ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 06, pp.52430-52434, June, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

TOTAL PHENOLIC, TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF LEAVES EXTRACTS OF *CHENOPODIUM ALBUM* L. AND *ATRIPLEX HORTENSIS* L.

***Adhav Rahul and Deokule Subhash**

Department of Botany, Savitribai Phule Pune University, Pune - 411007, India

ARTICLE INFO

Article History:
Received 22nd March, 2017
Received in revised form
03rd April, 2017
Accepted 19th May, 2017
Published online 30th June, 2017

Key words:

A. hortesnsis, *C. album*, ABTS, Antioxidant, Total Phenolic and Flavonoids.

ABSTRACT

The current investigation was conducted on *Chenopodium album* L. and *Atriplex hortensis* L. to estimate the contents of total phenolics, total flavonoids and antioxidant activity by using four different solvent extracts. Total phenolic content was determined by using the Folin-Ciocalteu method while total flavonoid contents were estimated by using Aluminium chloride ($AlCl_3$) colorimetric assay. The antioxidant avtivity in the form ABTS 2, 2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) was evaluated with the help of spectrophotometric method. Among all the tested extracts the highest amounts of total phenolic content was observed in *A. hortesnsis* methanolic extract 32.33±0.021 gallic acid equivalent (GAE)/g. Similarly the highest flavonoid content was observed in *A. hortesnsis* ethanolic extract 1.75±0.026 mg of quercetin equivalent/g. The highest ABTS free radical scavenging activity was observed in *A. hortesnsis* ethyle acetate extract 98.6 %±0.015µg/ml.

Citation: Adhav Rahul and Deokule Subhash, 2017. "Total phenolic, total flavonoid content and antioxidant activity of leaves extracts of *Chenopodium album* L. and *Atriplex hortensis* L.", *International Journal of Current Research*, 9, (06), 52430-52434.

INTRODUCTION

Antioxidants are the compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. (Choe, and Min, 2009), redox active compounds which help to prevent radical formation or remove them before damage can occur or repair oxidative damage. They eliminate damaged molecules by directly scavenging or neutralizing free radicals or other reactive oxygen species (ROS) and reactive nitrogen species (RNS). Oxidative damage to cells and macromolecule is considered to be the cause of several diseases such as coronary heart disease, a cataractogenesis, various neurodegenerative diseases including alzheimer's disease, cancer and aging oxidative injury involves free radical induced damage from both endogenous and exogenous sources (Lobo *et al*., 2010). Review of literature revealed that the dietary antioxidants play an important role in preventing degenerative diseases associated with ageing such as cancer, cardio-vascular diseases, cataract, neurodegenerative diseases and immunological decline (Rahman, 2007). This is a great deal of interest in the natural antioxidants often referred to as "nutraceuticals" in view of their positive health effects. Previous literature survey have shown that the action of the natural antioxidants at the cellular and molecular level involves scavenging of free radicals and modulating apoptosis (Devasagayam *et al*., 2004). In view of this, nutraceuticals are becoming widely accepted as an adjunct to conventional therapies for enhancing the well being Bioprospecting or the search for newer bioactive compounds from the nations biodiversity for better health is a new thrust area in biotechnology. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients so they must be supplied in the diet (Abbasi *et al*., 2015). A paradox in metabolism is that, while the vast majority of ramified life on Earth requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species (Dreher, 1996). Oxygen (O_2) is a universal electron acceptor that allows aerobic organism to use energy stored in foodstuffs. Consequently, organisms contain a ramified network of antioxidant metabolites and enzymes that work together to prevent oxidative forfeiture to cellular components such as DNA, proteins and lipids (Vertuani *et al*., 2004). Many spices, fruits, vegetables and medicinal plants contain potential antioxidant compounds, such as vitamins A, C and E, β-carotene, α-tocopherol, carotenoids, flavonoids, isoflavones, anthocyanins, polyphenols, tannins and other phenolics constituents etc. (Ghasemi *et al*., 2009). Few compounds out of these were used

Corresponding author:* **Adhav Rahul,
Department of Botany, Savitribai Phule Pune University, Pune - 411007, India.

for experimental analysis, these are mentioned below. Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenolics are generally involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as contributing to plants' colours. They are ubiquitous in all plant organs and are therefore an integral part of the human diet.

