Evaluation of *in vitro* antioxidant activity of ethanol extract of *Carmona retusa* (Vahl.) Masam

C.P. Chandrappa[1], M. Govindappa[1*], N.V.Anil Kumar[2], R. Channabasava[1], N. Chandrasekar[3] and T.Umashankar[1]

[1]Department of Biotechnology, Shridevi Institute of Engineering and Technology, Tumkur, Karnataka, India.

[2]Department of Chemistry, Manipal Institute of Technology, Manipal University, Manipal, Karnataka, India.

[3]Department of Chemistry, Shridevi Institute of Engineering and Technology, Tumkur, Karnataka, India.

***Corresponding author:** dravidateja07@gmail.com

Abstract: Oxidants contribute to more than one hundred disorders in humans. The ethanol extract of *Carmona retusa* was screened for antioxidant activity using 1,1-Diphenyl-2-Picryl Hydroxyl (DPPH) scavenging, Ferric Reducing Antioxidant Power (FRAP), hydrogen peroxide scavenging capacity (H_2O_2), reducing power assay and determination of the total antioxidant capacity by phosphomolybdenum method. The ethanol extract exhibited maximum antioxidant activity in DPPH, hydrogen peroxide. The reducing power and FRAP increased with increasing concentration of the sample. The scavenging activity of *Carmona retusa* found maximum in the DPPH, FRAP, hydrogen peroxide scavenging and reducing power assay of 95.35%, 9.296%, 78.539% and 82.9% respectively at 400µg/mL concentration with IC_{50} values of 93.35, 3.36, 12.62 and 20.79 µg/ml respectively. Percentage of antioxidant capacity with respect to one gram of dried plant extract in phosphomolybdenum method was found to be 8.875. The antioxidant activity of the sample was comparable with that of the standard ascorbic acid. This is the primary and preliminary work in *Carmona retusa*.

Keywords: *Carmona retusa*, Antioxidant, DPPH, FRAP, Reducing power.

Introduction

Antioxidants are helpful in protecting cells from such oxidative damage and play an important role in protecting the body tissues against free radicals damage (Dharmendra *et al.,* 2013). Oxidant, commonly known as free radical, is a molecule with one or more unpaired electrons in the outer orbital. Many of these free radicals, which are the harmful byproducts generated during normal cell aerobic respiration in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology (Raghu *et al.,* 2011). Antioxidants prevent the human system by neutralizing the free radicals interactively and synergistically. Plants are rich source of free radical scavenging molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavanoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites which are rich in antioxidant activity (Jayanthi P and Lalitha P 2011).

Carmona retusa is an evergreen shrub to small tree belonging family Boraginaceae. *C. retusa* leaf decoction is being used to treat cough and stomach ache root as antidote (Shrisha *et al.,* 2011). Many plants have proved to successfully aid in various ailments leading to mass screening for their therapeutic components. Today, the search for natural compounds rich in antimicrobial, antioxidant and anti-inflammatory properties is escalating due to their medicinal importance in controlling many related chronic diseases (Govindappa *et al.,*. 2011). In the present investigation, the free radical scavenging activity of alcoholic extract of *C.retusa* was examined for various assays.

Materials and methods

Collection of plant materials

Fresh stem of *Carmona retusa* was collected from Hemagangothri Campus, Hassan district of Karnataka, India on 25th March 2012 and it was authenticated by Dr Sharanappa P, Department

of Studies in Biosciences, Hemagangothri, University of Mysore, Hassan, Karnataka. The collected stem was cleaned with deionized water and dried under shade for two weeks at room temperature (26±2ºC). Dried stem was grounded and filtered using 0.3mm mesh. The stem powder was stored in air tight container and maintained at 4°C until use.

Preparation of sample

Solvent system used for the extraction was ethanol. Flask extraction procedure was adapted for extraction. 25 grams of the powdered stem sample was soaked in the conical flask containing solvent, wrapped with aluminum foil and placed in shaker for 48 hours at 120-130 rpm Sample was concentrated in hot air oven at 50ºC (Anyasor *et al.*, 2010).

