Study the Antioxidant

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ABSTRACT

Many substances consumed by man either through foods, drinks and inhalation, even effect of exogenous material (ultraviolet radiation) on the skin may be destructive to the health and thus, shortening the life span of man. Free radicals when generated in the body system of man causes damage to which eventually leads to death at shorter time (Borek, 1991). Continuous usage of the same vegetable oil which is not even properly stored and re-using the already fried oil (rancid) lead to generation of free radicals through lipid peroxidation. The reasons sometimes could be for economic reason but then it is highly damaging to the health. Smoking and chronic alcoholism is socio-cultural problem in the world today because uncontrolled cigarette smoke intake reduces the level of many important antioxidants in the serum, which is detrimental to the health (Cheremisinoff, 1989). Report has shown that proper intake of antioxidant will help quench all these inevitably free radicals in the body thus, improving the health by lowering the risk of various diseases such as cancer. Antioxidants are also important in body lotions creams, so as to protect the skin from sun exposure and decrease skin roughness, wrinkles depth, ultraviolet induced skin cancer and skin swelling from sunlight. To cap it up, there is need for proper orientation on the necessity of proper intake of balance diet which will definitely supply the much needed antioxidants. The RDA has been previewed therefore, people will have lower health risks and tend to live longer and have fewer disabilities.

Keywords: health risks, Antioxidant, foods, drinks

Introduction

Antioxidants are chemicals that may shield cells from the harm that free radicals,which are unstable molecules, may do. In addition to stabilizing free radicals, antioxidants may also stop some of the harm that free radicals may otherwise do.Damage from free radicals may cause cancer (A. A. Hamid, 2010). According to Sies (1997) (Sies, 1997), antioxidants include molecules like beta-carotene,lycopene, and vitamins C, E, and A.

A substance that has the ability to inhibit or delay the oxidation of other moleculesis called an antioxidant. The chemical process of oxidation involves the transfer of electrons from a material to an oxidizing agent. Free radicals are a byproduct of oxidation events and can initiate cell-damaging chain reactions. By eliminating free radical intermediates, antioxidants put an end to these chain events. They also prevent further oxidation processes by being oxidized themselves. Thus, reducing substances like thiols, ascorbic acid, or polyphenols are frequently antioxidants (A. A. Hamid, 2010).

A chemical known as an antioxidant prevents other molecules from oxidizing. Achemical process known as oxidation occurs when a material transmits anelectron or hydrogen to an oxidizing agent. Free radicals can be produced via oxidation processes. These radicals can then initiate chain reactions, which can harm or even kill a cell when they happen inside of it. By eliminating free radicalintermediates, antioxidants put an end to these chain events and stop further oxidative reactions. They achieve this by oxidizing oneself. Reducing agents such thiols, ascorbic acid, or polyphenols are frequently antioxidants (Youssef, 2014).According to Halliwell and Gutteridge (1999), an antioxidant can refer to a variety of substances that, in small amounts, can either prevent or significantly delay the oxidation of easily oxidizable materials. Another definition of an antioxidant is any substance that, when present in lower concentrations than those of an oxidizable substrate, significantly delays or prevents the oxidation of thosesubstances.

Oxidation processes can be harmful even though they are essential for existence. According to Hamid et al. (2010), plants and animals possess a complex system of several antioxidants, including vitamin C and E, as well as enzymes, including catalase (CAT), superoxide dismutase (SOD), and other peroxidases. Numerous illnesses in humans, including cellular necrosis, cardiovascular disease, cancer, neurological disorders, Parkinson's dementia, Alzheimer's disease, inflammatorydisorders, muscular dystrophy, liver disorders, and even aging, are mostly caused by oxidative stress (Amit and Priyadarsini 2011). Furthermore, some micronutrients like vitamin C, β-carotene, and E are antioxidants that the body isunable to produce on its own and need to be added to a regular diet (Teresa et al.2011).

Definition of Antioxidant

Antioxidants are the molecules that prevent cellular damage caused by oxidationof other molecules. Oxidation is a chemical reaction that transfers electrons fromone molecule to an oxidizing agent. Oxidation reactions are known to produce free radicals. These free radicals are highly reactive species which contains one or more unpaired electrons in their outermost shell. Once they are formed, the chain reaction starts. Antioxidant reacts with these free radicals and terminates this chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves (Mamta, 2013).

Antioxidants can also act as prooxidants when these are not present at the right place at the right concentration at the right time (Touriño et al. 2008).

