

Total Antioxidant Profile of some Common Medicinal Plants of West Bengal

Puspal De^{1*}, Samipa Das¹, Madhumita J Mukhophadhyay²

¹Department of Genetics, Institute of Genetic Engineering, 30 Thakurhat Road, Kolkata- 700128, West Bengal, India

²Department of Biotechnology, Institute of Genetic Engineering, 30 Thakurhat Road, Kolkata- 700128, West Bengal, India

Available Online: 25th June, 2018

ABSTRACT

Background: Nature provides several phyto constituents since thousands of years and many of them are used as an alternative drug in Ayurvedic and folk medicine. The medicinal value greatly depends on their antioxidant property. So, these herbal extracts are essentially important for the remediation of reactive oxygen species. Aim: Determination of total antioxidant property of twelve common Indian plant extract used in our daily life for several ways. Method: The crude aqueous extract was prepared and then phospho-molybdenum method was adopted for the study of total antioxidant property. Result: Among twelve common plant extracts bramhi stands first for its antioxidant property and rest of them are depicted in their decreasing order – green tea, sajne, laal sag, basaka, coffee, black tea, pineapple, turmeric, licorice, betel leaf. Conclusion: A number of common plants, known to have medicinal values were assayed for their antioxidant property. Bramhi shows maximum efficacy in this respect, whereas all other medicinal plants assayed showed antioxidant property to some extent.

Keywords: Antioxidant property, phosphomolybdenum method, common medicinal plants, crude extract, reactive oxygen species.

INTRODUCTION

Nature provides huge medicinal bio-active components or phyto-chemicals for thousands of years and a wide spectrum of modern human drug is being isolated from natural resources. Traditional therapeutic process, folk medicine and ayurvedic medicine, which are formerly known as second line therapy are now become an important source of therapeutic agent^{1,2}. So there is an urgent need to develop new plant derived drug with better bioactive potential without or less side effects. Use of different herbs and plants as an alternative and complementary therapy is under a category of medicine that includes a variety of treatment approaches that fall outside the category of conventional medicine. These traditional herbal medicines, often known as a good source of nutraceuticals, possess various beneficial effects on human health. In recent years a significant amount of research regarding plant originated drugs is going on though it still needed to establish the safety and efficacy of these therapies and compared with mainstream medical therapies researches are still inadequate³.

The medicinal property of a particular plant can be determined by the presence of different natural compounds with bio-active potentials and the balanced proportion of these components give them curative or therapeutic characteristics. So, analysis of varied natural

compounds is the key of interest to the researchers. The beneficial property of a medicinal plant often depends on their property to scavenge damaging free radicals or their antioxidant potentiality⁴. Recent research revealed that plants or plant parts having greater antioxidant property means greater value in therapeutic sectors. On this scenario, the present study was conducted to determine the total antioxidant property of some common local plants and for that purpose different plant parts like leaves, rhizome, fruit, shoots, bark, stem, beans are selected for the study.

Twelve common local plants are chosen to study their total antioxidant property and this selection of plant species remains as an essential step. In the current study common plants from West Bengal were used which are randomly consumed as spice, mouth freshener, beverage, fruit, cultural and medicinal purposes. These plants were chosen with the objective to find out their antioxidant potential both qualitatively and quantitatively. The plant species along with their family and scientific name, parts used and common uses are clearly depicted in Table-1.

MATERIALS AND METHOD

Collection of Samples

All the fresh leaves, rhizome, fruit, shoots, total fresh foliage, bark, stem, beans were collected from local markets of Kolkata, India, during the month of December

Table 1: The common name, scientific name, family of the plant species and common use of the selected plants.

