Medicinal & Aromatic Plants
ISSN: 2167-0412

Reddy and Grace, Med Aromat Plants 2016, 5:3
DOI: 10.4172/2167-0412.1000250

Research Article Open Access

In Vitro Evaluation of Antioxidant Activity of Methanolic Extracts of Selected Mangrove Plants

Reddy ARK* and Grace JR

Centre for Research, Dr. VS Krishna Government Degree and PG College (A), Maddilapalem, Visakhapatnam, Andhra Pradesh, India

Abstract

Antioxidants are vital substances that have an ability to protect the body from various damages caused by free radical-induced oxidative stress. Plants are the source of a wide variety of natural free radical scavenging antioxidants. The objective of the present study is to carry out *in vitro* antioxidant tests to evaluate the antioxidant ability of Methanolic extracts of selected mangrove plans i.e., *Aegiceras corniculatum*, *Excoecaria agallocha* and *Lumnitzera racemosa*. In this study, *In vitro* antioxidant activity was estimated by assays like DPPH, Reducing power assay and total antioxidant activity methods. The *in vitro* antioxidant studies revealed that, the extract of *Lumnitzera racemosa* has the highest phenolic content (38.80 ± 0.19 µg GAE/ 100 µg) and highest antioxidant potential. This is followed by *Aegiceras corniculatum (*24.06 ± 0.79 µg GAE/100 µg) and, *Excoecaria agallocha (*20.56 ± 0.58 µg GAE/100 µ). This study provides the preliminary data that can pave the way for further studies to validatee their medicinal uses and focus on bioassay guided fractionation and isolation of active compounds from the extracts.

Keywords: Mangroves; DPPH; Free radicals; Radical scavenging activity; Total antioxidant activity

Introduction

Free radicals are highly reactive molecules that contain one or more unpaired electrons. They either donate or take electrons from other molecules in an attempt to pair with their electrons and generate more stable species. Reactive oxygen species (ROS) are derivatives of oxygen [1] and are constantly produced in the body during various metabolic activities such as aerobic respiration and by various exogenous factors [2]. Some of the reactive oxygen species play a positive role in phagocytosis(oxygen burst), energy production and regulation of cell growth, intra cellular signaling etc. However, free radicals generated by sun light, UV light, ionizing radiation, chemical reactions and metabolic process have a wide variety of pathological effects. Reactive oxygen species generated in the organism are usually removed or neutralized by an efficient network of defense mechanism in the body. When the formation of these free radicals or reactive oxygen species exceeds the levels of defensing mechanism, it leads to the damage of tissues, bio molecules and further, leading to disease conditions especially degenerative diseases such as Aging, Diabetes, Arthritis, Carcinogenesis, and Cardio Vascular diseases [3-6].

Antioxidants are important substances that play a crucial role in delaying, intercepting, and preventing oxidative reactions catalyzed by free radicals and thus providing protection to humans [7]. Due to this special ability there is an icreased use of antioxidants for the balance of reactive oxygen species. Now a days, most of the antioxidants are manufactured synthetically. Several synthetic antioxidants such as Butylatedhydroxyl anisole (BHA), Butylated hydroxyl toluene (BHT), Tertiary butylated hydroxyl quinone (TBHQ), and Gallic acid esters are commercially available. Such synthetic antioxidants are known to have potential side effects and possess some degree of carcinogenicity when taken *in vivo* [8-12]. Hence their use is being restricted now-a-days. Antioxidant substances from plant materials are safe and terminate the action of free radicals thereby protecting the organism from various diseases. Therefore a special interest to screen medicinal plants for the presence of natural antioxidants has greatly increased. Plant derived natural copounds such as Flavonoids, Terpenes, Alkaloids etc. have received considerable attention in recent years due to their pharmacological properties including Antioxidant, Anti-microbial and Anti-inflammatory activities [13-18].