Phytochemical analyses

Phytochemical analysis of ethanol extract was carried out according to standard protocols (Chandrappa *et al.*, 2012).

DPPH radical scavenging assay

The antioxidant activity of the alcoholic extract of *Carmona retusa* was evaluated using the stable DPPH radical according to Hatano *et al.* method with some experimental modifications (Hatano *et al.*, 1988). Briefly 1.0 ml of 0.1 mM solution of DPPH radical was added to 1.5 ml of sample or standard solution in methanol containing different concentrations (50 µg - 400 µg). The reaction mixture was shaken and incubated for 30 minutes in the dark chamber. Absorbance was measured at 520 nm which shows the decrease in absorbance of the resulting solution with increase in concentration. Ascorbic acid was used as the standard antioxidant and antioxidant activity was expressed in terms of ascorbic acid equivalents.

The capacity to scavenge the DPPH radical was calculated using the following equation:

(%) scavenging = [(Ac - As)/Ac)] x 100

Where,

Ac - absorbance of the control and

As - absorbance of the test sample.

The IC_{50} values (alcoholic extract of *Carmona retusa* that causes 50% scavenging) were determined from the graph of scavenging effect percentage against the extract concentration. All determinations were carried out in triplicate.

Ferric reducing/antioxidant power (FRAP) assay

The antioxidant potential of alcoholic extract of *Carmona retusa* was determined using FRAP assay according to method of Benzie and Strain with some experimental modifications (Benzie and Strain 1996). The assay was based on the reducing power of a sample. A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}) producing a blue colour complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at 10:1:1 (v/v/v). The reaction mixture consists of 1.0 mL distilled water with different concentrations of alcoholic extract of *Carmona retusa* (50 µg-400 µg) and 600 µl of freshly prepared FRAP reagent. The reaction mixture was mixed thoroughly and incubated for 30 min under dark conditions. Absorbance was taken at 593 nm. Standard calibration curve was prepared using known concentrations of $FeSO_4.7H_2O$. Ascorbic acid was used as the standard antioxidant and antioxidant activity was expressed in terms of gram equivalent to Fe^{2+}. All determinations were performed in triplicates.

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging ability of ethanol extract of *Carmona retusa* was determined according to the method of Ruch *et al*. with some experimental modofications (Ruch *et al.*, 1989) A solution of hydrogen peroxide (40 mM) was prepared in 0.1 M phosphate buffer (pH

7.4). 2 ml of distilled water with different concentrations of plant extracts (50 µg-400 µg) were added to 1.2 mL of 40 mM hydrogen peroxide solution. After 10 minutes of incubation, absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing the phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the standard antioxidant and hydrogen peroxide scavenging activity was expressed in terms of ascorbic acid equivalents. All determinations were performed in triplicates.

The percentage of hydrogen peroxide scavenging of both extracts and standard compounds were calculated:

% Scavenged [H_2O_2] = [(Ac – As)/Ac] x 100

Where,

Blank – Containing only phosphate buffer without hydrogen peroxide

Ac - absorbance of the control without samples / standard

As - absorbance presence of the sample / standards.

Reducing power assay

The reducing capacities of alcoholic extract of *Carmona retusa* was determined by Oyaizu *et al.* method with some experimental modifications (Oyaizu 1986). The reaction mixture consists of 1 ml distilled water with different concentrations (50 µg - 400 µg) of alcoholic extract of *Carmona retusa*, 1.0 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1.0 mL of 1% potassium ferricyanide (w/v). The mixture was incubated at 50°C for 20 min. After cooling to room temperature 1.0 mL of 10% trichloroacetic acid (w/v) was added and the mixture was centrifuged at 3000 x g for 10 min. The upper layer (1.0 mL) was mixed with 1.0 mL of distilled water and 1.0 mL of 0.1% ferric chloride, and the absorbance was measured at 700 nm. Ascorbic acid was used as a standard. Blank as phosphate buffer and Control was prepared without adding standard or test compound. Higher absorbance indicates higher reducing power of the sample.

