5 mL phosphate buffer (0.2 M, pH 6.6) and 0.5 ml potassium hexacyanoferrate (0.1%), following 50ºC incubation in a water bath for 20 minutes. After incubation, 0.5 mL of TCA (10%) was added to end the reaction. The upper portion of the solution (1 mL) was mixed with 1 ml distilled water, and 0.1 mL $FeCl_3$ solution (0.01%) was added. The reaction mixture was left for 10 min at room temperature and the absorbance was measured at 700 nm against a suitable blank solution. All tests were performed six times. A higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as a positive control.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was used to estimate the reducing capacity of plant extracts, according to the method of Benzie and Strain [28]. The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3.6H_2O$ and 25 mL of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed at 37ºC. 900 µl FRAP reagent was mixed with 90 µl water and 30 µl of the extract. The reaction mixture was incubated at 37ºC for 30 minutes and the absorbance was measured at 593 nm.

RESULTS AND DISCUSSION

Natural antioxidants such as phenols, flavonoids, alkaloids and tannins are increasingly attracting attention because they are having qualities of disease-preventing, health-promoting and anti-ageing substances [29].The beneficial effects derived from phenolic compounds have

been attributed to their antioxidant activity [30]. Phenolics content are very important plant constituents because they can act as reducing agents, hydrogen donors and metal chelator [31]. They also act as radical scavenger due to their hydroxyl groups. Flavonoids show their antioxidant action through scavenging or chelating process [32]. Previous studies revealed that the *Cayratia trifolia* is medicinally important and used in the treatment of various diseases [33].

Total phenol, tannin, alkaloid, flavonoid and saponin contents were estimated in stem ethanolic extract of *Cayratia trifolia* which is showed in table 1. Maximum amount of phenols were found in stem ethanolic extract of *Cayratia trifolia*. The highest tannin content was found in stem (54.52 ± 0.3 mg/g) extract of *Cayratia trifolia*. Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. The values of flavonoid content is 26.07 ± 0.40 mg/g. Alkaloid shows highest amount in fruit when compared with other parts of the plant. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [31].

Quantitative analysis of stem ethanolic extracts of *Cayratia* *trifolia*

S.No	**Parameters**	**Contents**
1	Phenol (mg/g)	34.97 ± 0.4
2	Tannin (mg/g)	54.52 ± 0.3
3	Saponin (mg/g)	39.52 ± 0.50
4	Flavonoid (mg/g)	26.07 ± 0.40
5	Alkaloid (mg/g)	33.74 ± 0.68

Values are expressed as Mean±SD (n=3)

DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. DPPH is usually used as a substrate to evaluate the antioxidant activity of antioxidants [34]. It has been reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect or scavenge them, forms the pathological basis of several chronic disease conditions [35, 36]. The IC_{50} value of stem ethanolic extract of *Cayratia trifolia* and ascorbic acid were found to be 430 µg/mL and 345 µg/mL respectively which are shown in Figure 1.