Sl. No.	Plant	Family	Binomial Name	Parts Used for Study	Common Uses
1.	Turmeric	Zingiberaceae	<i>Curcuma longa</i>	Rhizome	-Widely used as a spice in various cuisines. -Used in Indian traditional medicine (also called Siddha or Ayurveda). -Used to make a poor fabric dye.
2.	Betel Leaf	Piperaceae	<i>Piper betle</i>	Leaves	-Primarily used as a flavor wrapper for chewing of areca nut or tobacco as a mouth freshner. -Also used in cooking, usually raw, for its peppery taste.
3.	Laal Sag	Amaranthaceae	<i>Amaranthus gangeticus</i>	Shoots or foliage	-Used in various cuisines specially in Bengali cuisine.. -Used as ornamental plants
4.	Nisindha	Lamiaceae	<i>Vitex negundo</i>	Leaves	-Used as a cough remedy. -Also used for treating stored garlic against pests.
5.	Brahmi	Plantaginaceae / Scrophulariaceae	<i>Bacopa monnieri</i>	Leaves with small steam	-Used in traditional Ayurvedic treatment for epilepsy and asthma. -Also used in Ayurveda for ulcers, tumors, ascites, enlarged spleen, inflammations, leprosy, anemia, gastroenteritis etc.
6.	Basaka	Acanthaceae	<i>Justicia adhatoda</i>	Leaves	-Has a number of medicinal uses in Siddha Medicine, Ayurvedic and Urani systems of medicine.
7.	Sajne	Moringaceae	<i>Moringa oleifera</i>	Leaves and soft foliage	-Used in various cuisines. -The bark, sap, roots, leaves, seeds and flowers are used in traditional medicine. -Its leaf powder is an effective soap for hand washing because of anti-septic and detergent properties from phytochemicals in the leaves. -Moringa seed cakes are used to filter water using flocculation to produce potable water.
8.	Green Tea	Theaceae	<i>Camellia sinensis sinensis,</i>	Leaves	-The leaves are used in traditional Chinese medicine and other medical systems to treat asthma, angina pectoris, peripheral vascular disease, and coronary artery disease. -Also consumed as beverage.
9.	Black Tea	Theaceae	<i>Camellia sinensis sinensis,</i>	Leaves	-The leaves are used in traditional Chinese medicine and other medical systems to treat asthma, angina pectoris, peripheral vascular disease, and coronary artery disease. -Also consumed as beverage.
10.	Coffee	Rubiaceae	<i>Coffea arabica</i>	Beans	-Mostly consumed as beverage.
11.	Licorice	Fabaceae	<i>Glycyrrhiza glabra</i>	Bark or steam	-Used as a flavoring agent for tobacco. -Used in a wide variety of candies or sweets. -Used by brewers to flavor and colour porter classes of beers.
12.	Pineapple	Bromeliaceae	<i>Ananas comosus</i>	Fruit	-Its flesh and juice are edible around the world.

to April and all the plants and plant parts were properly identified.

Chemicals

All the chemicals and reagents used were of analytical grade.

Preparation of Extract

All the fresh samples were washed twice through running tap water followed by distilled water and finally air dried. Separately all the samples were blended to fine powder after thorough drying. The shade dried powder of samples was labeled properly and stored in room temperature for future use. One gram dried powder from each samples

Table 2: Anti-oxidation activity of aqueous extract of selected medicinal plants.

Sl. No.	Medicinal plant	Concentration ($\mu\text{g/ml}$)	Antioxidation activity (%)
1.	Turmeric (<i>Curcuma longa</i>)	100	48.23 \pm 0.43
		50	37.64 \pm 0.34
2.	Betel leaf (<i>Piper betle</i>)	100	20.80 \pm 0.81
		50	16.70 \pm 1.15
3.	Laal sag (<i>Amaranthus gangeticus</i>)	100	67.90 \pm 0.73
		50	20.40 \pm 1.01
4.	Nisindha (<i>Vitex negundo</i>)	100	62.76 \pm 0.29
		50	28.57 \pm 0.37
5.	Bramhi (<i>Bacopa monnirrie</i>)	100	91.35 \pm 0.23
		50	71.15 \pm 0.19
6.	Basaka (<i>Justicia adhatoda</i>)	100	67.54 \pm 0.85
		50	41.23 \pm 0.52
7.	Sajne (<i>Moringa oleifera</i>)	100	72.86 \pm 0.51
		50	58.54 \pm 0.84
8.	Green tea (<i>Camellia sinensis</i>)	100	86.31 \pm 0.61
		50	64.40 \pm 0.30
9.	Black tea (<i>C. sinensis</i>)	100	57.01 \pm 0.76
		50	49.20 \pm 0.91
10.	Coffee (<i>Coffea arabica</i>)	100	65.11 \pm 0.11
		50	49.32 \pm 0.74
11.	Licorice (<i>Glycyrrhiza glabra</i>)	100	41.17 \pm 0.44
		50	36.47 \pm 0.26
12.	Pineapple (<i>Ananas comosus</i>)	100	52.69 \pm 0.57
		50	47.99 \pm 0.27