Plant phenols, a diverse group of phenolic compounds (Flavonols, Anthocyanins, phenolic acids, etc.) possess strong antioxidant activity and may help to provide the protection to the cells against the oxidative damage caused by free radicals. They are well known as "radical scavengers", "metal chelators", "reducing agents" hydrogen donors and singlet oxygen quenchers. They have an ideal structural chemistry for the removal of free radicals in the body. Anti oxidative properties of poly phenols arise from their high reactivity as hydrogen or electron donors. The current research efforts are channeled towards exploiting the antioxidant abilities of natural phenolics. Hence there is a growing interest all over the world for discovering the untapped reservoirs of medicinal plants [19,20].

Mangroves are the unique plant communities that grow in estuarine swamps with high salinity, high temperature, low nutrients and high radiation. They survive in high environmental stress as they have unique properties to combat stress. Exposure to these stress situations results in the formation of reactive oxygen species in these plants. In order to reduce the adverse affects of these ROS, the mangrove plants produce antioxidant enzymes and various defense compounds including poly phenolic compounds [5,6,21]. The potential of mangrove plants as a source of new bio active principles is still unexplored. Further, there have been no detailed i*n vitro* studies on antioxidant properties of mangrove medicinal plants from Corangi reserve forest, Kakinada, East Godavari district, Andhra Pradesh, India. Hence the current study is aimed on the evaluation of Methanolic leaf extracts of three mangrove species i.e., *Aegiceras corniculatum*, *Excoecaria agallocha* and *Lumnitzera*

***Corresponding author:** Reddy ARK, Research Scholar, MSc Biotechnology, Centre for Research, Dr. VS Krishna Government Degree and PG College (A), Maddilapalem-530 013, Visakhapatnam, Andhra Pradesh, India, Tel: +919491433536; E-mail: akhilbio@gmail.com

Received April 02, 2016; **Accepted** April 26, 2016; **Published** April 29, 2016

Citation: Reddy ARK, Grace JR (2016) *In Vitro* Evaluation of Antioxidant Activity of Methanolic Extracts of Selected Mangrove Plants. Med Aromat Plants 5: 250. doi:10.4172/2167-0412.1000250

racemosa for their antioxidant potential. The selected mangrove plants are commonly used as folklore medicine by local people.

Plant material

In Our present study, the fresh leaves of *Aegiceras corniculatum*, *Excoecaria agallocha* and *Lumnitzera racemosa* were collected from Corangi Reserve Forest, Kakinada, East Godavari district, Andhra Pradesh, India. Geographical location of Corangi Reserve Forest is between 16°39' N longitude- 17°N longitude and 82°14' E latitude – 82°23'E latitude. The collected leaves were washed thoroughly with tap water in the laboratory to remove dust and shade dried in a well – ventilated place at room temperature. The dried leaves were ground to a coarse powder and subjected to solvent extraction.

Extraction

Methanol was used as a solvent to prepare the crude extract. The plant material was first soaked for 12 Hrs in 500 ml of methanol and then subjected to extraction by refluxing for 6 to 8 Hrs below the boiling point of the solvent. The extract was further subjected to evaporation at a reduced pressure using rotary evaporator. The concentrated extract was further dried at 37°C for 3 to 4 days in order to facilitate complete evaporation of the solvent.

Total Phenolic Content (TPC) estimation (Folin-Ciocalteau method): The amount of total phenolics in plant extract was estimated by using spectrophotometric method. Methanolic solution of the plant extract at the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolc solution of extract, 2.5 ml of 10% Folin-Ciocalteu reagent was dissolved in water and 2.5 ml of 7% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml of methanol, 2.5 ml of 10% F.C reagent was dissolved in water and 2.5 ml of 7.5% $NaHCO_3$. The samples were thereafter incubated. The absorbance was determined using spectrophotometer at 650 nm. The same procedure was repeated for the standard solution of gallic acid at different concentrations and calibration line was constructed. Based on the measured absorbance, the concentration of phenolic content of the plant extract was estimated from the calibration line. The amount of total phenolics in extracts was expressed in terms of gallic acid equivalents mg of GAE/gram extract.

Methods for antioxidant analysis

The antioxidant activity of prepared plant methanol extracts was investigated by using DPPH assay, Reducing power assay and Total antioxidant capacity methods.