Atriplex hortensis L. is a hardy, annual plant, belongs to family Amaranthaceae. *A. hortensis* is also known as garden orache, red orach, mountain spinach. Leaves are prefered as a raw or cooked (Hedrick, 1972) or used like a spinach. They have a bland flavour and are traditionally mixed with sorrel leaves in order to modify the acidity of the latter (Facciola, 1990). Another report says that the flavour is stronger than spinach (Phillips, 1995). The leaves are diuretic, emetic and purgative (Polunin, 1969; Duke, 1983) They are also said to be a stimulant to the metabolism and an infusion is used as a spring tonic and a remedy for tiredness and nervous exhaustion (Launert, 1989). They have been suggested as a folk remedy for treating plethora and lung ailments (Duke, 1983). The leaves are said to be efficacious when used externally in the treatment of gout (Grieve, 1984). *Chenopodium album* L. belongs to family Amaranthaceae. A number of uses have been reported on *C. album*. The leaves and tender branches may be used as a vegetable, and also in India it is used for the preparation of curd, locally known as Raita (Maheshwari, 1963). It may also be used as a fodder for livestock. The leaves may be taken in the form of an infusion or decoction as a laxative and anthelminthic. It has also been recommended by Hindu physicians as a treatment for hepatic disorders and splenic enlargement (Chopra *et al*., 1958). In the present investigation it has been decided to evaluate the antioxidant properties, as well as to estimate the total phenolic and flavonoid content of the selected plant leaves extracts.

MATERIALS AND METHODS

Plant materials were collected from in and around Pune District. Efforts were made to collect plant material in flowering and fruiting conditions for the correct botanical identification. The collected plants were identified with the help of The flora of the presidency of Bombay (Cooke, 1904), Flora of Kolhapur District (Yadav and Sardesai, 2002), Flora of Maharashtra State Vol II (Singh *et al*., 2002). The identified plants were authenticated from the authorities of Botanical Survey of India, Pune-1, Maharashtra State.

Preparation of extract

Freshly collected plant samples (50 gm) were chopped and placed in the filter Paper (No. 89) in a classical Soxhlet apparatus and successively extracted with 170 ml of chloroform, ethanol, methanol and ethyl acetate solvents for 3 hrs. Extracts were cooled at room temperature. The extracts were filtered through whatman filter paper No. 1, and the filtrate was concentrated under reduced pressure by rotary evaporator. These extracts were used in the present study.

Determination of total phenol content

Total phenol content was determined by using standard protocol of (Madaan *et al*., 2011) with some modifications. For the preparation of Standard gallic acid graph, 10 mg of gallic acid was dissolved in 100 ml of 50% methanol (100 µg/ml) and then further diluted to 3.1, 6.25, 12.5, 25, 50 and 100 µg/ml. One ml aliquot of each dilution was taken in a test tube and diluted with 10 ml of distilled water. Then 1.5 ml Folin Ciocalteu's reagent was added and allowed to incubate at room temperature for 5 min. Four ml of 20% (w/w) Na_2CO_3 was added in each test tube, the final 25 ml total reaction volume was adjusted with distilled water and left to stand for 30 min at room temperature. Absorbance of the standard was measured at 765 nm using UV/VIS spectrophotometer (Sican 2301, inkarp) against blank, i.e. distilled water. One ml aliquot of the sample was taken in a test tube and diluted with 10 ml of distilled water. Then 1.5 ml Folin Ciocalteu's reagent was added and allowed to incubate at room temperature for 5 min. Four ml of 20% (w/w) Na_2CO_3 was added, final volume adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature. Absorbance of the sample was measured at 765 nm against blank, i.e. distilled water. The total phenol content was calculated from the regression equation prepared from a range of concentrations of gallic acid and optical densities for the concentrations.