(Data represented as \pm SD, where N=3)

were taken in each pre-labeled conical flasks and 40 ml of double distilled de-ionized water was added in each of them. The mixtures were kept in the BOD shaker incubator at 30°C temperature in 120 rpm overnight and were filtered through Whatman filter no. 1(Sigma-Aldrich) after 24 hrs.

Total antioxidant activity

The total antioxidant activity of these extract were evaluated according to Prieto *et al.*, 1999.⁵ 0.3 ml of each aqueous extract (50, 100 $\mu\text{g/ml}$) were mixed with 3 ml assay mixture which contain 4 mmol/L ammonium molybdate, 0.6 mol/L sulphuric acid and 28 mmol/L sodium phosphate. The mixture along with test samples was incubated at 95°C for 90 min in water-bath. After cooling to 25°C, absorbance of the final solution was measured at 695 nm wave length in spectrophotometer (Beckman). Vehicle (Distilled water) was used as blank and ascorbic acid as positive control.

Analysis of Data

The percentage of antioxidant property was calculated by using the following formula:

$$\% = \frac{[(\text{OD sample} - \text{OD blank}) / (\text{OD ascorbic acid} - \text{OD blank})] \times 100}{1}$$

All the experiments were performed thrice and the percentage of total antioxidant obtain from every experiment were calculated as mean standard deviation with the help of Microsoft Excel.

RESULT

In the present study, 100 and 50 $\mu\text{g/ml}$ concentrations of aqueous sample's extracts were used for the total antioxidant assay. The results obtained were depicted in Table-2.

From the experimental result it is observed that "bramhi" (*Bacopa monnirrie*) has highest amount of antioxidant property whereas betel leaf (*Piper betel*) shows the lowest amount of antioxidation property among the selected medicinal plants in both 50 and 100 $\mu\text{g/ml}$ concentrations of aqueous extract. The comparative analysis of total antioxidant property by phospo-molybdenum method of the twelve selected medicinal plants is represented graphically in Figure-1. The total antioxidant property was also changed when the concentration was double and this could be varied in different plant. The Figure: 2 show the change of the inclination of line graph against the concentration difference.

DISCUSSION

Any molecular species that contains an electron in its atomic orbital is known as free radical and they can be capable of independent existence within the body^[6]. These unpaired electrons have been shown some common properties that are mainly found in most of the radicals. The conformations of free radicals are mainly unstable and this instability in its orbital, contributes for its highly reactive nature. They can donate and receive electron from other molecules and thus behave like an oxidant and reductant. These free radicals are produced in the human body by normal metabolic process or by the exposure of external physical and chemical agents like X-rays, ozone, cigarettes smoking, pollutants, industrial effluent etc^{6,7}. Among 5% of the inhaled oxygen converted to the free radicals during the cellular respiratory mechanism. These highly reactive species destabilize cellular membrane; react with proteins, DNA, and biologically relevant lipids and carbohydrate and all other important micro or macro molecules in the body leading cell damage and disruption of homeostatic pathways⁸.