Evaluaion of antioxidant activity by DPPH radical scavenging method: The antioxidant activity of the plant extracts and the standard was measured on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picryl hydroxyl (DPPH) free radical activity method with minor modifications. The stable DPPH radical method is a widely used, relatively quick, most accepted and precise method for the evaluation of the free radical scavenging activity of the plant extract. 1, 1-diphenyl-2-picryl hydroxyl (DPPH) is a stable free radical and accepts an electron or hydrogen to become a stable molecule. Antioxidant on interaction with DPPH, transfer an electron or hydrogen atom to DPPH and thus neutralizing its free radical character. The degree of discoloration of DPPH indicates the scavenging activity of the plant extract. The reduction capacity of DPPH radical is determined by the decrease in its absorbance at 517 nm. The decrease in absorbance of DPPH radical caused by antioxidant because of the reaction between antioxidant and radical progress which results in scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When an antioxidant reacts with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor and is reduced to the DPPHH and as consequence, the absorbance at 517 nm decreases from the DPPH to DPPH-H form; results in decolorization (yellow colour) with respect to the number of electrons captured. More the decolorization more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of an extract Hence DPPH is usually used as substance to evaluate the antioxidant activity [22,23].

The diluted working solutions of the test extracts were prepared in methanol. Aascorbic acid was used as reference compound (100 µg/ml solution). DPPH of 0.004% was prepared in methanol and a 3 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. The mixtures were kept in dark for 30 min at room temperature. After incubation, the absorbance was spectrophotometrically measured at 517 nm against a blank. The inhibition percentage was calculated using the following formula:

$$\text{Percentage of Inhibition of DPPH activity} = (A-B)/A \times 100$$

Where, A is the absorbance of control and B is the absorbance of sample at 517 nm.

Evaluation of antioxidant activity by reducing power assay: The capacity of each plant extracts to reduce the ferric –ferry cyanide complex to the ferrous - ferry cyanide complex was determined by the method of Oyaizu [24] with some modifications. In brief, 2.5 ml of different plant extract solutions were mixed with 2.5 ml of phosphate buffer (PH-6.6) and 2.5 ml of 1% potassium ferry cyanide. The mixture was incubated at 50°C for 20 min. After incubation 2.5 ml of 10% TCA was added to mixture and was centrifuged for 10 min at 1000 g. 2.5 ml of upper layer of solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. Then the absorbance was measured at 700 nm by using UV spectrophotometer. Increase in absorbance of the reaction mixture indicates increased reducing power. All the tests were performed in triplicates and the results were expressed as mean. Similar method was adopted for Ascorbic acid which was used as a positive control.

Evaluation of Total Antioxidant Capacity (TAC) by phosphomolybdenum method: The total antioxidant capacity of plant extract was measured by spectrophotometric method of Prieto et al. [25] at different concentrations. Methanol extracts were prepared in water and combined in eppendorf tube with 1 ml of reagent solution (0.6M H_2SO_4, 28mM sodium phosphate, 4 mM ammonium molybdate) the tubes were incubated for 90 min at 95°C. The mixture was cooled to room temperature and the absorbance was read at 695 nm against a blank.

Statistical Analysis

All experimental measurements were carried out n triplicate and are expressed as average of three analyses ± standard deviation.

Results and Discussion

Nowadays, there is a considerable interest in the use of medicinal and aromatic plants for the search of natural antioxidants for their potential use in pharmacy and medicine. Mangrove plants provide an important source for the search of novel drugs as they are stress tolerant plants and rich in bioactive compounds. The bioactive compounds of mangrove plants can be used as potent source of modern drugs against various life

S No	Plant name	Common Name	Part used	Traditional use
1	*Aegiceras corniculatum*	Guggilum	Leaves	Used in the treatment of Rheumatism, Arthritis, Inflammation, Asthma & Diabetes
2	*Excoecaria agallocha*	Tilla	Leaves	Fish & Arrow head poison, cure for fish sting, cure for tooth ache & ulcer
3	*Lumnitzera racemosa*	Tanduga	Leaves	Cure for health problems in infants

Table 1: Ethno medicinal details of the selected mangrove plants.

threatening diseases. Keeping this in mind we have attempted to make a study on antioxidant potentials of selected mangrove plants. The Ethanomedicinal details of the selected mangrove plants of the present study were described in Table 1.