The relative percentage reducing power of the sample was calculated by using the formula (Xican and Chan 2012).

$$(A-A_{min}) / (A_{max} - A_{min}) \times 100$$

Here, A_{max} = absorbance of maximum absorbance tested,

A_{min} = absorbance of minimum absorbance tested and

A = absorbance of sample.

Assays were carried out in triplicate.

Determination of total antioxidant capacity by phosphomolybdenum method

The total antioxidant capacities of alcoholic extract of *Carmona retusa* was evaluated by the phosphomolybdenum method of reducing transition metal ions reported by Prieto *et al* with some experimental changes (Prieto *et al.*, 1999). 1.0 mL alcholoic extract of *Carmona retusa* with various concentrations (50µg - 400 µg) were added to 2.0 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixtures were capped and incubated in a water bath at 95 °C for 90 min. After cooling the mixture to room temperature, the absorbance was measured at 695 nm against a blank. Phosphate buffer (1.0 mL) in the place of plant extract was used as the blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid. The calibration curve was prepared by ascorbic acid with methanol. All assays were done in triplicate.

Results

Phytochemical analysis

Phytochemical analysis was performed for ethanol extract of *Carmona retusa*. We used different solvents but got many phytochemicals in ethanol, that is why we only representing ethanol. Table 1 indicates that ethanol extract yielded phytochemicals viz. alkaloids, flavonoids, saponins, phenols, tannins, cardiac glycosides, terpenoids and cardenolides. Anthraquinones and phlobatannins are not contained in the extract.

DPPH radical scavenging assay

The ethanol extract of *Carmona retusa* exhibited a maximum DPPH scavenging activity of 95.35% at 400 µg/ml whereas for ascorbic acid (standard) was found to be 97.13% at 400 µg/ml. and minimum at 50 µg/ml. The IC_{50} of the alcholic extract of *Carmona retusa* and ascorbic acid were found to be 93.35µg/ml and 96.88µg/ml respectively and are presented in Table 2.

FRAP assay

The ferric reducing antioxidant activities of ethanol extract of *Carmona retusa* and ascorbic acid are presented in table 3. From the results it is clear that the FRAP activity of *C.retusa* has shown maximum 9.296 at the concentration of 400 µg/ml and minimum 3.72 at the concentration of 50 µg/ml. The reducing power of extract of *C.retusa* was very potent and it increases as the concentration of sample increases. The IC_{50} of the ethanol extract of *C. retusa* and ascorbic acid were found to be 3.36µg/ml and 9.96µg/ml respectively and are presented in Table 3. Earlier reports on the antioxidant activity of *C. retusa* are not present in the literature. Therefore, it is very difficult to compare our results with that of previous studies.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity of the alcoholic extract of *C.retusa* and ascorbic acid are presented in Table 4. *C.retusa* extract showed a maximum activity of 78.53% inhibition very

much comparable to that of ascorbic acid with an activity of 89.64% at the same concentration of 500µg/ml. The IC_{50} value of the extract was 12.7µg/ml, whereas the standard exerted an IC_{50} of 42.963µg/ml.

Reducing power assay

The reducing power of the alcoholic extract of *C.retusa* and ascorbic acid were analyzed. Increase in the O.D determines the increase in the reducing power. In our present study the plant extract showed a good reducing power increase in concentration dependent manner and the IC_{50} value of the extract was 20.79µg/ml, whereas the standard exerted an IC_{50} of 49.568µg/ml (Table 5).

Determination of total antioxidant capacity by phosphomolybdenum method

The ethanol extract of *C.retusa* showed maximum total antioxidant capacity and are presented in table 6.