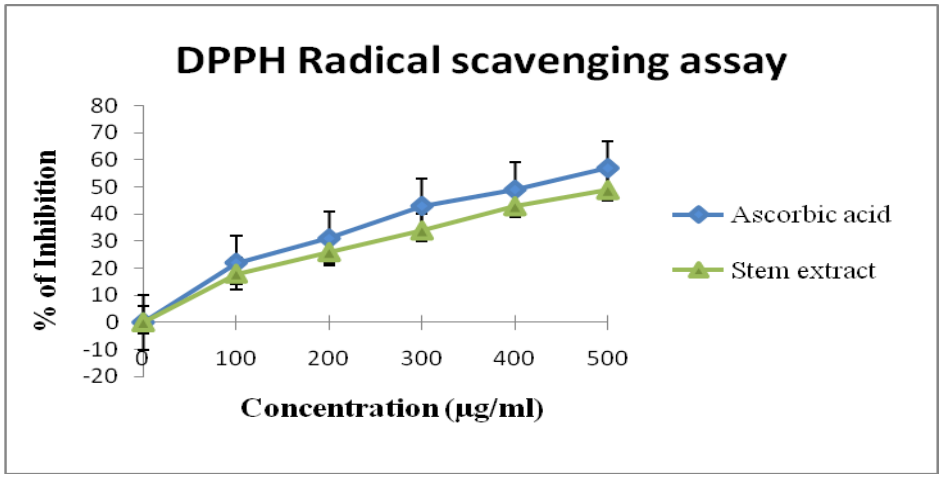

Figure 1-DPPH radical scavenging activity

$ABTS^+$ radical, a protonated radical has characteristic absorbance maximum at 734 nm which decreases at the scavenging of proton radical which is known as excellent substrate for peroxidases frequently used to study antioxidant properties of natural compounds [37]. Figure 2 depicts the $ABTS^+$ radical scavenging activity of ethanolic extract as well as standard compound. The percentage inhibition of the extract was found to be 315 µg/mL (stem) and 275 µg/mL (ascorbic acid). Nitric oxide is a very unstable species, so under aerobic condition it can react with O_2 to produce its stable products such as nitrate and nitrite through intermediates NO_2, N_2O_4. In the presence of a scavenging test compound, the amount of nitrous acid will decrease and can be measured at 546nm [38].The nitric oxide radical scavenging activities of *Cayratia trifolia* extract were shown in Figure 3. The IC_{50} value of the stem ethanolic extract was found to be 290 µg/mL and standard ascorbic acid 225 µg/mL respectively.