Any molecules that are stable enough to donate rampaging free radicals to neutralize or reduce the damaging capacity of it, is known as anti-oxidants⁹. The anti-oxidants are mainly of low molecular weight and they can interact with free radicals and terminate its chain reaction with cellular molecules before damage. By the radical's scavenging property, anti-oxidants can delay or inhibit the cellular damage^{7,8,9}. Micro-nutrients like vitamin E, vitamin C, β -carotene can scavenge the free

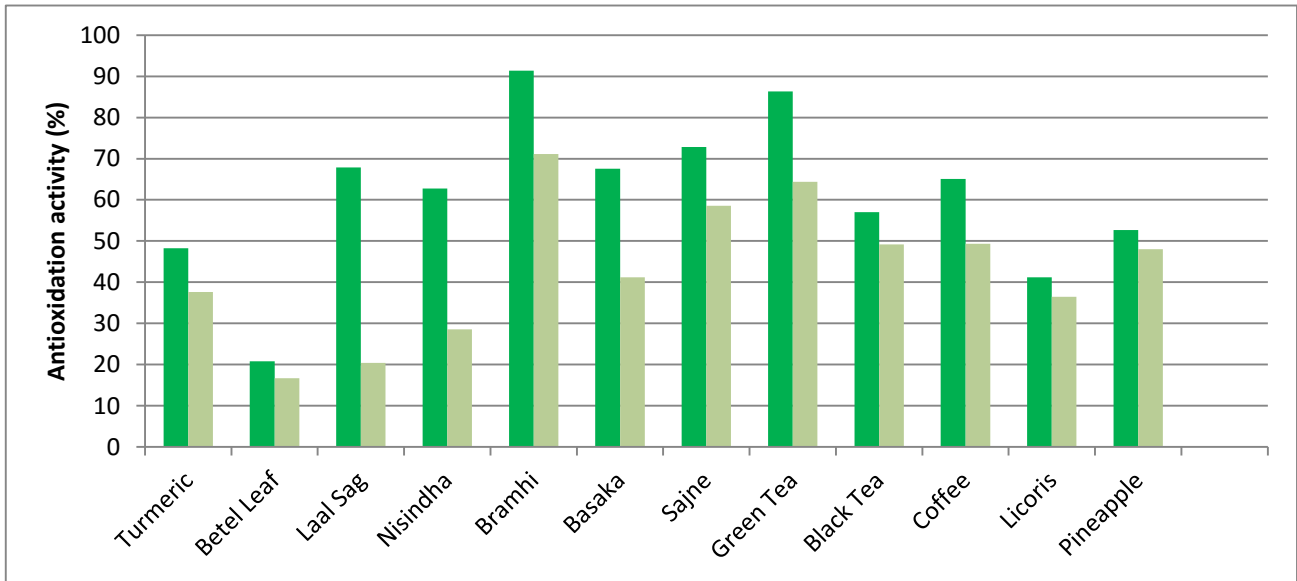


Figure 1: Comparative analysis of total antioxidants in different medicinal plants. Dark green color shows the antioxidant activity in 100µg/ml concentrations and light green color shows the 50µg/ml concentrations.

