CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1479234

Available online at: http://www.iajps.com *Review Article*

A REVIEW ON FREE RADICALS AND ANTIOXIDANTS

Running title: A review on free radicals and antioxidants

Reena S R *, Dr A S William Arputha Sundar, Dr S M Sandhya, Dhanya S, Lakshmi Gopal

Sreekrishna College of Pharmacy and Research Centre, Trivandrum-695502, India.

Abstract:

Antioxidants are used in food to protect it from deleterious effects of oxidation and are also employed as dietary supplements to neutralize the adverse effects of oxidative stress. Many of the natural antioxidants of interest are of plant origin and belong to the phenolic and polyphenolic class of compounds as well as carotenoids and antioxidant vitamins, among others. The activity of antioxidants and their mechanism of action is dictated by the structural features of the molecules involved, the system in which they are present as well as processing and storage conditions, among others. While much research has been carried out on natural sources of antioxidants, their widespread use is hindered by regulations, which only permits the use of those that have an RDI (required daily intake) such as vitamins. However, green tea, rosemary and other spices or their extracts thereof, and mixed tocopherols are often used in foods as flavouring agents or under other disguised forms to bypass these unwarranted regulatory issues.

Keywords: *Antioxidants, free radicals, enzymatic antioxidants*

Corresponding author:

Reena S R,

SreeKrishna college of Pharmacy and Research Centre,
Parassala, Trivandram
Kerala, India 695502
Email: reenachinchu88@gmail.com
Contact: 9747143253

QR code

Please cite this article in press Reena S R et al., ***A Review on Free Radicals and Antioxidants .,*** *Indo Am. J. P. Sci, 2018; 05(11).*

INTRODUCTION:

Antioxidants are naturally occurring substances that combat oxidative damage in biological entities. An antioxidant achieves this by slowing or preventing the oxidation process that can damage cells in the body. This it does by getting oxidized itself in place of the cells. Thus an antioxidant can also be termed as a reducing agent. Antioxidants are considered as important in the fight against the damage that can be done by free radicals produced due to oxidative stress. Although the human body has its own defenses against oxidative stress, these become weak with age or in the case of an illness.

FREE RADICALS

Free radicals are chemical species that have a single unpaired electron in their out. Ageing is universal but complex biological process with proverbial and unambiguous manifestations characterized by impairment of various functions and decreased ability to respond to "stress". Free radicals are implicated in aging and causation of several diseases. However these free radicals are essential intermediates of normal physiological reactions. The most important free radicals, reactive oxygen species (ROS) include oxygen free radicals or oxygen-centered free radicals and non radical species. Thus ROS includes both radical and non radical reactive species as follows

Table 1:Reactive oxygen and nitrogen species radicals and non radicals

Free radicals	Examples
Reactive oxygen species(radicals)	Molecular oxygen (O_2), superoxide ($O_2^{\bullet -}$), hydroperoxyl (HO_2), (RO), hydroxyl (HO •).
Reactive oxygen species (non radicals)	Singlet oxygen ($1O_2$), hydrogen peroxide (H_2O_2),hypochlorous acid(HOCl),Ozone(O_3)
Reactive nitrogen species (radicals)	Nitric oxide (NO•), nitrogen dioxide (NO_2)
Reactive nitrogen species(non radicals)	Nitrous acid (HNO_2), dinitrogen tetraoxide (N_2O_4), dinitrogen trioxide (N_2O_3), peroxynitrous acid (ONOOH), nitronium cation alkyl peroxynitrates (ROONO)

DIFFERENT TYPES OF FREE RADICALS

Superoxide radical:

This is a relatively poor reactive radical made by addition of one electron to the oxygen molecule. Superoxide is made deliberately as a part of the mechanism by which foreign organisms are killed. The super oxide radical has a longer life span and relatively diffuses to a significant distance. Oxidative metabolism of xenobiotics involving hepatic cytochrome P450 enzyme system, recovery from injury thus produced super oxide is converted to H2O2 by SOD (Superoxide dismutases). This is further converted to water by catalase or to OH radical which is highly reactive and destructive. H_2O_2 also converted into singlet oxygen. Though the singlet oxygen is not a free radical, it acts as a catalyst in generation of free radicals by transferring energy to a new molecule.