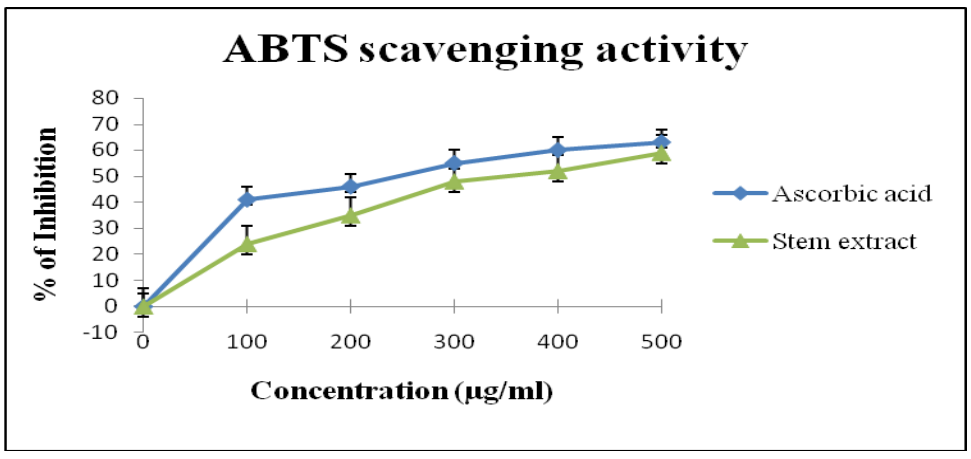

Figure 2-$ABTS^+$ radical scavenging activity

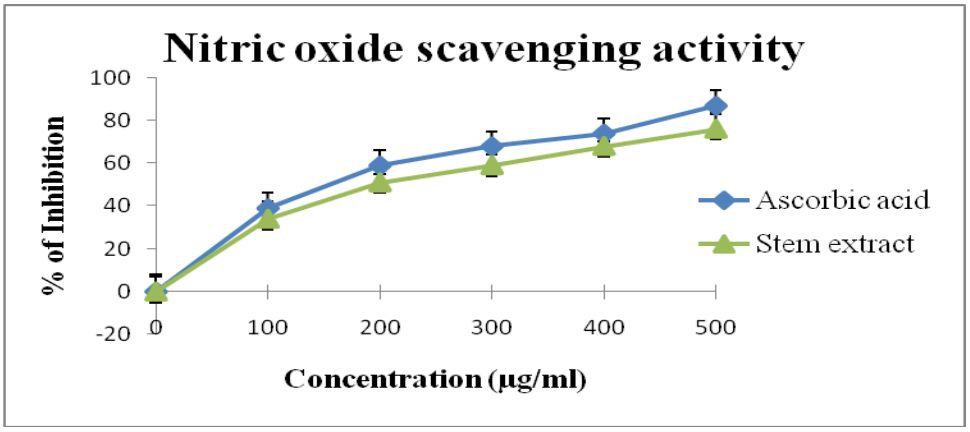

Figure 3-Nitric oxide radical scavenging activity

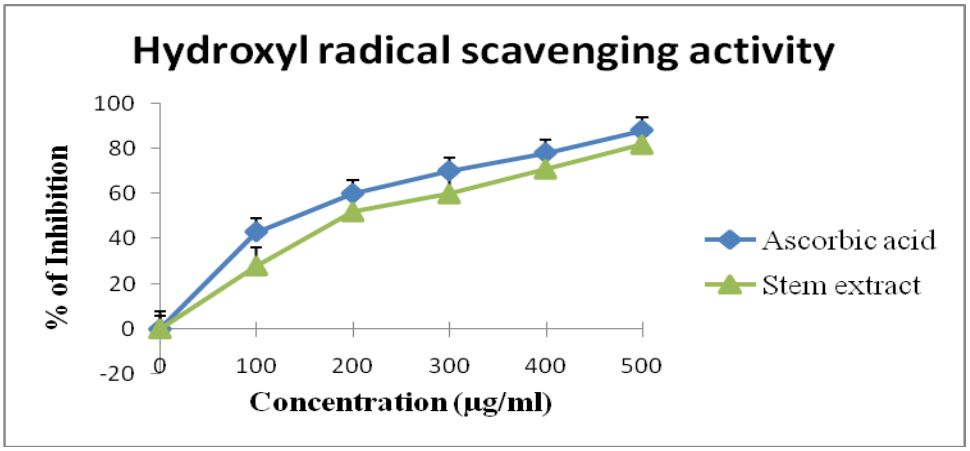

Figure 4-Hydroxyl radical scavenging activity

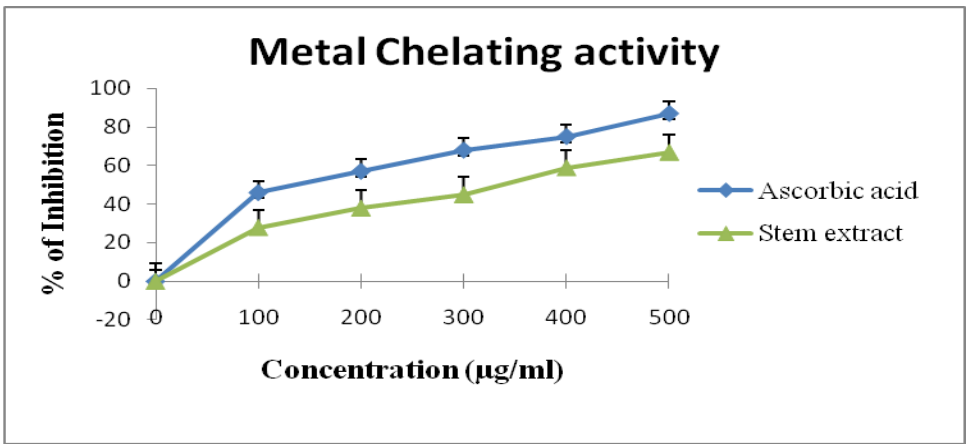

Figure 5-Metal chelating activity

The presence of transition metal ions in a biological system could catalyse the Haber-Weiss and Fenton type reactions, resulting in generation of hydroxyl radicals (OH). However, these transition metal ions could form chelates with the antioxidants, which results in the suppression of OH generation and inhibition of peroxidation processes of biological

molecules [39].The hydroxyl radical scavenging activities of ethanolic extract of *Cayratia trifolia* was showed in figure 4. The IC_{50} value of stem ethanolic extract of *Cayratia trifolia* and ascorbic acid were found to be 245 µg/mL and 215 µg/mL respectively. Metal chelating activity was given in figure 5. The % inhibition of stem ethanolic extract of *Cayratia trifolia* and ascorbic acid were established to be 265 µg/mL and 265 µg/mL.