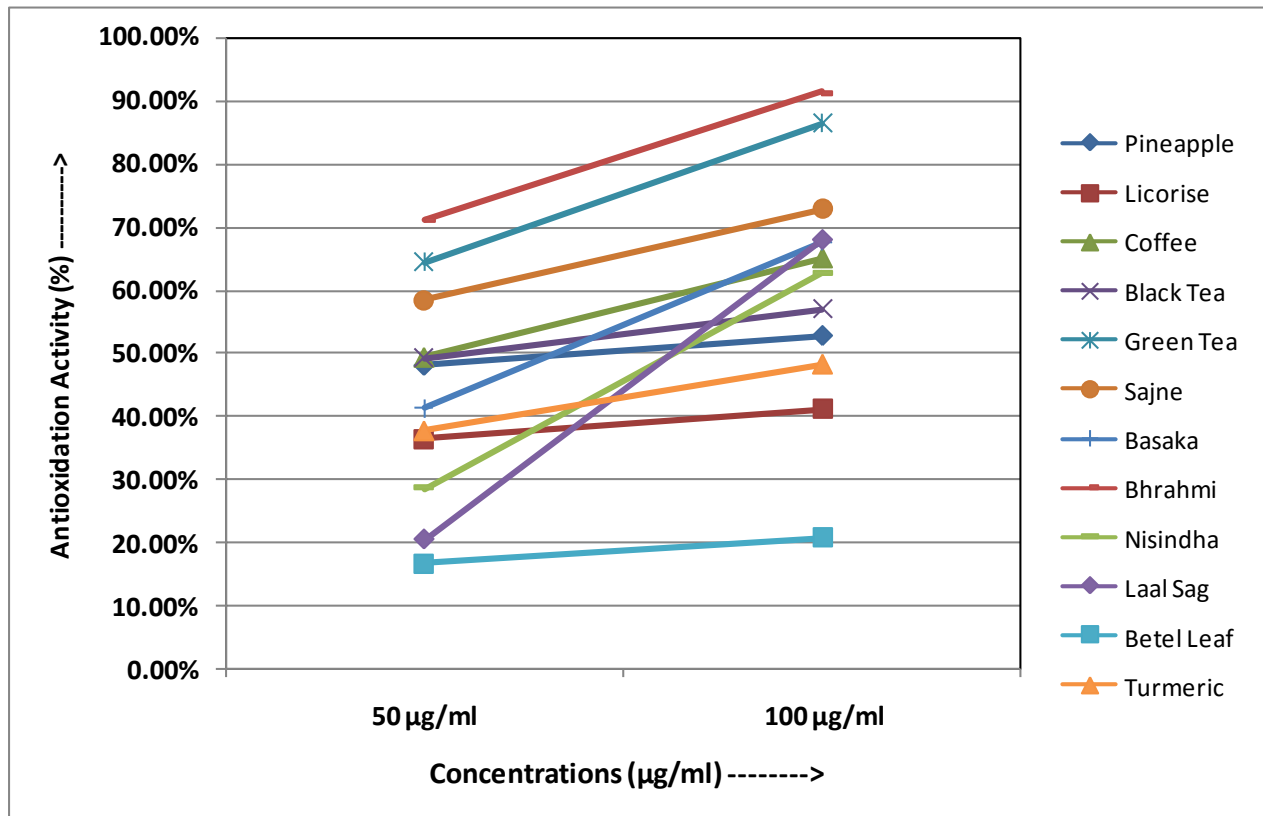


Figure 2: The change of the inclination of line graph against the concentration difference.

radicals and act as an anti-oxidant and also few antioxidants can be produced during metabolic processes¹⁰. As these micro-nutrients are not produced within our body, they have to be supplied in the diet. All the natural or supplemented anti-oxidants should have a number of properties like electron donation, hydrogen donation, radical scavenging, active oxygen quenching, peroxide decomposition, enzyme inhibition and metal chelating activity¹¹. The mechanism of action of any anti-

oxidant is mainly classified into three basic defense mechanisms. In the first line of defense they mainly suppress the formation of free radicals. Secondly, they scavenge the active radicals to suppress chain reaction and in the third line, help in repair the *de novo* anti-oxidants¹².

Balance between free radicals and anti-oxidants plays a major role to maintain the body homeostasis but the equilibrium state is disrupted by the excess production of

free radicals due to stress and other related factors^{13,14}. These excess free radicals cannot be neutralized totally by the naturally produced cellular anti-oxidants. As a result, these excess free radicals can damage different important cellular components leading different diseases severe to chronic. Thus, it is essential to consume synthetic and natural anti-oxidants as a food or supplement to restore the equilibrium^{6,7}. However some synthetic or industrial anti-oxidant have some physical properties like instability at elevated temperature, volatility and even carcinogenicity due to prolonged use¹⁵. In this situation consumer's preference have been shifted to an alternative source of anti-oxidant and use of natural phyto-constituents present in the medicinal as well as dietary plant could be preferred for low cost, high compatibility and less or no harmful effects on human body. Many naturally occurring phyto-originated anti-oxidant compounds have been identified to scavenge free radicals or reactive oxygen species (ROS)^{16,17}. In this present study, some common everyday used local herbs and their parts also showed high to moderate total anti-oxidant properties.