Determination of total flavonoid content

The total flavonoid content was determined by following the protocol of (Kamtekar *et al*., 2014) and was calculated from the regression equation prepared from a range of concentrations of quercetin and optical densities for the concentrations. Total flavonoid content was estimated by using Aluminium chloride ($AlCl_3$) colorimetric assay. One ml of sample aliquots and one ml standard quercetin solution (100, 200, 400, 600, 800, 1000 µg/ml) was positioned into test tubes separately and 4ml of distilled water and 0.3 ml of 5% sodium nitrite solution was added into each test tube. After 5^{th} minutes, 0.3 ml of 10 % aluminum chloride was added. At 6^{th} minute, 2 ml of 1 M sodium hydroxide was added. Finally, volume was making up to 10 ml with distilled water and mix well. At the end of reaction yellowish orange colour was appeared. The absorbance was measured spectrophotometrically at 510 nm. The blank was performed using distilled water. Quercetin was used as standard. The samples were performed in triplicates.

ABTS free radical scavenging Activity

ABTS radical scavenging activity was performed by following the method of (Re *et al*., 1999) with some modifications. ABTS (Hi-Media) radical cation was produced by reacting ABTS stock solution 7 mM with 2.45 mM potassium persulfate (final concentration) by dissolving in distilled water (1:1) ratio and allowing the mixture to incubate 16-20 hrs. for the formation of ABTS radical cation at room temperature. Before use, this solution was diluted with ethanol to get an absorbance of 0.700 at 734 nm. Dilutions of plant extracts in various solvents were made to obtain concentrations of 100 to 400 µg/ml. The plant extract at various concentrations with 1ml of ABTS solution was homogenized and its absorbance was recorded spectrophotometrically (Sican 2301, inkarp). At 734 nm. Against blank i.e. Ethanol. Trolox was used as

positive control. As for the antiradical activity, ABTS scavenging activity was expressed as IC_{50} (µg/ml). The percent inhibition of ABTS radical scavenging activity was calculated using the following formula: ABTS scavenging activity (%) = (A0 –A1) /A0 ×100 Where A0 is the absorbance of the negative control, and A1 is the absorbance of the sample. All tests were performed in triplicate, and the average value and standard deviation were calculated.