Table 1. Phytochemical analysis of ethanol extract of *Carmona retusa*

Phytochemical	Ethanol extract
Alkaloids	++
Flavonoids	+++
Saponins	+++
Phenols	+
Tannins	+++
Anthraquinones	-
Cardiac glycosides	++
Phlobatannins	-
Terpenoids	+
Cardenolides	+

+ = presence, - = absence, ++ = moderate presence and +++ = more presence

Repeated each experiment thrice

Table 2. DPPH scavenging activity of ethanol extract of *Carmona retusa* and ascorbic acid

Concentration of alcoholic extract of Carmona retusa and ascorbic acid (µg/ml)	(%) scavenging	
	Alcoholic extract	Ascorbic acid
50	93.50	96.44

100	93.73	96.90
200	93.81	97.97
300	94.19	97.97
400	95.35	97.13
IC_{50} Value (µg/ml)	93.35405	96.88338

Repeated each experiment thrice

Table 3. Total antioxidant activity of ethanol extract of *Carmona retusa* and ascorbic acid

Concentration of alcoholic extract of *Carmona retusa* and ascorbic acid (µg/ml)	**Gram Equivalent to Fe^{2+}**	
	Alcoholic extract (µg)	**Ascorbic acid (µg)**
50	3.72	9.968
100	4.816	10.192
200	7.168	10.36
300	8.288	10.416
400	9.296	10.64
IC_{50} Value (µg)	3.362	9.967

Repeated each experiment thrice

Table 4. Hydrogen peroxide scavenging activity of alcoholic extract of *Carmona retusa* and ascorbic acid

Concentration of alcoholic extract of *Carmona retusa* (µg/ml)	**% of inhibition of sample**	**% of inhibition of diclofenac sodium (Std)**
50	15.4612	41.075
100	25.232	52.1751
200	36.9913	65.7219
300	59.375	76.1562
400	78.539	89.6484
IC_{50} Value (µg)	12.622	42.963

Repeated each experiment thrice

Table 5. The relative percentage reducing power of ethanol extract of *Carmona retusa* and positive control ascorbic acid.

Concentrations of sample/ascorbic acid (µg/ml)	**% reducing power of plant extract**	**% reducing power of Ascorbic acid (Std)**
50	22.18	46.68

100	33.9	57.88
200	42.9	75.92
300	63.3	84.68
400	82.9	100
IC_{50} Value (µg)	20.792	49.568

Repeated each experiment thrice

Table 6. Total antioxidant activity of ethanol extract of *Carmona retusa* and positive control ascorbic acid.

Concentration	**Total antioxidant capacity(µg/ml)**	**Total antioxidant capacity/gram of dried plant extract (gram equivalent to ascorbic acid)**	**% of antioxidant capacity with respect to dried plant extract**
1mg	88.75	88.75 mg	8.875

Repeated each experiment thrice

Discussion

Many reports are showing that the plant phytochemicals possessing strong antioxidant activity. In the present investigation, the ethanol extract also showing strong antioxidant activity due to the presence of phytochemicals. The earlier reports have shown highest antioxidant activity due to presence of alkaloids (Cardoso *et al.*, 2004), flavonoids (Braca *et al.*, 2002), saponins (Gulsin *et al.*, 2004), phenols (Liao *et al.*, 2000)), tannins (Reddy *et al*,. 2007) and Terpenoids (Grassmann 2005).

The presence of flavonoids in the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers (Polterait 1997). The measurement of the scavenging of DPPH radical allows one to determine exclusively the intrinsic ability of substance to donate hydrogen atom or electrons to this reactive species in a homogenous system. The method is based on the reduction of methanolic DPPH solution

because the presence of antioxidant substances having hydrogen-donating groups such as phenols and flavonoid compounds due to the formation of non-radical DPPH-H form (Mortada *et al*,. 2013). The result showed that DPPH radical scavenging activity has exhibited potent scavenging activity in a concentration dependent manner. Antioxidants are thought to be highly oxygen specific mediated tissue impairments. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. The Phenolic compounds (phenols, flavonoids and terpenoids) identified in the present study might be responsible to some extent for total antioxidant properties of *Carmona retusa* (Sadananda *et al*,. 2011).