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chainreactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Hamid AA, 2010).

Antioxidants act by delaying or preventing the oxidation of other chemicals. Thefirst studies on the role of antioxidants in biology focused on their use in preventing unsaturated fats from going rancid. However, the milestone that led tothe understanding of the role of antioxidants for living organisms was theidentification of vitamins A, C, and E and the understanding of the mechanism of lipid peroxidation prevention by vitamin E . The classification of antioxidants,along with the most representative examples, is shown in the diagram. Antioxidants are usually classified into enzymatic and nonenzymatic. Among them, there are various compounds with different modes and places of action and different final effects. This diversity determines the individual role of each of

them in the body. It should be emphasized that the network of interacting antioxidant enzymes, such as superoxide dismutase enzymes (SODs), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRd), shows the highest antioxidant defense effectiveness (Jolanta Flieger, 2021).

Classification of Antioxidants

There are different attributes to classify the antioxidants. The first attribute is based on the function (primary and secondary antioxidants). The second attribute is based on enzymatic and non-enzymatic antioxidants:

1- Primary antioxidants: -

They are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products. Antioxidants of this group are mainly phenolics, in structure and include the following: Antioxidant minerals, antioxidant vitamins and phytochemicals which include favonoides, catechins, carotenoids, β-carotene, lycopene, diterpene of, black pepper, thyme, garlic, cumin and their derivatives (Hurrel, 2003).

2- secondary antioxidants: -

These are phenolic compounds that perform the function of capturing freeradicals and stopping the chain reactions. The compounds include: Butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG) (Youssef, 2014).

Notwithstanding, according to Ratnam, et al. (2006), antioxidants can be dividedinto two classes namely enzymatic antioxidants and nonenzymatic antioxidants. Some of these antioxidants are endogenously produced which include enzymes, low molecular weight molecules and enzyme cofactors. Among nonenzymatic antioxidants many are obtained from dietary sources. Dietary antioxidants can beclassifed into various classes of which polyphenols present the largest class.

Polyphenols consist of phenolic acids and favonoids. The other classes of dietary antioxidants include vitamins, carotenoids, organosulfural and minerals. Fig (1) illustrates the classifcation of antioxidants whereas Fig (1) indicates the broad scope of antioxidants (Youssef, 2014).