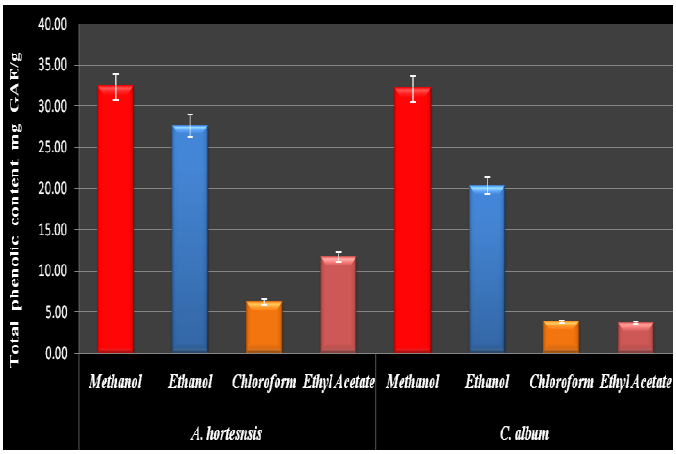
Determination of IC_{50} values

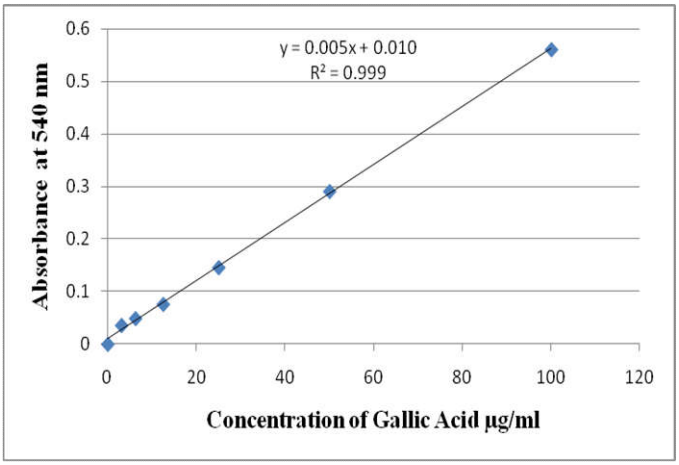
Regression equations were prepared from the concentrations of the extracts and percentage inhibition in different systems of assay. IC_{50} values (Concentration of inhibitor sample required to inhibit) were calculated from these regression equations. A lower IC_{50} value indicates higher inhibitory activity.

RESULTS AND DISCUSSION

Determination of total phenol content

Quantitative estimation of total phenols was done on the basis of a standard curve of gallic acid and linearity of the calibration curve was achieved between 3.1 to 100 mg/ml concentration for gallic acid (r^2 =0.99). The results showed that, the total phenolic contents in the selected plants species for the study varied considerably and ranged from 3.71 to 32.33 mg GAE/g (Table 1 and graph 1). The highest phenolic content was observed in *A. hortesnsis* leaf Methanolic extract i.e. 32.33 mg GAE/g. and lowest phenolic content was observed in *C. Album* ethyl acetate extract i.e. 3.71 mg GAE/g. It is also reported that phenolics compounds and flavonoids are natural products which have been shown to possess various biological properties related to antioxidant mechanisms (Shirwaikan *et al*., 2004). Polyphenols have the function to scavenge the free radicals in human body and to help maintain healthy body by scavenging or removing the reactive oxygen species (ROS) (Ghasemzadeh *et al*., 2012). In general, the higher total phenolic content resulted in higher antioxidant capacity (Farasat *et al*., 2014).





Graph 1. Total Flavonoid content and standard curve of extinction against GA concentration

ABTS free radical scavenging activity

In the present study, the results showed inhibition percentage and IC_{50} values ranges from 14.3 to 98.6 and 2.0 to 9.9 respectively which are depicted in (Table 2 and Graph 3) Among the four different concentrations of standard Trolox (100, 200, 300, 400 µg/ml) used in the study, showed 72.70%, 78.04%, 83.83% and 91.72 % scavenging activity respectively, where highest scavenging activity of trolox was recorded 91.72% at 400 µg/ml concentration. Among the plants studied the highest ABTS free radical scavenging activity in terms of inhibition percentage was observed in *A. hortesnsis* ethanol extract i.e. 98.6 % µg/ml with IC_{50} value 2.0 µg/ml. The lowest inhibition percentage was observed in *C. album* chloroform extract i.e. 29.3 µg/ml with IC_{50} value 7.3 µg/ml. In the present investigation it was observed that both the standard trolox and *A. hortesnsis* extracts showed dose dependent activity.