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine [Fe3+-TPTZ] complex and producing a coloured ferrous tripyridyltriazine [Fe2+-TPTZ]. Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom.Frap assay treats the antioxidants in the sample as a reductant in a redox-linked colorimetric reaction (Nisha *et al*,.2012).

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} to form hydroxyl radical and this may be the origin of many of its toxic effects (Nisha *et al.*, 2012).

Reducing capacity is considered as a significant indicator of potential antioxidant activity of a compound or sample. The presence of reductants (i.e. antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 655 nm, the amount of Fe^{2+} can be monitored. Higher absorbance indicated higher reducing power (Indu and Seenivasan 2013).

Total antioxidant capacity by phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample to be analyzed and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid (Ramesh *et al.*, 2011).

Conclusion

Results of our study indicated that the ethanol extract of *Carmona retusa* showed free radical scavenging ability in all the assays conducted. This study suggests the contention that traditional medicines remain a valuable source in the potential discovery of natural product pharmaceuticals. In conclusion of our studies, we revealed that ethanol extract of *Carmona retusa* have significant antioxidant activities as compared with standard ascorbic acid. A potent antioxidant activity of ethanol extract may be due to the presence of various phytochemicals such as flavonoids, saponins, phenols, tannins, cardiac glycosides. However, further investigations are needed to determine the bioactivities in this sample of *Carmona retusa*.

Acknowledgment

Authors are thankful to **Dr. M.R.Hulinaykar**, Managing Trustee, Sri Shridevi Charitable Trust (R.) and **Dr. K.Sukumaran**, Principal, Shridevi Institute of Engineering and Technology, Tumkur for providing all facilities. Authors are also thankful to **Dr. P.Sharanappa**, Associate Professor, Department of Studies in Biosciences, Hemagangothri, University of Mysore, Hassan, Karnataka for the identification of our plant.

Reference

1. Dharmendra, K.K., Parikshit, J. and Archana, R.J. (2013).” Phytochemical investigation and *in vitro* antioxidant activities *Indigofera cordifolia* seed extracts”, International Journal of Pharmacy and Pharmaceutical Sciences, 5:1, 71-75.
2. Raghu, K.L., Ramesh, C.K., Srinivasa, T.R. and Jamuna, K.S. (2011). ” Total antioxidant capacity in aqueous extracts of some common vegetables”, Asian Journal of Experimental Biological Science, 2: 58-62.
3. Jayanthi, P. and Lalitha, P. (2011). “Reducing power of the solvent extracts of *Eichhornia crassipes* (Mart.) Solms”, International Journal of Pharmacy and Pharmaceutical Sciences, 3: 126-128.
4. Shrisha, D.L., Raveesha, K.A. and Nagabhushan. (2011). “Bioprospecting of selected medicinal plants for antibacterial activity against some pathogenic bacteria”, Journal of Medicinal Plants Research, 5: 4087-4093.
5. Govindappa, M., Nagasravya, S., Poojashri, M.N., Sadananda, T.S., Chandrappa, C.P., Gustavo Santoyo, Sharanappa, P. and Anil Kumar, N.V. (2011). “Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity and phytochemical screening of water extract of *Wedelia trilobata* (L.) Hitchc”, Journal of Medicinal Plants Research, 5: 5718-5729.
6. Anyasor, G.N., Ogunwenmo, K.O., Oyelana, O.A. and Akpofunure, B.E. (2010). “Phytochemical Constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl. (Costaceae), African Journal of Biotechnology, 9: 4880-4884.
7. Chandrappa, C.P., Govindappa, M. and Anil Kumar, N.V. (2012). “Phytochemical screening and antibacterial property of *Carmona retusa* (Vahl.) Masam”, International Journal of Pharma Medicine and Biological Sciences, 1: 91-98.
8. Hatano, T., Kagawa, H., Yasuhara, T. and Okuda, T. (1988). “Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects”, Chemical and Pharmaceutical Bulletin, 36: 1090-2097.
9. Benzie, I.F.F. and Strain, J.J. (1996). “The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay”, Analytical Biochemistry, 239: 70–76.