Nitric oxide free radical:

The physiological free radical nitric oxide (NO•) made by vascular endothelium as a relaxing factor has many useful physiological functions, but excess nitric oxide can be toxic to the living organism due its affinity for O. Upon reaction with O nitric oxide radical is converted to an unstable peroxy nitrite free radical which is responsible for its cytotoxic effect

$$NO + O_2^- \rightarrow ONOO^- \rightarrow NO_2^- + OH^-$$

Hydroxy free radical:

This is highly reactive radical, formed by two ways in biological process: by radiolysis of water and by reaction of hydrogen peroxide with ferrous ions. If once generated it attacks whatever it is next to. And its lifetime in vivo is very short because this radical reacts at its site of formation, usually leaving behind a legacy in the form of a propagating free radical chain reaction leading to cell damage.

Formation of free radicals:

Free radicals in the body are generated by multiple mechanisms and are often initiated by removal of a H atom from other molecules (during lipid peroxidation). The potentially imprudent derivatives of oxygen, endorsed as ROS are incessantly generated within the human body as a consequence of endogenous metabolic process and/or exogenous chemicals**.** Free radical formation occurs continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions. Enzymatic reactions which serve as sources of free radicals include those involved in the respiratory chain, phagocytosis, prostaglandin synthesis and in the cytochrome P450 system. The other endogenous mechanisms involved in the generation of free radicals are energy liberating recations of

Mitochondria, Xanthine oxidase, reactions involving iron and other transition metals, arachidonate pathways, peroxisomes, exercise, inflammation, ischaemia/ reperfusion etc. Free radicals also arise in non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. Exogenous factors involved in the generation of free radicals are Cigarette smoke, Environmental pollutants, Radiation, Ultraviolet light, certain Drugs, Pesticides, Anesthetic and Industrial solvents and Ozone etc.

Generally in living organisms, free radicals may be initiated within cells in the following ways

- Absorption of radiant energy: Ionizing radiation for ex. Ultraviolet, X rays can hydrolyze water into hydroxyl (OH) and hydrogen (H) free radicals.
- Enzymatic metabolism of exogenous chemicals or drugs: for ex. Carbon tetrachloride can generate CCl3 free radicals.
- The redox reactions that occur during normal metabolic process: During normal respiration molecular oxygen is sequentially reduced by the addition of four electrons to generate water by the oxidative enzymes in the endoplasmic reticulum, cytosol, mitochondria, peroxisomes, and liposomes. During this process, small amounts of toxic intermediates are produced that includes super oxide anion radical (O_2-), hydrogen peroxide (H_2O_2) and hydroxyl ions. Rapid burst of super oxide production occurs in activated polymorphonuclear leukocytes during inflammation. Some intracellular oxidases such xanthine oxidase generate superoxide radicals as a consequences of their activities.

Transition metals: Iron and copper the transition metals donate or accept free electrons during intracellular reactions and catalyze free radical formation as in the Fenton reactions.

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH + OH^-$$
$$H_2O_2 + CU^{+} \rightarrow CU^{2+} + OH + OH^-$$

Nitric Oxide: It is an important chemical mediator generated by endothelial cells, macrophages, neurons and other cell types. It can act as free radical and can able be convert to highly reactive peroxynitrite anion (ONOO) as well as NO_2 and NO_3 - in the presence of super oxide.

Advantages of free radicals

Free radicals, however, are not always harmful. They also serve useful purposes in the human body. Several observations indicate that the oxygen radical in living systems are probably necessary compounds in the maturation processes of cellular structures. Further, white blood cells release free radicals to destroy invading pathogenic microbes as part of the body's defense mechanism. Hence, the complete elimination of these radicals would not only be impossible, but also harmful.