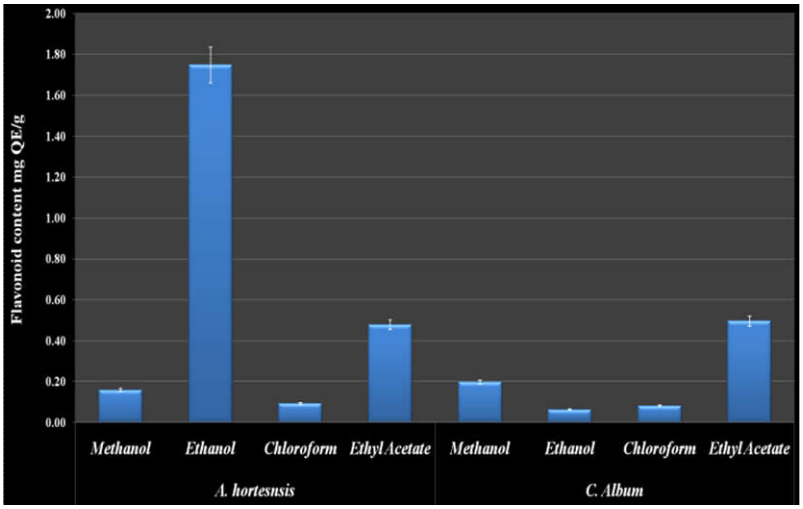
Table 1. Total phenol (mg gallic acid equivalent (GAE)/g) and Flavonoid content (mg of quercetin equivalent (QE) /g.)

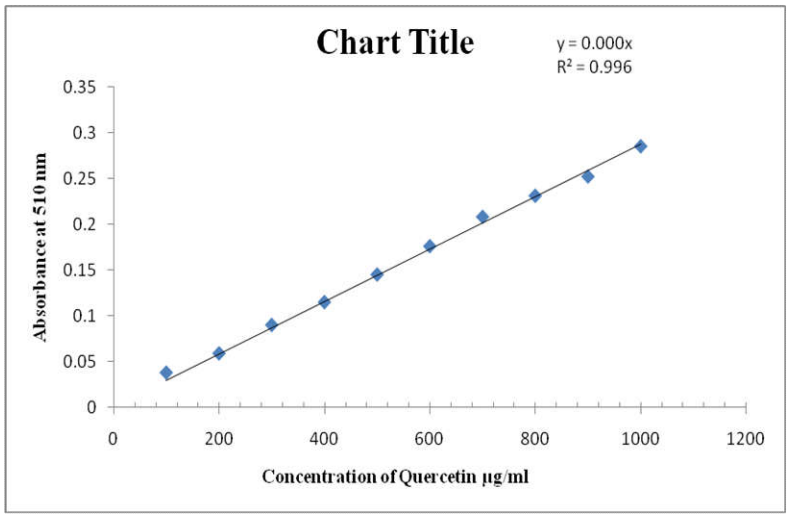
S.No.	Plant Name	Extract	Total Phenol content mg GAE/g	Total Flavonoid content mg QE/g
1	*A. hortesnsis*	Methanol	32.33±0.021	0.16±0.018
		Ethanol	27.63±0.017	1.75±0.026
		Chloroform	06.18±0.010	0.09±0.035
		Ethyl Acetate	11.67±0.019	0.48±0.026
2	*C. album*	Methanol	32.14±0.009	0.20±0.019
		Ethanol	20.32±0.015	0.06±0.062
		Chloroform	03.85±0.009	0.08±0.081
		Ethyl Acetate	03.71±0.012	0.50±0.049

*Each value represents a mean ± SE (n = 3).

Determination of total flavonoid content

Quantitative estimation of total flavonoid was done on the basis of a standard curve of quercetin and linearity of the calibration curve was achieved between 100 to 1000 mg/ml concentration of quercetin (r^2 =0.996). The results showed that the total flavonoid contents in the selected plants species for the study varied considerably and ranged from 0.06 to 1.75 mg of quercetin equivalent/ g (Table 1 and Graph 2). The highest flavonoid content was observed in *A. hortesnsis* leaf ethanolic extract i.e. 1.75 quercetin equivalent/ g. and lowest flavonoid content was observed in *C. Album* ethanol extract i.e. 0.06 quercetin equivalent/ g.