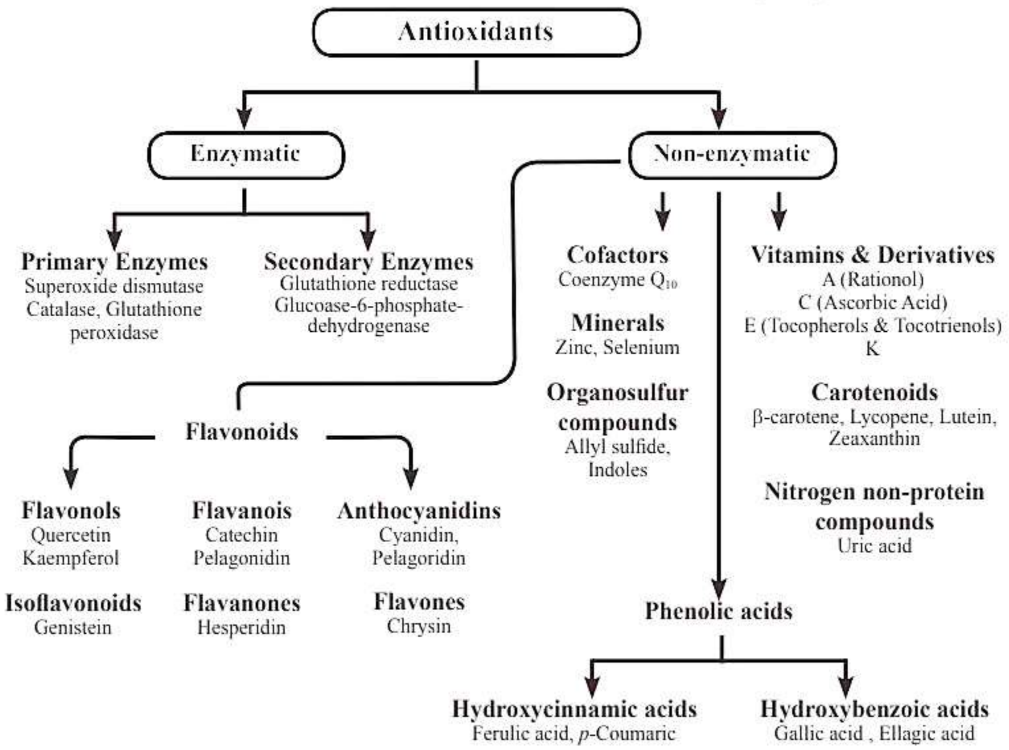

Figure 1 Classification of antioxidants

It should be emphasized that there is a great difference between antiradical and antioxidant activity. The antiradical activity characterizes the ability of compounds to react with free radical while antioxidant activity represents the ability to inhibit the process of oxidation. Consequently, all the tested systems using a stable free radical (DPPH, ABTS, etc) give information on the radical scavenging or antioxidant activity, although in many cases this activity doesn't correspond to the antioxidant activity. In order to obtain information about the real antioxidant activity with respect to lipids or food stabilization, it is necessaryto carry out the study on the real products, although it may be seen this as a complex problem (Youssef, 2014). According to Frankel & Finley (2008), agreement on standardized test methods allows for:-

1- Guidance for appropriate application of assays.

2- Full comparisons of foods or commercial products.

3- A means to control variation within or between products.

4- Provision of quality standards for regulatory issues and health claims.

Too many analytical methods result in inconsistent inappropriate application and interpretation of assays (Prior et al., 2005). Therefore, a variety of in-vitro chemical methods are being used to determine the antioxidant activity of productsand ingredients (Prior, 2005).

Types of Antioxidants

Ascorbic acid

Ascorbic acid or "vitamin C" (Figure 1) is a monosaccharide antioxidant found in both animals and plants. As one of the enzymes needed to make ascorbic acidhas been lost by mutation during human evolution, it must be obtained from the diet and is a vitamin. Most other animals are able to produce this compound in their bodies and do not require it in their diets. In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins. Ascorbic acid is a reducing agent and canreduce and thereby neutralize, reactive oxygen species such as hydrogen peroxide(Hamid AA, 2010).

Glutathione

The free radical mechanism of lipid peroxidation: Glutathione is a cysteine- containing peptide found in most forms of aerobic life. It is not required in the diet and is instead synthesized in cells from its constituent amino acids.

Glutathione has antioxidant properties since the thiol group in its cysteine moietyis a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants (Meister and Anderson, 1983). Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants. In someorganisms glutathione is replaced by other thiols, such as mycothiol in the Actinomycetes, or by trypanothione in the Kinetoplastids (Hamid AA, 2010).

Melatonin

Melatonin is a powerful antioxidant that can easily cross cell membranes and theblood-brain barrier. Unlike other antioxidants, melatonin does not undergo redoxcycling, which is the ability of a molecule to undergo repeated reduction and oxidation. Redox cycling may allow other antioxidants (such as vitamin C) to actas pro-oxidants and promote free radical formation. Melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end-productsupon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant (Tan et al., 2000).

Tocopherols and tocotrienols (vitamin E)

Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties. Of these,

- tocopherol (Figure 2) has been most studied as it has the highest bioavailability,with the body preferentially absorbing and metabolizing this form (Hamid AA, 2010). It has been claimed that the -tocopherol form is the most important lipid- soluble antioxidant and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidized -tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants,such as ascorbate, retinol. This is in line with findings showing that - tocopherol, but not water-soluble antioxidants, efficiently protects glutathione peroxidase (GPX4)-deficient cells from cell death. GPX4 is the only known enzyme that efficiently reduces lipid-hydro peroxides within biological membranes (Packer etal., 2001).