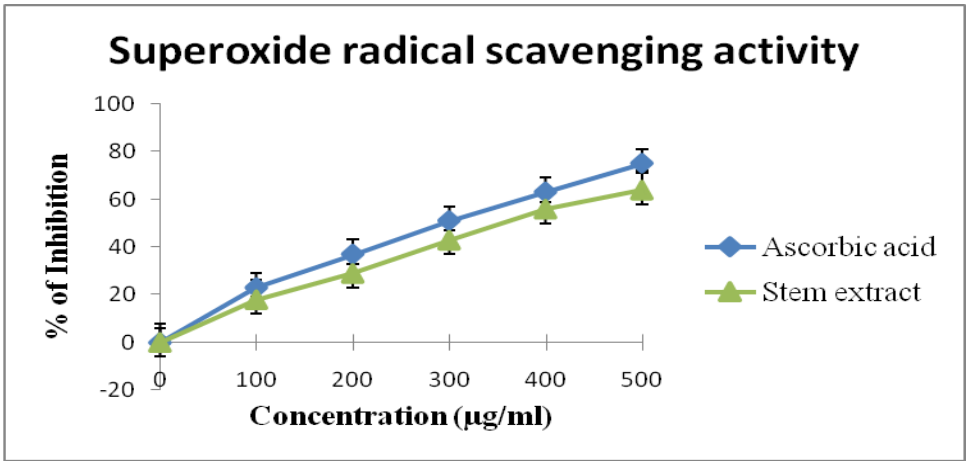

Figure 6-Superoxide radical scavenging activity

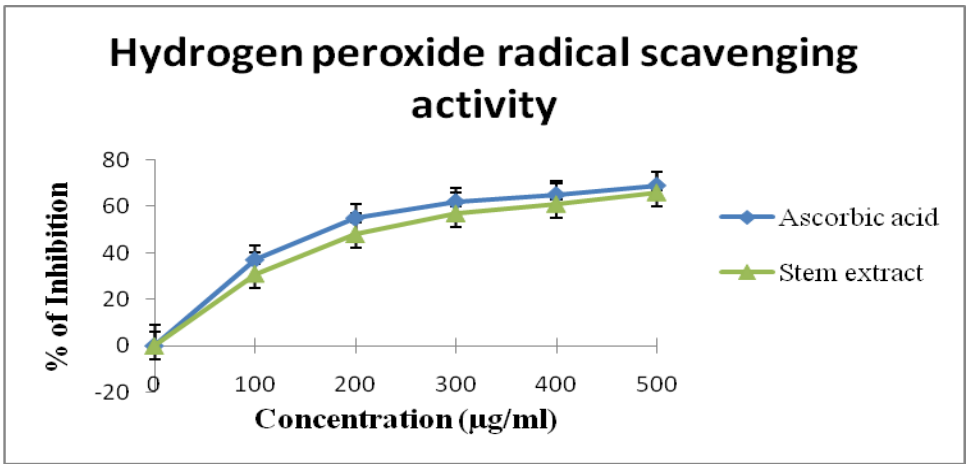

Figure 7-Hydrogen peroxide radical scavenging activity

In biochemical systems, superoxide radical can be converted into hydrogen peroxide by the action of superoxide dismutase and the H_2O_2 can subsequently generate extremely reactive hydroxyl radicals in the presence of certain transition metal ions on by UV photolysis. Hydroxyl radicals can attack DNA molecules to cause strand scission [40]. The IC_{50} value of stem ethanolic extract of *Cayratia trifolia* and ascorbic acid is 345 µg/mL and 295 µg/mL (Figure 6) respectively. Figure 7 showed hydrogen peroxide radical scavenging activity and the % inhibition is 225 µg/mL (stem) and 205 µg/mL (ascorbic acid).