Total phenolic content

The quantitative estimation of total phenol was carried out by using Folin-Ciocalteu reagent in terms of gallic acid equivalent. It involves the oxidation of phenols in alkaline solution by the yellow molybdotungastophosphoric hetero polyanion reagent and colorimetric measurement of the resultant molybdotungesto phosphate blue [26]. This blue coloured pigment has a maximum absorption depending on the qualitative and /or quantitative composition of phenol mixtures besides the PH of the solution, usually adding sodium carbonate [27]. The calibration line with gallic acid at different concentrations was shown in Figure 1. Total phenolic content (mg gallic acid equivalent per gram dry extract weight) of each plant extract was shown in Table 1. The plant extracts which displayed the lowest and highest content of total phenols are the extracts of *Excoecaria agallocha* (20.56 ± 0.58 µg GAE/100 µg plant extract), *Aegiceras corniculatum* (24.06 ± 0.79 µg GAE/100 µg of plant extract) *Lumnitzera racemosa* (38.80 ± 0.19 µg GAE/ 100 µg of plant extract), the relatively higher content of *Lumnitzera racemosa* and *Aegiceras corniculatum* might explain the high antioxidant property, (the amount of total phenolic content was given in Table 2 and the graphical representation was shown in Figure 1) and Positive correlations were established between the total phenolic contents in these plant extracts and their antioxidant activities. Phenolic compounds are commonly found in plants and have been reported to possess several biological activities including a strong antioxidant activity [28,29] phenolic compounds have an important role in stabilizing lipid oxidation and are associated with the antioxidant activity. The antioxidant capacity of phenolic compounds is very often attributed to their radical scavenging ability mediated by hydroxyl groups [30].

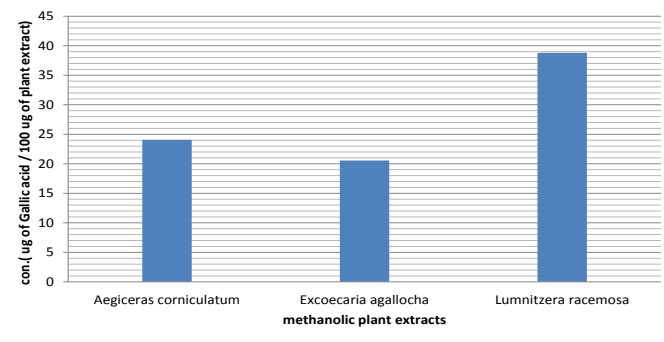

Figure 1: Total phenolic content by FC reagent.

S No	Plant Extract	Total Phenolics Expressed in Gallic Acid Equivalents (µg/100 µg of Plant Extract)
1	*Aegiceras corniculatum*	24.06 ± 0.79
2	*Excoecaria agallocha*	20.56 ± 0.58
3	*Lumnitzera racemosa*	38.80 ± 0.19

Results are expressed as mean ± SD (n=3) of three parallel measurements.

Table 2: Total phenolic content of selected plant extracts.

DPPH assay

DPPH has been used extensively as a free radical to evaluate reducing substances [31] and is a useful reagent for investigating the free radical scavenging activities of compounds [32]. Our results show high DPPH scavenging activity. Many researchers have reported positive correlation between free radical scavenging activity and total phenolic contents, which also matches with our findings.

Among the three mangrove species and standard, tested for i*n vitro* antioxidant activity using the DPPH method, the crude Methanolic extracts of *Aegiceras corniculatum*, *Excoecaria agallocha* and *Lumnitzera racemosa* with inhibition percentage of 75.13 ± 0.52, 41.43 ± 0.34 and 79.1 ± 0.62 (at 1 mg concentration) respectively. The percentage of inhibition of DPPH radical at different concentration was given in Table 2. The *Lumnitzera racemosa* extract showed highest antioxidant activity and *Excoecaria agallocha* extract showed lowest antioxidant activity. The percentage of inhibition of scavenging activity of DPPH free radical was shown in Figure 2 and Table 3.