Recent research reveal that for the prevention of chronic disease dietary nutrition plays a vital role and this concept mainly open the research area of preventive medicine and functional food in a great advance. Functional foods contain dietary fibers, vitamins, minerals, natural anti-oxidants, essential fatty acids, and oligosaccharides which help in reduction of the risk of disease and decrease the enhanced physiological function caused by disease progression¹⁸. As the main objective of this study was to look for the antioxidant properties in the commonly available edible fruit, vegetable or herbs, crude plant extract are used instead of specific isolated phyto-chemicals. In Indian alternative therapeutic system like Ayurveda, Unani, Siddha, combination of phyto-chemicals are proved to be more effective for the remission of disease condition than a single drug, commonly used in traditional therapeutic process¹⁹.

Literature review showed the medicinal properties and related antioxidant properties of the phytochemicals isolated from all those plants under study^{6,20,21,22,23}. Percentage of anti-oxidation and free radical scavenging activity showed to vary in different study possibly due to difference in extraction methods, concentrations, doses and assay techniques. The free radical scavenging activity against specific agent like nitrous oxide, nitrous oxide, hydrogen peroxide, hydroxyl radicals, ferric ions, superoxide anions also affects the test results²⁴. So, to determine and compare the antioxidant property of twelve selected plants needs single extraction method, specific concentration for study and particular assay techniques to establish their antioxidant potential through qualitative and quantitative study. The aqueous extraction method for preparing plant extract and determination of total anti-oxidant property by phospho molybdenum method was selected for the current study^{5,25}. This spectrophotometric quantitative determination method has been adopted by various laboratories to estimate the total antioxidant capacity of different plant extract. Molybdenum, the

principle molecule of this method, reduces its valency from VI to V by changing its color from light green to dark bluish green which is directly proportional to the antioxidant property of the particular extract or atom. The method has been optimizing with molar absorbance coefficient for the quantification of antioxidant in recurrent linear fashion Prieto-1999⁵.

The experimental result reveals that bramhi (*Bacopa monnieri*) possess the highest and betel leaf (*Piper betle*) the lowest anti-oxidant property. Green tea (*Camellia sinensis sinensis*) and sajne (*Moringa oleifera*) possess the second and third position respectively. This is followed by basaka (*Justicia adhatoda*), laal sag (*Amaranthus gangeticus*), coffee (*Coffea Arabica*), nisindha (*Vitex negundo*), black tea (*Camellia sinensis sinensis*), pine apple (*Ananas comosus*) and turmeric (*Curcuma longa*). In case of lower dilution (one fifth) the anti-oxidant properties can be altered in nisindha (*Vitex negundo*) and laal sag (*Amaranthus gangeticus*).

CONCLUSION

It could be concluded from the present study that these herbs and plant parts are engaged in scavenging free radicals by the antioxidant compounds present in their extracts themselves. Although the results from this study are considered preliminary, they are of immense importance as plant-based medicines are slowly gaining popularity over the world. In conclusion, Bramhi, green tea and sajina were found to exhibit higher antioxidant potential than other tested samples. Though the actual performance of these plants in physiological systems requires detail research, their capabilities as antioxidants through the preliminary qualitative and quantitative screening of this study has been established. Increasing intake of these dietary antioxidants may help us to maintain an adequate antioxidant status in the body to protect us against damaging reactive oxygen species.

FUNDING STATEMENT

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. All the research work done by the affiliated institutions funding.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing interests.

CONTRIBUTORSHIP STATEMENT

Dr. Madhumita J Mukhopadhyay helped for concept, work plan and data summarization. Puspal De performed all the experiments, laboratory work and analysis. Samipa Das helped in sample collection and laboratory experiments. All authors approved the final manuscript.

DATA SHARING STATEMENT

We cannot share any unpublished data with other laboratory or person.

ACKNOWLEDGEMENT

All authors acknowledge Director and Vice Principal of Institute of Genetic Engineering for funding and affiliation. They are also thankful to other laboratory members and other associated persons of IGE for their enthusiastic participation.