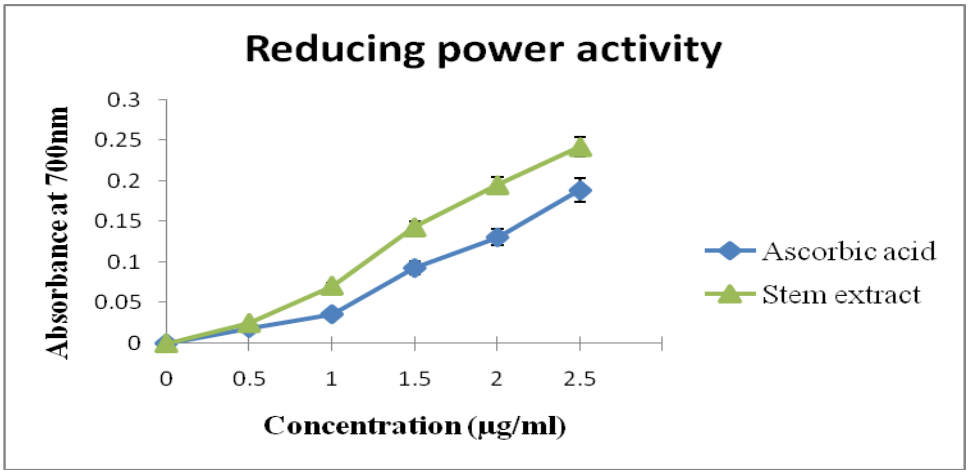

Figure 8-Reducing power activity

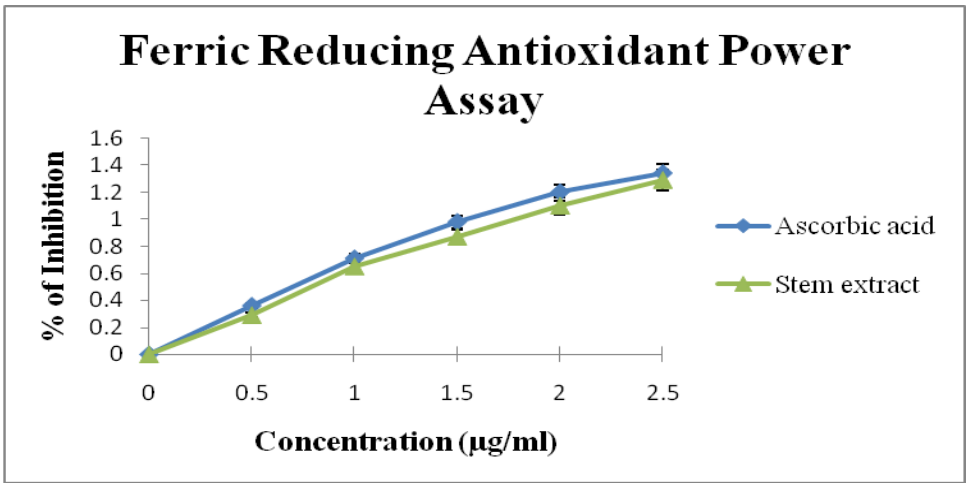

Figure 9-Ferric reducing antioxidant power activity

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the 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 [41]. The Figure 8 represents the reductive capabilities of and stems ethanolic extract of *Cayratia trifolia*. In the concentration range investigated, all the extracts demonstrated reducing power that increased linearly with concentration. The ferric reducing antioxidant power was studied which is shown in figure 9. FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidant present, it can be reported that ethanolic extract may act as free radical scavenger, capable of transforming reactive free radical species into stable non-radical products.