Reducing power assay

Fe (III) Reduction is often is used as an indicator of electron donation activity, which is an important mechanism of antioxidant action of phenolics. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation process, so that they can acts as primary and secondary antioxidants. In this case, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of

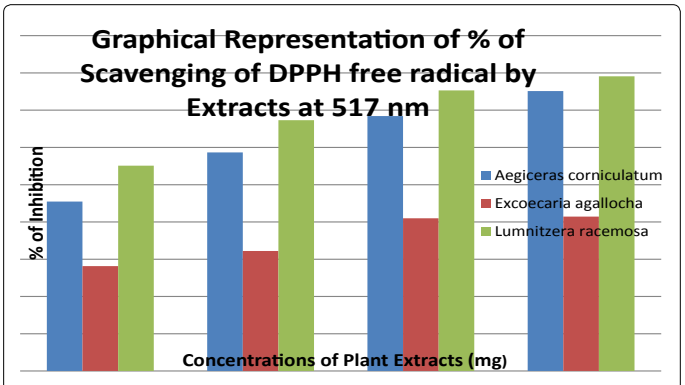

Figure 2: Percentage of inhibition of DPPH radical by plant extracts.

S No	Plant Species	% of Dpph Inhibition At Different Concentrations (Mg/Ml)			
		0.25	0.5	0.75	1 mg
1	*Aegiceras corniculatum*	45.48 ± 0.55	58.67 ± 0.43	68.42 ± 0.76	75.13 ± 0.52
2	*Excoecaria agallocha*	28.15 ± 0.37	32.21 ± 0.59	41 ± 0.16	41.43 ± 0.34
3	*Lumnitzera racemosa*	55.1 ± 0.48	67.32 ± 0.32	75.31 ± 0.74	79.1 ± 0.62

Results are expressed as mean ± SD (n=3) of three parallel measurements.

Table 3: Percentage of DPPH inhibition of plant extracts at different concentrations.

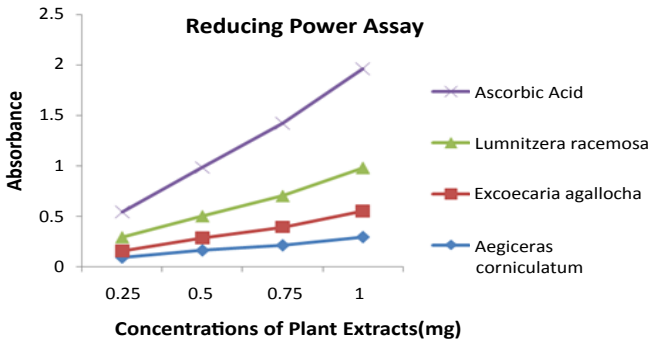

Figure 3: Reducing power assay by plant extracts.

each compound. Presence of reducers causes the conversion of the Fe^{3+} to Fe^{2+}. Amount of Fe^{2+} complex can be then monitored by measuring the formation of Perl's prusan blue at 700 nm. Increasing the absorbance at 700 nm indicates an increase in reducing ability. A higher absorbance indicates a higher reducing power. It was found that the reducing power of all the studied plant extracts increased with increase in their concentrations. Among these three plant extracts, the reducing power was found to be highest in *Lumnitzera racemosa* which was significantly followed by *Excoecaria agallocha* and *Aegiceras corniculatum* extracts. In this study, a positive relationship between reducing power and total phenolic content of plant extract was observed with in *Lumnitzera racemosa*. The graphical representation of reducing power assay was shown in Figure 3. The reducing capacity of a compound may serve as indicator of its potential antioxidant capacity [33]. The reducing capacity of compounds has been attributed to various factors such as prevention of chain reaction, chelating metals, reducing capacity and radical scavenging ability etc. [34].