Disadvantages of free radicals

Free radical–mediated cell injury / Free radical reactions:

The effects of these reactive species are wide-ranging but the important reactions that particularly relevant to cell injury includes;

- Lipid peroxidation :-
  Polyunsaturated fatty acids of membrane are attacked repeatedly and severely by oxygen derived free radicals to yield highly destructive PUFA radicals-lipid hydroperoxy radicals and lipid hypoperoxides, termed as lipid peroxidation. The lipid peroxides is propagated to other sites causing widespread membrane damage and destruction of organelles.
- Oxidation of proteins :-
  Oxygen derived free radicals cause cell injury by oxidation of protein macromolecules of the cells, cross linking of liable amino acids as well as fragmentation of polypeptides directly. The end result is degradation of cytosolic neutral proteases and cell destruction.
- DNA damage :-
  Free radical cause breaks in the single strands of the nuclear and mitochondrial DNA. This result in cell injury, it may also cause malignant transformation of cells.
- Cytoskeletal damage :-
  ROS are also known to interact with cytoskeletal elements and interfere in mitochondrial aerobic phosphoryalation and thus cause ATP depletion.

Free radical diseases

Free radicals involved reactions are expected to produce progressive adverse changes that accumulate with age throughout the body. Such normal changes with age are relatively common to all. However, superimposed on this common pattern are patterns influenced by genetics and environmental differences that modulate 'free radical' diseases. These are manifested as diseases at certain ages determined by genetic and environmental factors the oxidative damage a decisive etiological factor concerned in quite a large number of chronic human diseases.

However, the human body evolved a clearly defined antioxidant systems like GSH, SOD, Catalase etc. But due to excessive generation of free radicals may over power the endogenous antioxidant mechanisms

leading to various human ailments. Therefore it was hypothesized that strengthening the inbuilt defence by exogenous supplements may be beneficial. This hypothesis was met with some success and hence, there is a growing interest on antioxidants and their uses in preventing/treating human diseases.

ANTIOXIDANTS

The human body has several mechanisms to counteract damage by free radicals and other reactive oxygen species. These act on different oxidants as well as in different cellular compartments. Under normal state of affairs the ROS generated are detoxified by the Antioxidants nearby in the body and there is symmetry between the generated ROS and the antioxidants present.

Antioxidant may be any substance, which significantly delays or prevents oxidation of the oxidizable substrate at low concentrations. Antoxidants are effective because they are ready to give up their own electrons to a free radical and act as 'free radical scavengers". When a free radical gains the electron from an antioxidant and remain stable, no longer needs to attack the cell and the chain reaction of oxidation is broken. After donating an electron the antioxidant though becomes a free radical remains harmless and stable because of its ability to accommodate the change in electrons without becoming reactive.

The antioxidant systems are grouped as enzymatic antioxidants and non enzymatic antioxidants as fallows

- **Enzymatic antioxidents:**
  Examples**:** Glutathione peroxidases (GPx), Superoxide dismutases (SOD) and Catalase (CAT) etc.
- **Non-Enzymatic antioxidents:**
- Endogenous non enzymatic antioxidants
  Examples: GSH etc.
- Exogenous non enzymatic antioxidants
  Examples: Vitamin E, Vitamin C and the Carotenoids

Enzymatic antioxidants

They quench of harmful oxidants and act as first line of defence of the body. Several essential minerals including selenium, copper, manganese and zinc are necessary for the formation or activity of these enzymes. Hence, if the nutritionals supply these minerals are inadequate, enzymatic defenses against free radicals may be impaired.

Superoxide dismutase

SOD is an endogenous intracellular enzyme present essentially in every cell in the body. Superoxide dismutase (SOD) is a metalloenzyme whose active center is occupied by copper and zinc, sometimes by iron and manganese, plays a major role in the protection of cells against oxidative damage. SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide.