REFERENCES

- Daniel A. Dias, Sylvia Urban and Ute Roessner. A Historical Overview of Natural Products in Drug Discovery. *Metabolites* 2012; 2: 303-336.
- Atanas G. Atanasov, Birgit Waltenberger, Eva-Maria Pferschy-Wenzig, Thomas Linderd, Christoph Wawroscha, Pavel Uhrine et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnol Adv.* 2015 December; 33(8): 1582–1614.
- Puspal D, Subhradeep S, Madhumita JM. Study the antioxidant and *In vitro* Anti-inflammatory activity by membrane stabilization method of *Amaranthus gangeticus* leaf extract. *J of Pharmacognosy and Phytochemistry* 2017; 6(4): 103-105.
- M Greenwell, PKSM Rahman. Medicinal Plant: their use in anticancer treatment. *Int. J. Pharm. Sci. Res.* 2015, 6(10): 4103-4112.
- Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem.* 1999; 269(2): 337-341.
- V. Lobo, A. Patil, A. Phatak, and N. Chandra. Free radicals, antioxidants and functional foods: impact on human health. *Pharmacog Rev.* 2010 Jul-Dec; 4(8): 118–126.
- Lien Ai Pham-Huy, Hua He, Chuong Pham-Huy. Free Radicals, Antioxidants in Disease and Health. *Int J Biomed Sci* 2008; 4 (2): 89-96.
- Khalid R. Studies on free radicals, antioxidants, and co-factors. *Clinical Interventions in Aging* 2007;2(2) 219–236.
- Suja C, Shuhaib B, Muhammed Abdurahman, Hunaida Khathoom, Simi K. A Review on Dietary Antioxidants. *Research J. Pharm. and Tech.* 9(2): Feb., 2016; Page 196-202.
- Gurbani K, Rahul K, Shruti B, Archana S, Dipti S. Dietary antioxidants and their indispensable role in periodontal health. *Journal of food and drug analysis* 24 (2016) 239-246.
- Jian-ML, Peter HL, Qizhi Y, Changyi C. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *J. Cell. Mol. Med.* Vol 14, No 4, 2010 pp. 840-860.
- Sangh P, Sanjay P. A Review on Herbal Antioxidants. *Journal of Pharmacognosy and Phytochemistry* 2012; 1(4): 26-37.
- Marian V, Dieter L, Jan M, Mark TD. Cronin MM, Joshua T. Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology* 39 (2007) 44–84.
- Ergul BK. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Kurutas Nutrition Journal* (2016) 15:71
- Papas AM. Diet and antioxidant status. *Food Chem Toxicol.* 1999;37:999-1007.
- Aruoma OI. Methodological consideration for characterization for potential antioxidant actions of bioactive components in plants foods. *Mutat Res.* 2003;532:9–20. [PubMed].
- Nisreen H, Anil K. Reactive Oxygen Species and Natural Antioxidants: A Review. *Advances in Bioresearch Volume 3 [4] December 2012: 164- 175.*
- Avrelija C, Walter C. The Role of Functional Foods, Nutraceuticals, and Food Supplements in Intestinal Health. *Nutrients* 2010, 2, 611-625.
- Agarwal DP. Review: Complementary and alternative medicine: an overview. *Pal, S.K. 2002. Current Science* 82(5): 518-524.
- Furuta S, Nishiba Y, Suda I. Fluorometric assay for screening antioxidative activities of vegetables. *J Food Sci.* 1997;62:526–8.
- Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. *J Agric Food Chem.* 1996; 44:701–5.
- Lin JK, Lin CH, Ling YC, Lin-Shian SY, Juan IM. Survey of catechins, gallic acid and methylxantines in green, oolong, puerh and black teas. *J Agric Food Chem.* 1998; 46:3635–42.
- Devasagayam TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in Human Health: Current status and future prospects. *J Assoc Physicians India.* 2004;52:794–803.
- Brown JE, Rice-Evan CA. Luteolin-rich Artichoke extract protects low density lipoprotein from oxidation in vitro. *Free Radic Res.* 1998;29:247–255.
- Puspal D, Monidipa D, Madhumita JM. The Study of Antioxidation, Membrane Stabilization, Anti Proteindenaturation Property and Analysis of Phytochemicals in Three Species of Tagetes Leaf and Flower Extract. *International Journal of Pharmacognosy and Phytochemical Research* 2017; 9(8); 1159-1165.