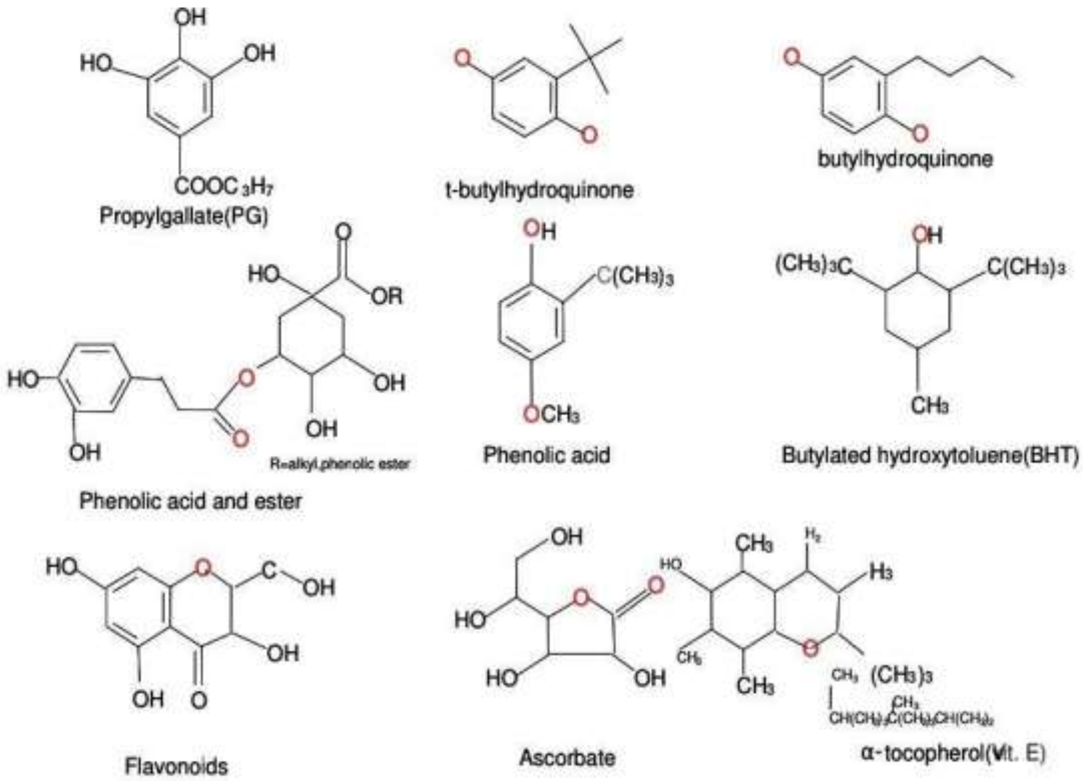

Figure 2 Structures of some antioxidants

Adverse Effects of Antioxidants

The most popular "antioxidants" forms include vitamins, such as vitamin A (retinol, retinoic acid), vitamin C (L-ascorbic acid, ascorbic acid, ascorbate), vitamin E (α-tocopherol), β-carotene, minerals, like Se, and naturally-occurring polyphenols, each one has a different effect on body cells. Vitamins and β- carotene have conjugated double bonds and key functional groups responsible for their antioxidant role and quality as pigments in several foods, like fruits and vegetables. Below, we briefly summarize the adverse effects of these popular antioxidants, often consumed as supplements at much higher doses than those found in foodstuffs. While their adverse effects are known in the medical community, they are not well-known among the population, who believe that natural products cannot be toxic. Czernichow investigated the effect of antioxidant supplementation for 7.5 years on metabolic syndrome (MetS) incidence and even the epidemiologic association between baseline serum antioxidant concentrations and MetS prospective risk (Czernichow, et al., 2009). No beneficial effects of antioxidant supplementation were observed in a generallywell-nourished population. Baseline serum antioxidant concentrations of β- carotene and vitamin C, however, were negatively associated with MetS risk. Baseline serum zinc concentrations were positively associated with the risk of developing MetS. Park found that there was no association between dietary intakes of vitamins A, C, and E and colon cancer risk in this pooled analysis of thirteen prospective cohort studies. However, total vitamins A, C, and E intakes were each one inversely associated with colon cancer risk. Multivitamin use, particularly in combination with a single vitamin A, C and/or E supplements use,was inversely associated with colon cancer risk. A low dietary intake of antioxidant vitamins and minerals raises the incidence of cardiovascular diseasesand cancer. After 7.5 years, low-dose antioxidant supplementation lowered total cancer incidence and all-cause mortality in men but not in women. In fact, supplementation may be effective in men only because of their low basal status of certain antioxidants, especially of β-carotene (Bahare Salehi, 2018)

Free Radicals vs. Oxidative Stres

Free radicals can be defined as highly reactive species that contain an unpaired electron in the valence shell. They can donate this electron but also accept it fromother molecules, acting as an oxidant or reducing agent. In the human body, reactive forms (RS) come from metabolic processes involved in the respiratory chain, phagocytosis, prostaglandin synthesis, and the cytochrome P-450 system (Flieger, 2021).