Catalase

Catalase is mostly present in all major body organs, especially in liver. It is an enzyme, which can function either in the catabolism of H_2O_2 or in the peroxidatic oxidation of small substrates. Catalase (present in the peroxisomes) serves to protect the cell from the toxic effects of hydrogen peroxide, a byproduct of fatty acid. It promotes the conversion of hydrogen peroxide into molecular oxygen and water without production of the free radicals.

Glutathione peroxidase

Glutathione peroxidase is a tetrameric protein 88,000-D and it has 4 atoms of selenium (Se) bound as seleno-cysteine. Selenium acts as cofactor for the enzyme. (GPx) plays an important role in the defense mechanisms of higher animals against oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione as the reducing substrate.

Non Enzymatic antioxidants

A. Endogenous non enzymatic antioxidants

Endogenous non-enzymatic antioxidants such as GSH and total thiol play a vital role in scavenging ROS. Tripepetide glutathione (gluyamylcysteinyglycine) executes multiple functions. The GSH is the main storage form of sulfur and it acts as a potent detoxifier of xenobiotics through GSH-cinjugation. Together with its oxidized form (GSSG) glutathione maintains a redox balance in the celluar compartments. This property is of great biological importance since it allows fine-tuning of the cellular redox environment under normal conditions and upon the onset of stress. Due to redox properties of the GSH/GSSG pair and reduced SH-group of GSH, they can participate in scavenging the free radicals.

GSH acts as antioxidant under oxidative stress. A central nucleophilic cysteine residue is responsible for high reductive potential of GSH. It scavenges cytotoxic H2O2, and reacts non-enzymatically with other ROS : Singlet oxygen, Superoxide radical and Hydroxyl radical. The central central role of GSH in the antioxidative defence is due to its ability to regenerate another powerful water-soluble antioxidant ascorbic acid, via the ascorbate-glutathione cycle.

B. Exogenous non enzymatic antioxidants

The second line of defense against free radical damage is the presence of antioxidants found in the diet. These antioxidants act as a cooperative network, employing a series of redox reactions. Although about 4000 antioxidants have been identified, the best known are Vitamin E, Vitamin C and the Carotenoids. Many other non-nutrient food substances generally display antioxidant properties and, thus may be important for health. Thu variety of antioxidants in food contribute to prevention nutrients have specific activities and they often work synergistically to enhance the overall antioxidant capability of the body .

Some important antioxidants are given below

Amino acids:

Tryptophan	Cystein	Methionine
Histidine	Cystine	Arginine etc.

B-Vitamins:

Thiamin	Betaine	Folic acid
Riboflavin	Di,ethyl glycine	Phytates
Niacin	Inositol	Choline
Pantothenic acid	Biotin	Pyridoxine etc.

Phytosterols:

β – Sitosterol	Campesterol	Stigmastenol
Sitosterol	Stigmasterol	Branosterol
Avinsterol	Acylsterol glucoside	Gramisterol
Sterol glucoside	Oligoglycosylsterol	Isofucosterol
28 – Homotyphasterol	Monoglycosylsterol	Obtusifoliol
Cellotetraosylsitosteol	Methyl sterol	6-Deoxycastasterone
28–Homosteasteronic acids	Dimethylsterol	β–Amyrin etc.

Enzymes:

Glutathione peroxidase	Polyphenol oxidase	
Methionine reductase	Catalase Aspartate	amino transferase
Superoxide dismutase	Coenzyme Q 10 etc.	

Polysaccharides:

Cycloartenol – ferulic acid glycoside	Arabinoxylan	Glycoprotein
Deferulic acid complex	Xyloglucan	Hemicelluloses
Deferulic acid – calcium comlex	Proteroglycan	Arabinogalactan
Arabinofuranoside etc		

Phospholipids:

Phosphatidylserine	Lysophophatidylcholine
Phosphatidylcholine	Lysophosphatidylethanolamine
Phosphatidylethanolamine etc	

Carotenoids:

α - Carotene	Lycopene

β - Carotene Lutein Zeaxanthine etc.