CONCLUSION

In the present study, quantitative analysis and free radical scavenging activities of stem ethanolic extract of *Cayratia trifolia* was investigated. The extract was found to possess more secondary metabolites and it exhibit radical scavenging activities, Based on the results it can be concluded that, the stem ethanolic extract of *Cayratia trifolia* which contains high amount of secondary metabolites and exhibits free radical scavenging activities. In future this plant extract are significant sources of natural antioxidant, which may be helpful in preventing the progress of various oxidative stresses and as a possible food supplement or in pharmaceutical industry.

ACKNOWLEDGEMENTS

The authors are thankful to our Chancellor, Chief Executive Officer, Vice-Chancellor and Registrar of Karpagam University for providing facilities and encouragement.

REFERENCES

1. Gulcin I. The antioxidant and radical scavenging activities of black pepper seeds. Int J Food Sci Nutr, 2005; 56: 491-499.
2. Rimbach G, Fuchs J, Packer L. Application of nutrigenomics tools to analyze the role of oxidants and antioxidants in gene expression. In: Rimbach G, Fuchs J, Packer L (eds.), Nutrigenomics, Taylor and Francis Boca Raton Publishers; FL, USA: 2005, pp. 1-12.
3. Rajeshwar Y, Gupta M, Mazumder UK. Antitumor and in vivo antioxidant status of *Mucuna pruriens* (Fabaceae) seeds against Ehrlich ascites carcinoma in Swiss albino mice. Iranian J Pharm Ther, 2005; 4: 46-53.
4. Ghoshal S, Tripathi VK, Chauhan S. Active constituents of *Emblica officinalis*. Part I, the Chemistry and antioxidative effects of two hydrolysable tannins, emblicanin A and B. Indian J Chem, 1996; 35 (B): 941-948.
5. Erkan N, Ayranci G, Ayranci E. Antioxidant activity of rosemary (*Rosmarinus officinalis*) extract, Black seed (*Nigella sativa*) essential oil , carnosic acid, rosmarinic acid and sesamol. Food Chem, 2008; 110:76-82.
6. Youwei Z, Jinlian Z, Yonghong P. A comparative study on the free radical scavenging activities of some fresh flowers in southern China. LWT Food Sci Technol, 2008; 41:1586-1591.

7. Saha MR, Hasan SMR, Akter R, Hossain MM, Alam MS, Alam MA, et al. In vitro free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi* Linn. Bangladesh J Vet Med, 2008; 6:197-202.
8. Erasto, P, Grierson, DS, Afolayan, AJ. Antioxidant constituents in *Vernonia amygdalina* leaves. Pharmaceutical Biology, 2007; 45: 195-199
9. Edoga HO, Okwu DE, Mbaebie BO. Phytochemicals constituents of some Nigerian medicinal plants. Afr J Biotechnol, 2005; 4: 685-688.
10. Scalbert A, Manach C, Remesy C, Morand C. Dietary polyphenols and the prevention of disorders. Critical reviews in Food Science and Nutrition, 2005; 45: 287-306.
11. Chen Z, Ren H, Wen J. Vitaceae, Flora of china. (Beijling) and Missouri Bot. Science Press, 2010; 12: 33.
12. Chellaperumal P, Sophia D, Arulraj C, Ragavendran P, Starlin T, Gopalakrishnan VK. *In vitro* antioxidant activities and HPTLC analysis of ethanolic extract of *Cayratia trifolia* (L.). Asian Pacif J Trop Dis, 2012; S952-S956.
13. Kumar D, Gupta J, Kumar S, Arya R, Kumar T, Gupta A. Pharmacognostic evaluation of *Cayratia trifolia* (Linn.) leaf. Asian Pacif J Trop Biomed, 2012; 6-10.
14. Gupta K, Sharma M, Review on Indian Medical Plant. Delhi, India: ICMR, 2007; 5: 879-82.
15. Singleton VL, Rossi JA, Calorimetre of total phenolics with phosphomolbdic phosphor tugnstic acid reagents. Amer J Enol Viticul, 1965; 16: 144-158.
16. Schendrel SH, Methods in food analysis. Academic Press, 1970; 749-756.
17. Obdoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Glob J Pure Appl Sci, 2001; 8b: 203-208.
18. Ordon LE, Gomez JD, Vattuone MA, Isla MI, Antioxidant activity of *Sechium edule* (Jacq.) Swart extracts. Food Chemistry, 2006; 97: 452-458.
19. Harborne JB. Phytochemical Methods. 1st edn., London: Chapman and Hall,1973; pp. 288-504.
20. Blois MS. Antioxidant determinations by the use of stable free radical. Nature, 1958; 81: 1199-2000.
21. Elizabeth K, Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharm, 1990; 58: 237-240.
22. Fontana M, Mosca L, Rosei MA. Interaction of Enkephalines with oxyradicals. Biochem Pharmacol, 2001; 61:1253-1257.