The most reactive species found in biological systems include the hydroxyl radical (OH), which is formed by attaching three electrons to an oxygenmolecule, e.g., as a result of the Fenton reaction, and the superoxide radical (O),which is formed mainly in mitochondria, as a byproduct of electron transport in the respiratory chain. Other reactive forms of oxygen (ROS), nitrogen (RNS), andchlorine occurring as free radicals and nonradicals that as oxidizing agents can beeasily converted into radicals are listed in Table 1 (Apak R., 2016).

Reactive Species	Form	Example
Reactive oxygen species (ROS)	Radical	$HO^{\bullet}$, $^{1}[O]_2$, $O_2^{\bullet-}$ $HOO^{\bullet}$, $ROO^{\bullet}$, $RO^{\bullet}$, $CO_2^{\bullet-}$, $CO_3^{\bullet-}$
	Non-radical	O_3, H_2O_2, HOCl, HOI, HOBr, ROOH, CO, ONOOH, $ONOO^-$, O_2NOO^-, $HOOCO_2^-$, (O_2 1Dg)
Reactive nitrogen species (RNS)	Radical	$NO^{\bullet}$, $NO_2^{\bullet}$, $NO_3^{\bullet}$
	Non-radical	ROONO, RO_2ONO, $CH_3C(O)OONO_2$, N_2O_4, N_2O_3, N_2O_5, HNO_2 NO_2Cl NO^-, NO^+
Reactive chlorine species	Radical	$Cl^{\bullet}$
	Non-radical	ClBr, Cl_2, ClO_2
Reactive sulfur species	Radical	$S^{\bullet}$
	Non-radical	H_2S, RSSR, RS(O)SR, RSOH, $RS(O)_2SR$, RSR'

Table 1 Examples of reactive species. Reproduced with permission from Graves, D.B., [J. Phys. DAppl. Phys.]; published by IOP Publishing, 2012

ROS/RNS generated in oxygen metabolism are necessary in the regulation of gene expression, cell proliferation, apoptosis, the processes of protein phosphorylation or calcium concentration in cells, activation of proteins controlling cell division, and elimination of microorganisms. Free radicals are also generated under the influence of external sources, such as exposure to X- rays, ozone, smoking, air pollution, and industrial chemicals. There is a balance in the cell between RS production and its neutralization by defense systems. Under physiological conditions, this balance is slightly shifted in favor of prooxidative conditions, providing continuous, mild oxidative stress (Flieger, 2021).

Each disturbance of this particular balance may lead to the development of oxidative stress, i.e., a state in which the oxidizing potential increases to a level that threatens the stability of cellular structures . Under oxidative stress, biologically important macromolecules such as DNA, proteins, carbohydrates, and lipids are damaged. The excess of free radicals changes their structure and thus the physiological functioning of the cell by disrupting redox signaling and the accumulation of cytotoxic compounds, such as malonyl dialdehyde or 4- hydroxynonenal (Pisoschi A.M., 2015).

There is evidence that free radicals can accumulate throughout the body with age,initiating the aging process, as well as various neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, muscular dystrophy, and atherosclerosis. An imbalance between ROS and the antioxidant defense system has also been recognized in the induction of diabetes and age-related eye disease.Currently, it is believed that oxidative stress has a significant negative impact alsoon inflammatory diseases, cancer, ischemic diseases, immunodeficiency syndrome, hypertension, alcoholism, smoking-related diseases, and many others.Oxidative stress was first described and defined by Sies in 1991 (Flieger, 2021).

The reasons for the occurrence of oxidative stress may be (i) an increase in the rate of ROS production, (ii) deficiencies of low-molecular-weight antioxidants, and (iii) inactivation of enzymes with antioxidant activity. Increased and/or prolonged state of oxidative stress may cause serious damage to the cell and even lead to its death . Therefore, the current discussions focus on the role of free radicals in the pathogenesis of many diseases and the usefulness of antioxidants in their potential therapy (Flieger, 2021).

Antioxidants are produced by the protective system of various organisms in order to respond to the destructive effects of free radicals. Antioxidants are able to reduce the damage caused by ROS/RNS and even chlorine. The action of the protective system may limit the negative effects of free radicals by preventing theformation of reactive radicals or by interrupting free radical reactions (Flieger, 2021).

Reactive Oxygen Species

Reactive oxygen species (ROS) is a term which encompasses all highly reactive,oxygen-containing molecules, including free radicals. Types of ROS include thehydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage.

ROS are generated by a number of pathways. Most of the oxidants produced by cells occur as:

- A consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system.
- Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed, and by which foreign proteins (antigens)are denatured.
- Xenobiotic metabolism, i.e., detoxification of toxic substances.