Polyphenols:

Ferulic acid β – Coumaric acid

Lipoic acid Methyl ferulate

Sinapic acid Isovitexin Proanthocynidins etc.

Tocophenols and Tocotrienols :

α – Tocopherol α – Tocotrienol β – Tocopherol

β – Tocotrienol β – Tocotrienol ↓-Tocopherol

↓- Tocotrienol ∂ - Tocopherol ∂ - Tocotrienol

Demethyl – tocotrienol Didesmethyl tocotrienol

Gamma Oryzanol:

Cycloartenol trans - ferulate Cycloartanol trans – ferulate

Cycloartenol cis - ferulate Cycloartanol cis – ferulate

Cycloeucalenol trans – ferulate Cycloeucalenol cis - ferulate

24 Methylenecycloartanol Tran -ferulate β – Sitosterol trans - ferulate

24 – Methylenecycloartanol cis ferulate β – Sitosterol cis – ferulate

24 – Methylcholesterol trans -ferulate β – Sitostenol trans – ferulate

24 – Methylcholesterol cis -ferulate β – Sitostenol cis - ferulate

Stigmasterol trans - ferulate Campesterol trans – ferulate

Stigmasterol cis - ferulate Campesterol cis – ferulate

Dietary antioxidants:

Numerous studies have shown the protective effects of antioxidant nutrients on various health problems. Dietary antioxidants like Vitamin E, C and Carotenoids are of significance in the prevention/protection/retardation of progression of diseases. There is now convincing evidence that food containing antioxidants may be of major importance in disease prevention.

Phenolic compounds as antioxidants:

Phenolics are the diverse secondary metabolite that includes flavonoids, tannins, hydroxycinnamate esters, lignin, coumarins etc. which are ubiquitously present in a broad range of medicinal plants and dietary products. These polyphenols possess a high antioxidant potential due to their ideal structural chemistry for free radical scavenging activity.

Medicinal plants as antioxidants:

As plants produce a lot of phytoantioxidant compounds to combat the oxidative stress, these phytochemicals serve as an important source with significant antioxidant activity. It has been observed that phytochemicals like tannic acid, flavonoids, tocopherol, curcumin, ascorbate, carotenoids, polyphenols, etc. have potent antioxidant properties. Besides, the use of modern medicines of synthetic origin is believed to impart dramatic results in a short span in the therapeutic field. But it has a hidden drawback of serious afterward consequences on the health on prolonged treatment due to various pathological, pharmacological and chrono-pathological parameters of the mankind.

Hence, in spite of overwhelming influence of modern medicine and tremendous advances made on the production of synthetic drugs, traditional and medicaments designated now a days as herbal drugs with phytoantioxidants, have retained their place in therapy. As a result of this, plants and drugs by plants extract are an important part of our everyday diet and

their constituents with nutritional value have been intensively studied for decade.

ANTIOXIDANT CAPACITY ASSAYS

2, 2,-Diphenyl-1-picrylhydrazyl Activity:

DPPH is one of a few stable and commercially available organic nitrogen radicals bearing no similarity to the highly reactive and transient peroxyl radicals involved in various oxidative reactions in vivo. This assay is based on the measurement of the reducing ability of antioxidants towards DPPH•. The ability can be evaluated by electron spin resonance or by measuring the decrease of its absorbance. The measurement of the loss of DPPH color at 515 nm following the reaction with test compounds is what the antioxidant assays are based on Prior (2005). DPPH radical, with a deep violet color, receives a hydrogen atom from the antioxidant and is converted to a colorless molecule. Using this reagent, the free radical scavenging ability of the antioxidant can be determined by spectrophotometric methods. It has been reported that inhibition of free radical formation by different antioxidants can be measured using very stable free radicals such as 2,2-diphenyl-1-picrylhydrazyl. DPPH assay has its own advantages like simple, rapid and needs only a UV vis spectrophotometer to carry out the experiment.