Total antioxidant capacity

The total antioxidant capacity of methanolic plant extracts were evaluated by the phosphomolybdenum method according to the procedure described by Prieto etal is based on the reduction of mo (vI) to mo (v) by the sample and the subsequent formation green phosphate / mo (V) at acidic PH. This method is purely quantitative since the total antioxidant activity is expressed as the number of the equivalents of ascorbic acid. The methanol extracts of three different plant extracts showed potent antioxidant activity. The extract of *Lumnitzera racemosa* exhibited highest total antioxidant capacity and this is followed by *Aegiceras corniculatum* and *Excoecaria agallocha*. The graphical representation of total antioxidant capacity was shown in Figure 4.

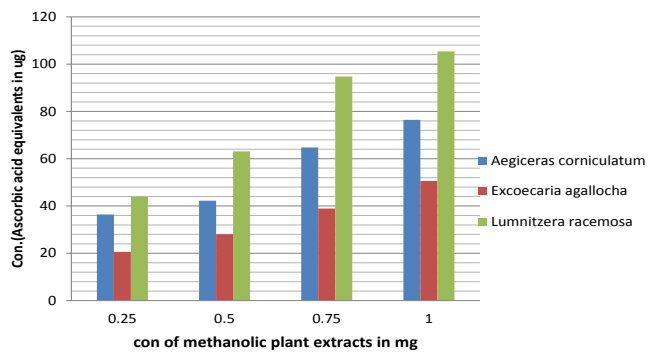

Figure 4: Total antioxidant capacity of selected plant extracts.

Conclusion

In order to characterize and analyze antioxidant activity of plant extracts, it is desirable to subject the extracts for the tests that evaluate the range of activities such as scavenging of the free radicals or reactive oxygen species, inhibition of membrane LPO and metal ion chelation. Antioxidant rich plant extracts serves as sources of nutraceuticals that reduce the oxidative stress and therefore prevent or slow down the degenerative diseases. In the present study, an effort has been made to explore the antioxidant property of the methanol extracts of selected mangrove plants. All the extracts demonstrated significant antioxidant properties as determined by the scavenging assay, reducing power assay and total antioxidant capacity. However, the methanol extract of *Lumnitzera racemosa* exhibited highest phenolic content and antioxidant potential. The present study indicates the potential of the extracts as a source of natural antioxidants with potential applications to reduce oxidative stress with consequent health benefits.

References

1. Fresquet F, Pourageaud F, Leblais V, Brandes RP, Savineau JP, et al. (2006) Role of reactive oxygen species and gp91phox in endothelial dysfunction of pulmonary arteries induced by chronic hypoxia. Brit J Pharmacol 148: 714-723.
2. Kikuzaki H, Nakatani N (1993) Antioxidant effects of some ginger constituents. Journal of Food Science 58: 1407-1410.
3. Hallwell B, Gutteridge JMC (1999) Free radicals in Biology and Medicine. 3rd edn, Oxford University Press, Oxford, UK, p: 543.
4. Finkel J, Holbrook NJ (2000) Oxidants, Oxidative stress and the biology of aging. Nature 408: 239-247.
5. Das M, Mukherjee SB, Shaha C (2001) hydrogen peroxide induces apoptosis like death in Leishmania donovani promomagstigotes. J Cell Sci 114: 2461-2469.
6. Naskar K, Guha bakshi DN (1995) Vegetarian pattern of the sundarbans. In mangrove swamps of the sundarbans. An ecological perspective. Naya proksah: Calcutta, India, p: 27.
7. Vilioglu YS, Mazza G, Gao L, Oomah BD (1998) Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. J Agri food chem 46: 4113-4117.
8. Barlow SM (1990) Toxicology aspects of antioxidants used as food additives. In Hudson BJF (Ed), Food antioxidants. Elsevier, USA, pp: 253-307.
9. Branen AL (1975) Toxicology and Biochemistry of butylated hydroxyanisole and butylated hydroxyl toluene. Journal of American Oil chemical Society 52: 59-63.
10. Chan HWS (1987) Autioxidation of unsaturated lipids. Academic press, London, p: 296.
11. Namiki M (1990) Antioxidants/Antimutagens in Food. Critical Reviews in Food science and Nutrition 29: 273-300.
12. Prkorny J (1991) Natural Antioxidants for food use. Trends in food science Technol 9: 223-227.
13. Sahu SC, Dhal NK, Mohanty RC (2010) "Potential Medicinal plants used by the Tribal of Deogarh district, Orissa, India" studies on Ethano. Medicine 4: 53-61.
14. Newman DJ, Cragg GM (2007) Natural Products as source of new drugs over the last 25 years. Journal of Natural Products 70: 461-477.
15. McChesney JD, Venkataraman SK, Henri TT (2007) Plant Natural products: Back to the future or in to Extinction? Phyto chemistry 68: 2015-2022.
16. Li JW, Vederas JC (2009) Drug discovery and Natural products: end of an ear or an endless Frontier? Science 325: 161-165.
17. Marrinova D, Atanassova R (2005) Total phenolics and Total flavonoids in Bulgarian fruits and Vegetables. Journal of the University of Chemical Technology and Metallurgy 40: 255-260.
18. Pereira DM, Valentaop PJA, Andrade PB (2009) Phenolics: From Chemistry to Biology. Molecules 14: 2202-2211.
19. Rammurthy PK, Bono A (2007) Antioxidant activity, Total phenolic and