Consequently, things like vigorous exercise, which accelerates cellular metabolism; chronic inflammation, infections, and other illnesses; exposure to allergens and the presence of "leaky gut" syndrome; and exposure to drugs or toxins such as cigarette smoke, pollution, pesticides, and insecticides may all contribute to an increase in the body's oxidant load (PERCIVAL, 1997).

Mechanism of action of antioxidants

The compound that reduces *in vitro* radicals does not really work as an antioxidant in the *vivo* system. This is because FR is fragile and easily spread. Some have very short life spans, in nanoseconds, so it is difficult for an antioxidant to be present at a time and place where oxidative damage is produced.Additionally, the reaction between antioxidants and FR is a secondary reaction. Therefore, they depend not only on the concentration of free antioxidants and radicals but also on factors related to the chemical structure of reagents, mediumand reaction conditions.

Metabolism of hydroxycinnamates: Hydroxycinnamic acids are among the most abundant antioxidant foods, found in many vegetables, fruits, grains and beverages. These compounds can be present in plants in free form, or as compounds (e.g. as esters in chlorogenic acid, rosmarinic acid, etc.), and are widely known as structural elements in the biosynthesis of many phenoliccompounds of plants such as lignans or flavonoids.

Metabolism of resveratrol: Resveratrol is probably the most popular antioxidant in the diet. This stilbene is best known for the "magic" that combinesthe skin of green grapes and red wine, and it occurs in low amounts in several foods that include a variety of berries, tomato skin, peanuts, pistachios, cocoa, etc.

Metabolism of luteolin: Luteolin is a type of catechol-B- ring that contains flavone, which is found in many plants, vegetables and medicinal plants, and is essential for many health benefits including anti-inflammatory, neuro- protective and chemo-preventive effects (Haedi, 2022).

Measurement of antioxidant activity

The methods and instruments used to measure the activity of the antioxidants have made remarkable progress in the past few decades. Early methods measurethe efficiency of the antioxidants against the formation of particular species of oxidation products and thus, are based on measuring lipid oxidation. Thus far, various chemical tests coupled with highly sensitive and automated detection technologies have been used to evaluate antioxidant activity by special methods, like for instance scavenging activity against different types of free radicals or ROS, reducing power and metal chelation, among others. Oxidation substrates have also been extended from food model systems to chemical compounds, biological materials, cellular lines and even living tissues (Shahidi F., 2015).

A high number of tests are available for the direct measurement of the transfer ofthe hydrogen atom or the transfer of electrons from antioxidants to free radicals. The antioxidant activities reported in this method group are generally associatedwith their capacity to neutralise certain types of radical species, out of which somemay be artificial and biologically irrelevant. As a result, these methods have the disadvantage that they do not reflect the situation in an oxidant food or an in vivo case. Nevertheless, the data regarding the hydrogen atom transfer or the data regarding the donating capacity of the electrons obtained by these methods provide important information on their intrinsic antioxidant potential with minimal environment interference. These tests do not require a lipid substrate andnormally use a chemical system containing an oxidant (free radicals or other ROS), an oxidising substrate (some tests do not need it) and antioxidants under investigation (Apetrei, 2021).

A standardised method for antioxidant activity of a food component should meetthe following ideal requirements (Prior R.L., 2005):

- The radical source used must be biologically relevant;
- It is desirable for it to be simple;
- The method used must have a defined endpoint and chemical mechanism;
- Both the instruments used and the chemicals must be readily available;

- Reproducibility within the cycle and between days is appropriate;

- It allows analysis for both hydrophilic and lipophilic antioxidants, usingdifferent radical sources;
- The method must be applicable for quality control analyses.

It should be emphasised that antioxidant activity must not be tested on the basis of a single method. Several antioxidant procedures should be performed in vitro to determine antioxidant activities for the sample of interest. Taking this into account, it is difficult to compare one method completely with another. Therefore,the methods of analysis must be checked before choosing one for the purpose ofresearch (Apetrei, 2021).

The various methods for evaluation of the antioxidant capacity fall into three distinct categories namely, spectrometry, electrochemical assays and chromatography (Moharram H.A., 2014) as presented in Table 2.

Table 2

Different techniques used to measure antioxidant activity.