Reducing Power (RP):

RP increases according to the increase in absorbance. As more Fe3+ are reduced to the ferrous form or when more electrons are donated by antioxidant components; the colour of Perl's Prussian blue will be darker, resulting in an increased absorbance reading . Reductants also react with certaiperoxides, thus preventing the formation of peroxide. The RP is found to be dependent on extract concentration. A report says RP of tea extracts increased markedly with increasing extract concentration.

Ferric Thiocyanate (FTC) Capacity :

Real food Systems generally consists of multiple phases in which lipid and water co exists with some emulsifier. Hence an antioxidant assay using heterogenous systems such as oil-in water emulsion is one of the model systems for such evaluation, satisfying above conditions. The linoleic acid emulsion system/thiocyanate method has been used here for evaluation under above conditions. During peroxidation in an incubator the absorbance values increased owing to oxidation products, which react to form ferric thiocyanate the colour of red blood . Antioxidants can hinder the oxidation and consequently, the increase in absorbance will be less.

β-carotene bleaching assay:

Mechanism of bleaching of β-carotene is a free radical mediated phenomenon resulting from hydroperoxides formed from linoleic acid. β-carotene, in this model system undergoes rapid discolouration in the absence of an antioxidant. The linoleic acid free radical formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups attacks the highly unsaturated β-carotene molecule and loses its chromophore and characteristic orange colour.

CONCLUSION:

Modifiable risk factors, life style choices about diet, smoking, exercise and antioxidant supplementation, substantially influence how long we will live. The bonus years we earn by making good choices can double the number of healthy years a sixty-year-old will enjoy. Today, our understanding of the antioxidant network, and how it modulates genes critical to healthy aging, has matured. The corollary of The Free Radical Theory of Aging - that supplementation with antioxidants may reduce the rate of aging - has a persuasive theoretical basis, extensive experimental and epidemiological evidence, and convincing support from the regrettably few intervention studies that have been funded and reported.

I am not a doctor, but as a scientist who studies antioxidants, and a man in the second half of life, I find the weight of scientific evidence compelling. We can all reasonably expect to enjoy a longer, healthier old age, adding life to our year

REFERENCES:

1. Hui Y.H. Antioxidants: Technical And Regulatory Considerations In Bailey's Industrial Oil And Fat Products Wiley Interscience Publications New York 3, 1996, Pp 523-539.
2. Cook, N. C. And Samman S. Flavonoids - Chemistry, Metabolism, Cardio Protective Effects And Dietary Sources. Nutr Biochem 7, 1996, Pp 66-76.
3. Huang, M.T And Ferraro T. Phenolic Compounds In Food And Cancer Prevention. In H.T .Huang, C.T, Ho And C.Y. Lee (Eds.) Phenolic Compounds In Food And Their Effects On Health II.ACS Symposium Series 507, 1992, Pp 8-34.
4. Al-Saikhan, M.S, Howard, L.R, Miller, J.C., Jr. Antioxidant Activity And Total Phenolics In Different Genotypes Of Potato (Solanum Tuberosum L.). J Food Sci 60, 1995, Pp 341-347.