flavonoids content of Morinda citrifolia fruit extract from various extraction process. Journal of Engineering Science and Technology 2: 70-78.

20. Patel VR, Patel PR, Kajal SS (2010) Antioxidant activity of some selected medicinal plants in western region of India. Advances in Biological Research 4: 23-26.
21. Cotelle N, Bemier JL (1996) Antioxidant Properties of Hydroxyl flavones. Free Radical Biol Med 20: 35-43.
22. Sieniwska E, Baj T, Glowniak K (2010) Influence of the preliminary sample preparation on the tannins content in the extracts obtained from Mutellina purpurea poir. Annales universitatis Mariae Curie- Skedowska 23: 47-54.
23. Luis A, Domingue F, Gil C, Duarte AP (2009) Antioxidant activity of extracts of Portuguese shrubs: Pterospartum tridentatum, Cytisus scoparius and Erica spp. Journal of Medicinal plant research 3: 886-893.
24. Oyaizu M (1986) Studies on product of browning reaction prepared from glucose amine. Japanese journal of Nutrition 44: 307-315.
25. Prieto P, Pineda M, Anguilar M (1999) Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Anal Biochem 269: 337-341.
26. Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, et al. (2001) Antioxidant principles from Bauhinia terapotensis. J Nat Prod 64: 892-895.
27. Shazia U (2013) Screening for antioxidant and free radical scavenging potential of extracts of leaves and flowers of Calotropis gigantean. Asian J Pharm Clin Res 6: 97-100.
28. Chandini SK, Ganesan P (2008) *In vitro* antioxidant activities of three selected brown sea weeds of India. Food Chem 107: 707-713.
29. Duh PD, Tu YY (1999) Antioxidant activity of water extract of Harng jyur (Chrysanthimum morifolium Ramat). Lebnesmittel-wissenchaft and Techonoligie 32: 269-277.
30. Hatano T, Edamatsu R (1980) Effect of interaction of tannins with co-existing substances VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chem Pharma Bull 37: 2016-2021.
31. Duan XJ, Zhang WW (2006) Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. Food Chem 95: 35-43.
32. Meir S, Kanner J (1995) Determination and involvement of aqueous reducing compounds in oxidative defense systems of various sensing leaves. J Agric Food Chem 43: 1813-1815.
33. Diplock AT (1997) will the 'good fairies' please prove to us that vitamin E lessens human degenerative of disease? Free Radical Res 27: 511-532.
34. Yildirim A, Mavi A (2001) Determination of antioxidant and antimicrobial activities *Rumaxs crispus* L. extracts. J Agric Food Chem 49: 4083-4089.