Techniques	**Antioxidant CapacityAssay**	**Principle of theMethod**	**End-Product Determination**
	ORAC	Antioxidant reaction with peroxyl radicals, induced by 2,2′-azobis-2- amidino-propane (AAPH)	Loss of fluorescence offluorescein
	HORAC	Antioxidant capacity to quench OH radicals generated by a Co(II) based Fenton-like system	Loss of fluorescence offluorescein

	TRAP	Antioxidant capacityto scavenge luminol- derived radicals, generated from AAPH decomposition	Chemiluminescenc equenching
	CUPRAC	Cu (II) reduction to Cu (I) by antioxidants	Colorimetry
Spectrometry	FRAP	Antioxidant reaction with a Fe(III) complex	Colorimetry
	PFRAP	Potassium ferricyanide reduction by antioxidants and subsequent reaction of potassium ferrocyanide with Fe^{3+}	Colorimetry
	ABTS	Antioxidant reaction with an organic cation radical	Colorimetry
	DPPH	Antioxidant reaction with an organic radical	Colorimetry
	Fluorimetric Analysis	Emission of light by a compound, which has absorbed light orother	Recording of fluorescence excitation/emissi onspectra

Techniques	Antioxidant CapacityAssay	Principle of theMethod	End-Product Determination
		electromagnetic radiation of a different wavelength	
Electrochemical Techniques	Voltammetry	The reduction or oxidation of a compound at the surface of a working electrode, at the appropriate applied potential, resulting in the mass transport of new material to the electrode surface and in the generation of a current	Measurement of the current of the cathodic/anodic peak
	Amperometry	The potential of the working electrode is set at a fixed valuewith respect to a reference electrode	Measurement of the current generated by the oxidation/ reduction of an electroactive analyte
	Biamperometry	The reaction of the analyte (antioxidant) with the oxidised form of a reversible indicating redox couple	Measurement of the current flowing between two identical working electrodes, at a small potential difference and immersed in a solution containing the analysed sample and a reversibleredox couple

Chromatography	Gas chromatography	Separation of the compounds in a mixture is based on the repartition between a liquid stationary phase and a gas mobile phase	Flame ionisation or thermal conductivity detection
	High performance liquid chromatography	Separation of the compounds in a mixture is based on the repartition between a solid stationary phase anda liquid mobile phase with different	UV-Vis (e.g., diode array) detection, fluorimetric detection, mass spectrometry or electrochemical detection
Techniques	**Antioxidant CapacityAssay**	**Principle of theMethod**	**End-Product Determination**
		polarities, at high flow rate and pressure of the mobile phase	

ORAC—Oxygen Radical Absorption Capacity; HORAC—Hydroxyl Radical Antioxidant Capacity; TRAP—Total Peroxyl Radical Trapping Antioxidant Parameter; CUPRAC—Cupric Reducing Antioxidant Power; FRAP—Ferric Reducing Antioxidant Power; PFRAP—potassium ferricyanide reducing power; ABTS—2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid; DPPH—[2,2- di(4-tert-octylphenyl)-1-picrylhydrazyl]. (Apetrei, 2021)

Medicinal Applications of Antioxidants Anti-cancer agent in medicinal chemistry Lanthanides as anti-cancer agents

The application of inorganic chemistry to medicine is a rapidly developing field,Novel therapeutics and diagnostic metal complexes are now having an impact on medical practice. Advances in bio-coordination chemistry are crucial for improving the design of compounds to reduce toxic side effects and understand their mechanisms of action. A lot of metal –based drugs are widely used in the treatment of cancer (Xianquan S, 2005). The clinical success of cisplatin and other platinum complexes is limited by significant side effects acquired or intrinsic resistance. Therefore, much attention has focused on designing new coordination compound with improved pharmacological properties and a broaderrange of antitumor activity (Blot et al., 1993). Strategies for developing new anticancer agents include the incorporation of carrier groups that can target tumorcells with high specificity. Also of interest is to develop complexes that bind to DNA in a fundamentally different manner than cisplatin, in an attempt to overcome the resistance pathway that has evolved to eliminate the drug. This review focuses on recent advancement in developing lanthanide coordination complexes (Blot WJ, 1993).