10. Ruch, R.J., Cheng, S.J. and Klaunig, J.E. (1989). "Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea", Carcinogenesis, 10: 1003-1008.
11. Oyaizu, M. (1986). "Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine", Japanese Journal of Nutrition, 44: 307–315.
12. Xican, L. and Chan, C. (2012). "Systematic evaluation on antioxidant of Magnolol *in vitro*", International Research Journal of Pure and Applied Chemistry, 2: 68-76.
13. Prieto, P., Pineda, M. and Aguilar, M. (1999). "Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E", Analytical Biochemistry, 269: 337-341.
14. Cardoso, C.L., Castro-Gamboa, I., Silva, D.H.S., Furlan, M., Epifanio, R.A., Pinto, A.D.C., Rezende, C.M., Lima, J.A. and Bolzani, V.D.S. (2004). "Indole Glucoalkaloids from *Chimarrhis turbinata* and their evaluation as antioxidant agents and Acetylcholinesterase Inhibitors", Journal of Natural products, 67: 1882-1885.
15. Braca, A., Sortino, C., Politi, M., Morelli, I. and Mendez, J. (2002). "Antioxidant activity of flavonoids from *Licania licaniaeflora*", Journal of Ethnopharmacology, 79: 379-381.
16. Gulsin, I., Mshvildadze, V., Gepdiremen, A. and Elias, R. (2004). "Antioxidant Activity of Saponins Isolated from Ivy: α-Hederin, Hederasaponin-C, Hederacolchiside-E and Hederacolchiside-F", Thieme eJournals, 70: 561-563.
17. Liao, K.L. and Yin, M.C. (2000). " Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems: Importance of the partition coefficient", Journal of Agriculture and Food Chemistry, 48: 2266-2270.
18. Reddy, M., Gupta, S.K., Jacob, M.R., Khan, S.I. and Ferreira, D. (2007). "Antioxidant, antimalarial and antimicrobial activities tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum* L.", Planta Med,1-7.
19. Grassmann, J. (2005). "Terpenoids as plant antioxidants", Vitam Horm, 72: 505-535.
20. Polterait, O. (1997). "Antioxidants and free-radical scavengers of Natural Origin", Current organic chemistry, 1: 415-440.

21. Mortada, M., El-Sayed, Hanan, A., El-Nahas, El-Sayed, S., Adel, H., Eman, A. and El-W. (2013). "Investigation and antioxidant of phenolic compounds of the leaves of *Gleditsia triacanthos* L.", International Journal of Pharmacy and Pharmaceutical Sciences, 5: 172-177.
22. Sadananda, T.S., Nirupama, R., Chaithra, K., Govindappa, M., Chandrappa, C.P. and Vinay, R.B. (2011). "Antimicrobial and antioxidant activities of endophytes from *Tabebuia argentea* and identification of anticancer agent (lapachol)", Journal of Medicinal Plants Research, 16: 3643-3652.
23. Nisha, S., Vishnupriya, M., Sasikumar, J.M., Hephzibah, P., Christabel. and Gopalakrishnan, V.K. (2012). "Antioxidant activity of ethanolic extract of *Maranta arundinacea* L. tuberous rhizomes", Asian Journal of Pharmaceutical and Clinical Research, 5: 85-88.
24. Indu, H. and Seenivasan, R. (2013). "*In vitro* antioxidant activity of selected seaweeds from southeast coast of India", International Journal of Pharmacy and Pharmaceutical Sciences, 5: 474-484.
25. Ramesh, C.K., Raghu, K.L., Jamuna, K.S., Govinden, S.J. Ranghoo, S.V.M. and Vijay, A.B.R. (2011). "Comparative evaluation of antioxidant property in methanol extracts of some common vegetables of India", Scholars Research Library, 2: 86-94.