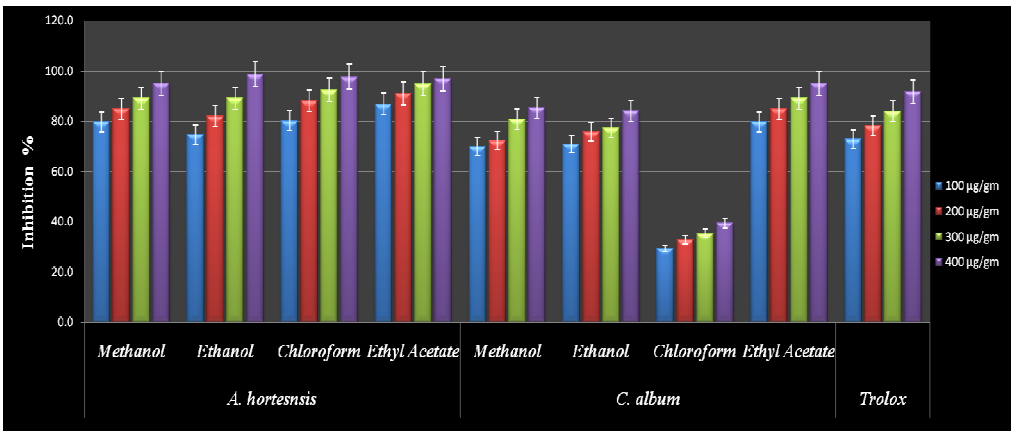


Graph 2. Total flavonoid content mg of quercetin equivalent/g. and standard curve of extinction against quercetin concentration

Table 2. ABTS free radical scavenging activity and IC_{50} values in different solvent extracts

S. No.	Plant Name	Extract	Inhibition Percentage µg/ml				IC_{50} Values (µg/ml)
			100	200	300	400	
1	*A. hortesnsis*	Methanol	79.7±0.014	84.8±0.030	89.1±0.017	95.0±0.012	4.8±0.032
		Ethanol	74.7±0.027	82.0±0.020	89.1±0.038	98.6±0.015	2.0±0.014
		Chloroform	80.2±0.017	88.1±0.014	92.5±0.017	97.6±0.057	4.4±0.022
		Ethyl Acetate	86.9±0.011	91.2±0.021	94.9±0.066	97.0±0.027	9.9±0.032
2	*C. album*	Methanol	69.7±0.049	72.3±0.05	80.7±0.013	85.3±0.078	2.4±0.016
		Ethanol	70.9±0.049	75.7±0.06	77.3±0.015	84.0±0.060	4.1±0.012
		Chloroform	29.3±0.080	32.9±0.08	35.3±0.075	39.4±0.072	7.3±0.025
		Ethyl Acetate	79.7±0.011	84.8±0.011	89.1±0.088	95.0±0.017	4.8±0.023
3	Trolox		72.7±0.016	78.0±0.018	83.8±0.020	91.7±0.025	2.5±0.017

*Each value represents a mean ± SE (n = 3).



Graph 3. ABTS free radical scavenging activity (Inhibition %) of different solvent extracts and standard trolox

Conclusion

This study revealed that all the tested solvent extracts possess antioxidant activity. *A. hortesnsis* exhibited high phenolics and flavonoid contents and also, high antioxidant activity with a low IC_{50}. Strong positive and significant correlations between ABTS radical scavenging and phenolics and flavonoid contents showed that, phenolic compounds, including flavonoids are the main contributors of antioxidant activity in *A. hortesnsis.* It has been also shown that the scavenging effects on the ABTS radical increased with the increasing concentration of the samples to a certain extent and hence are said to be strongly dependent on the extract concentration. The antioxidant activity exhibited by the extract of *A. hortensis* leaves could justify the ethnotherapeutic usage of this plant by the traditional healers. The results from the present study indicate that it would be highly economical for the production of potential antioxidant supplement (s).

Acknowledgements

The author is thankful to authorities of Savitribai Phule Pune University for providing facilities and granting research fellowship.

REFERENCES

Abbasi, A. M.; Shah, M. H. and M. A. Khan. 2015. Wild edible vegetables of lesser Himalayas. *Springer International Publishing*.