Lycopene as a potential anti cancer agent

Dietary chemoprevention has emerged as a costeffective approach to control most prevalent chronic diseases including cancer. In particular, tomato and products are recognized to confer a wide range of health benefits. Epidemiology studies have provided evidence that high consumption of tomatoes effectively lowers therisk of reactive oxygen species (ROS)-mediated diseases such as cardiovascular diseases and cancer by improving the antioxidant carotenoid reported to be more stable and potent singlet oxygen quenching agent compared to other carotenoids.In addition to its antioxidants properties, lycopene shows an array of biological effects including cardio protective, antiinflammatory, anti-mutagenic and anti- carcinogenic activities. The cancer activities of lycopene have been demonstratedboth in vitro and in vivo tumor models (Blot et al., 1993).

Selenium derivatives as cancer preventive agents

The role of selenium in the prevention of cancer has been recently established by laboratory experiments, clinical trials and epidemiological data. Consequently, selenium supplementation has moved from the realm of correcting nutritional deficiencies to one of pharmacological intervention, especially in the clinical domain of cancer chemoprevention and in the control of heart failure (Hamid AA,2010).

Lipoic acid, the antioxidant’s antioxidant

Lipoic acid protects against diseases of aging, this offer powerful antioxidant protection against three common afflictions (two of them potentially disastrous) association with the aging stroke, heart attack and cataracts. It does it by suppressing the action of free radicals in the cells of the brain, heart and eyes. Lipid acid has an unusual relationship with four other important antioxidants: glutathione, coenzymeQ10, vitamin C and vitamin E. Memory loss is not considered to be not considered to be a disease at least not until it is a component of a fullfledged dementia, such as Alzheimer’s disease-but it is certainly anotherhallmark of aging (Hamid AA, 2010).

Unlike lipoic acid other antioxidants are either primarily water-soluble or fat- soluble, but not both. This means that they have different (often overlapping) domain are free radical scavengers. What is good is that lipoic acid not only actsas a primary antioxidant in brain cells but serves to boost glutathione levels through the antioxidant network interactions (Hamid AA, 2010).

Diabetes, a terrible yet largely preventable disease, is practically epidemic in thewestern world, especially the United States, because of our tendency to obesity due to poor diet and lack of exercise. Since lipoic acid is the most versatile and powerful antioxidant in the entire antioxidants defense network (Hamid AA, 2010).

Gene therapy promises to be one of the most exciting and fruitful avenues of medical practice in the twenty-first century and it offer powerful antioxidant protection against common afflictions (Hamid AA, 2010).

Significance of antioxidants in red cells

Erythrocytes containing abnormal haemoglobin with high affinity for red cell. Since HbS is known to have high affinity for red cell membrane and sickle cells are particularly susceptible to membrane lipid peroxidation, the behaviour of erythrocyte antioxidant system has been evaluated in 20 subjects, heterozygous for sickle cell anaemia. These subjects have shown normal levels of reduced glutathione, increased superoxide dismutase and glutathione peroxidase activities and low catalase activity. These data suggest that such an unbalanced antioxidant system cannot prevent damage by the enhanced production of oxygen free radicals by membrane-bound HbS molecules (Hail N, 2008).

Antioxidants therapy in acute central nervous system injury

Free radicals are highly reactive molecules generated predominantly during cellular respiration and normal metabolism imbalance between cellular production of free radicals and ability of cells to defend against them is referred to as oxidative stress (OS) (Gutter, 1991). OS has been implicated as a potential contributor to acute central nervous system (CNS) injury by ischemic or hemorrhagic stroke or trauma. The production of reactive oxygen species (ROS) may increase, sometimes drastically leading to tissues damage via several different cellular molecular pathways. Radicals can cause damage to cardinal cellular components such as lipids, proteins and nucleic acid e.g DNA leading to subsequent cell death by modes of necrosis or apoptosis. The damage can becomemore widespread due to weakened cellular antioxidant defense systems. Moreover, acute brain injury increases the level of excitoxic amino acids (such as glutamate), which also produce ROS, thereby promoting parenchymatous destruction. Therefore, treatment with antioxidants may theoretically act as tissuedamage and improve both the survival and neurological outcome, several such agents of widely varying chemical structures have been investigated as therapeutic agents for acute CNS injury, although, a few of the antioxidants showed some efficacy in animal models or in small clinical studies. Better understanding of the pathological mechanisms of acute CNS injury would characterize the exact primary targets for drug intervention improved antioxidantdesign should take into consideration the relevant and specific harmful free radical (Hamid AA, 2010).

A vast amount of circumstantial evidence implicates oxygen-derived free radicals (especially superoxide and hydroxyl radicals) and high-energy oxidants such as peroxynitrite as mediators of inflammation, shock and ischemia/reperfusion injury (Hamid AA, 2010).

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