23. Garratt DC. The quantitative analysis of Drugs. Chapman and Hall ltd; 1964, p.456-458.
24. Ruch, RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 1989; 10: 1003-1008.
25. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (Acetoaminophen, Salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys, 1994; 315: 161-169.
26. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, and Rice-Evance C. antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med, 1999; 26: 231-237.
27. Oyaizu M. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. Jpn J Nutr, 1986; 44:307-315.
28. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power" The FRAP assay. Anal Biochem, 1996; 239: 70-76.
29. Ozyurt D, Ozturk BD, Apak R. determination of total flavonoid content of Urtica dioica L. by a new method. Proceedings book. Thrkey: Adnan menderes University, 4th AACD Congress; 2004.
30. Heim, KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships, J. Nutr. Biochem, 2002; 13: 572-584.
31. Dipankar C, Nikhil BG, Rhitajit S, Nripendranath M. Phytochemical analysis and evaluation of antioxidant and free radical scavenging activity of *Withania somnifera* root. 2012; 5: 193-199.
32. Kessler M, Ubeaud G, Jung L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. J Pharm Pharmacol, 2003; 55 (1): 131-142.
33. Kumar D, Kumar S, Gupta J, Arya R, Gupta1 A. A review on chemical and biological properties of *Cayratia trifolia* Linn.(Vitaceae). Pharmacogn Rev, 2011; 5: 184-188
34. Chang LW. Yen WJ. Huang SC. Duh PD. Antioxidant activity of sesame coat. Food Chem, 2002; 78: 347-354.
35. Mondal SK, Chakraborty G, Gupta M, Mazumder UK. *In vitro* antioxidant activity of *Diospyros malabarica* Kostel bark. Indian J Exp Biol, 2006; 44: 39-44.
36. Rajeshwar Y, Gupta M, Mazumder UK. Antitumor and in vivo antioxidant status of *Mucuna pruriens* (Fabaceae) seeds against Ehrlich ascites carcinoma in Swiss albino mice. Iranian J Pharm Ther, 2005; 4: 46-53.

37. Sanmugapriya K, Saravana PS, Payal H, Peer Mohammed S, Binnie W. Antioxidant activity, total phenolic and flavanoid contents of Artocarpus heterophyllus and Manilkara zapota seeds and its reduction potential. Int J Pharm Pharmaceu Sci, 2011; 3: 256-260.
38. Dharani B, Sumathi S, Sivaprabha J, Padma PR. In vitro antioxidant potential of Prosopis cineraria leaves. J Nat Prod Pln Resour 2011; 1: 26-32.
39. Chew YL, Goh JK, Lim YY. Assessment of in vitro antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. Food Chem, 2009; 116: 13-18.
40. Halliwell B and Gutteridge, JMC. Free radicals in biology and medicine. 3rd edition, oxford university press, oxfor, U.K, 1999.
41. Anjali S, Sheetal S. Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts; J Pharmac Phytochem 2013; 2: 22-29.