Chopra, R. N.; Chopra, I. C.; Handa, K. L. and L. D. Kapur. 1958. Indigenous Drugs of India. Calcutta, India: U. N. Dhur & Sons Private Limited.

Cook, T. 1904. The Flora of The Presidency of Bombay, 2: 586, 288.

Devasagayam, T.P.A.; Tilak, J.C.; Boloor, K. K.; Sane, K. S.; Ghaskadbi, S. S. and R. D. Lele. 2004. Free radicals and antioxidants in human health: current status and future prospects. *Japi*., 52(10):794-804.

Dreher, D. and A. F. Junod. 1996. Role of oxygen free radicals in cancer development. *European Journal of cancer*, 32(1):30-38.

Duke, J. 1983. Handbook of Energy Crops.

Facciola, S. 1990. Cornucopia - A Source Book of Edible Plants. *Kampong Publications* ISBN 0-9628087-0-9.

Farasat, M.; Khavari-Nejad, R. A.; Nabavi, S. M. B. and F. Namjooyan. 2014. Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. *Iranian Journal of Pharmaceutical Research*, 13 (1), pp.163-170.

Ghasemi K.; Ghasemi Y. and M. A. Ebrahimzadeh. 2009. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pakistan Journal of Pharmaceutical Sciences*, 22(3): 277-81.

Ghasemzadeh, A.; Omidvar, V. and H. Z. E. Jaafar. 2012. Polyphenolic content and their antioxidant activity in leaf extract of sweet potato (*Ipomoea batatas*). *Journal of Medicinal Plants Research*. 6(15):2971-2976.

Grieve, A. 1984. Modern Herbal. Penguin ISBN 0-14-046-440-9.

Hedrick, U. P. 1972. Sturtevant's Edible Plants of the World. *Dover Publications,* ISBN 0-486-20459-6.

Kamtekar, S., Keer, V. and Patil, V., 2014. Estimation of Phenolic content, Flavonoid content, Antioxidant and Alpha amylase Inhibitory Activity of Marketed Polyherbal Formulation. Journal of Applied Pharmaceutical Science Vol. 4(09), pp. 061-065.

Lau, N.C., Lim, L.P., Weinstein, E.G. and Bartel, D.P., 2001. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science, 294(5543), pp.858-862.

Lobo, V.; Patil, A.; Phatak, A. and N. Chandra. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*., 4(8): 118–126.

Madaan, R., Bansal, G., Kumar, S. and Sharma, A., 2011. Estimation of total phenols and flavonoids in extracts of Actaea spicata roots and antioxidant activity studies. *Indian journal of pharmaceutical sciences*, 73(6), p.666.

Maheshwari, J. K. 1963. The Flora of Delhi. New Delhi, India: CSIR.

Phillips, R. And M. Rix. 1995. Vegetables Macmillan Reference Books, London. ISBN 0 333 626400.

Polunin, O. 1969. Flowers of Europe - A Field Guide. *Oxford University Press,* ISBN 0192176218.

Rahman, K. 2007. Studies on free radicals, antioxidants, and co-factors. *Clinical interventions in aging*, 2(2):219.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, 26(9), pp.1231-1237.

Shirwaikan, A.; Rajendran, K. and K. Dinesh. 2004. In vitro antioxidant studies of Annona squamosa. *Indian J Exp Biol*., 142:803.

Singh, N. P. 2001. *Flora of Maharashtra state*. Botanical survey of India.(2), pp. 795, 798.

Soobrattee, M. A.; Neergheen, V. S.; Luximon-Ramma, A.; Aruoma, O. I. and T. Bahorun. 2005. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 579(1), pp.200-213.

Vertuani, S.; Angusti, A. and S. Manfredini. 2004. The antioxidants and pro-antioxidants network: an overview. *Current pharmaceutical design*, 10(14):1677-1694.

Yadav, S. R. and M. M. Sardesai. 2002. Flora of Kolhapur district. Shivaji University, Kolhapur.

****