5. Yen GC And Duh PD. Scavenging Effect Of Methanolic Extracts Of Peanut Hulls On Free-Radical And Active Oxygen Species. J Agric Food Chem 42, 1995, 629- 632.
6. Oomah, B.D And Mazza, G. Flavonoids And Antioxidative Activities In Buckwheat. J Agric Food Chem 1996,44 (7),1746-1750
7. Chen, Z.Y., P.T. Chan. K.Y. Ho, K.P. Fung And J. Wang. Antioxidant Activity Of Natural Flavonoids Are Governed By Number And Location Of Their Aromatic Hydroxyl Groups. Chem Phys Lipids 79, 1996, Pp 157-163.
8. Cao, G., E. Sofic And R.L. Prior. Antioxidant Capacity Of Tea And Common Vegetables. J Agric Food Chem 44, 1996, Pp 3426-3431. 64
9. Amarowicz, R.A. Troszynska, M. Karamac And H. Kozlowska. Antioxidative Properties Of Legume Extracts In Agri-Food Quality. An Interdisciplinary Approach, Edited By G.R. Fenwick, C. Hedley And S. Khokhar, The Royal Society Of Chemistry, Cambridge, 1996, Pp 376-379.
10. Zhou KK, Su L, Yu L. Phytochemicals And Antioxidant Properties In Wheat Bran. J Agric Food Chem 52, 2004, Pp 6108-6114.
11. Halliwell B And Gutteridge JMC. Oxygen Is A Toxic Gas An Introduction To Oxygen Toxicity And Reactive Oxygen Species. In: Free Radical In Biology And Medicine; Oxford University Press: United Kingdom. 1999, Pp 1-35.
12. Wallace SS. Oxidative Damage To DNA And Its Repair, In Oxidative Stress And Molecular Biology Of Antioxidant Defenses. Scandalios JG, Editor; Cold Spring Harbor Laboratory Press: Plainview New York. 1997, Pp 49-90.
13. Stratil P, Klejdus B, Kubán V. Determination Of Total Content Of Phenolic Compounds And Their Antioxidant Activity In Vegetables. Evaluation Of Spectrophotometric Methods. J Agric Food Chem 54, 2006, Pp 607-616.
14. Dugan, L. Lipids. In: Fennema, O.R(Ed).Principles Of Food Science. Part 1: Food Chemistry. New York: Marcel Dekker, Inc.1976, Pp 169-186.
15. Andres Moure, Jose M.Cruz, Daniel Franco, J.Manuel Dominguez, Jorge Sineiro, Herminia Dominguez, Maria Jose Nunez And Carlos Parajo. Natural Antioxidants From Residual Sources. Food Chem 72, 2001, Pp 145-171.
16. Onyeneho, S. N. And Hettiarachchy, N.S. Antioxidant Activity Of Durum Wheat Bran J Agric Food Chem 40, 1992, Pp 1496-1500.
17. Negro, C, Tommasi, L. And Miceli, A. Phenolic Compounds And Antioxidant Activity From Red Grape Marc Extracts. Bioresource Techn 87, 2003, Pp 41-44 65
18. Gordon, M.H. And X.C. Weng. Antioxidant Properties Of Extracts From Tanshen (Salvia Miltiorrhiza Bunge) Food Chem 44, 1992, P119.
19. Gamez-Meza N, J.A. Noriega-Rodriguez, L.A. Medina- Juarez, J. Ortegagarcia R. Cazarez-Casanova And Angulo-Guerrero, Antioxidant Activity In Soybean Oil Of Extracts From Thompson Grape Bagasse. J Am Oil Chem Soc 76(12), 1999, Pp 1445-1447.
20. Gordon, M.H. The Mechanism Of Antioxidant Action In Vitro. In: Hudson, B.J.F. (Ed) Food Antioxidants. England: Elsevier Science Publishers Ltd. 1990, Pp 1-18.
21. Johnsen S, Overvad K, Stripp, C, Tjonneland, A. Husted S.E. And Sorenson,H.T. Intake Of Fruits And Vegetables And The Risk Of Ischemic Stroke In A Cohort Of Danish Men And Women, Am J Clin Nutr 78, 2003, Pp 57-64.
22. Julkunen-Tiitto, R. Phenolic Constituents In The Leaves Of Northern Willows: Methods For The Analysis Of Certain Phenolics. J Am Food Chem 33, 1985, Pp 213-217.
23. Pin- Der Duh. Antioxidant Activity Of Budrock (Arctium Lappa Linn.): Its Scavenging Effect On Free Radical And Active Oxygen, J Am Oil Chem Soc 75, 1998, Pp 455-461.
24. Azizah A.H., Ruslawati, N.M.N And Swee Tee, T. Extraction And Characterization Of Antioxidant From Cocoa By–Products. Food Chem 64, 1999, Pp 199-202.