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Research Article

Review on Antioxidant Activity

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Abstract

Antioxidants are natural compounds that play a vital role in protecting cells from damage caused by free radicals .Free radicals are highly reactive molecules that can cause oxidative stress, leading to various diseases such as cancer, cardiovascular diseases, and aging. Antioxidants have become scientifically interesting compounds due to their many benefits such as anti-aging and anti-inflammatory[1]. This review article summarizes the mechanisms underlying the antioxidant activity of natural compounds, such as vitamins, flavonoids, carotenoids, and phenolic acids. The review article also provides a comparative analysis of various techniques used for measuring antioxidant activity of natural compounds. The review covers both chemical and biological assays, including the DPPH assay, ABTS assay, FRAP assay, ORAC assay, and comet assay, among others. Overall, this review aim is to crystallize the information on antioxidants regarding to its classification, mechanism of action, role in food processing and determination techniques.

Keywords: Free radicals, Oxidative stress, ROS, Enzymatic antioxidants, Non enzymatic antioxidants, In-vitro assay, In-vivo assay.

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Contents

1. Introduction

Antioxidants are groups of compounds that neutralizes free radicals and reactive oxygen species[1]. Free radicles are unstable molecules that can damage DNA, proteins, and other cellular components, leading to various diseases and aging. Antioxidants work by neutralising free radicles, reducing their harmful effects on the body. Antioxidants can found in many foods, including fruits, vegetables, nuts, and whole grains. Some common antioxidants include vitamins C and E, beta-carotene, selenium, and flavonoids[7][10]. These antioxidants can be obtained through a healthy diet, but some people may choose to take supplements to increase their antioxidant intake. Antioxidants may also improve skin health by reducing the effects of UV radiation and other environmental stressors.

However it should be emphasized that the antioxidant activity is different for different varieties and morphological parts of natural resources [1]. In addition, the activity of natural products is influenced by many other factors, such as climatic and soil conditions or harvest time. They hinder the standardization of natural products to a large extent. In recent years, a large body of evidence has been published that natural antioxidants increase the stability of edible oils, the stability of carotenoid dyes, and the aroma of fruit juices and that they work well as additives in meat products.

Antioxidant activity refers to the ability of a substance to neutralize free radicals and reduce oxidative stress, which can contribute to the development of chronic diseases such as cancer, heart disease, and Alzheimer's disease [2][13]. Several methods exist for evaluating antioxidant activity, each with its own strengths and limitations. One commonly used method is the Oxygen Radical Absorbance capacity (ORAC) assay, which measures the ability of a substance to neutralize free radicals in a test tube. Another method is the Trolox Equivalent Antioxidant Capacity (TEAC) assay, which measures the ability of a substance to scavenge free radicals in a similar way to the ORAC assay.

Free radicals are atoms, molecules, ions with unpaired electrons, which are highly active to chemical reactions with other molecules. In the biology system, free radicals are often derived from oxygen molecule, nitrogen and sulphur molecules. These free radicals are part of groups of molecules called reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulphur species (RSS) [10][13]. In order to prevent or reduce the ROS-induced oxidative damage, the human body and other organisms have developed an antioxidant defence system that includes enzymatic, metal chelating and free radical scavenging activities to neutralize these radicals after they have formed. In addition, intake of these dietary antioxidants may help to maintain an adequate antioxidant level in the body.

2. Classification

Antioxidants can be classified into several categories based on different attributes.

- The first attribute is based on the function(primary and secondary antioxidants)
- The second attribute is based on the enzymatic and non-enzymatic antioxidants[1][5][18].

Primary antioxidants:

Primary antioxidants are a group of molecules that play a crucial role in protecting cells from oxidative stress. They are called "primary" because they are the first line of defence against free radicals and reactive oxygen species (ROS) that can damage cells, proteins, lipids, and DNA. These antioxidants work by donating hydrogen atoms to free radicals, neutralizing their reactivity and breaking the chain reaction of oxidative damage [9]. Here are some primary antioxidants in detail:

Vitamin C (ascorbic acid):

Vitamin C is a water-soluble antioxidant that scavenges free radicals and ROS in the body. It can also regenerate other antioxidants, such as vitamin E, by donating electrons to reduce them back to their active form. Vitamin C is an essential cofactor for many enzymes involved in collagen synthesis, neurotransmitter production, and immune function. It also has anti-inflammatory properties and enhances iron absorption.

Vitamin E (alpha-tocopherol):

Vitamin E is fat-soluble antioxidant that protects cell membranes from oxidative damage. It prevents the propagation of lipid peroxidation by donating hydrogen atoms to lipid peroxyl radicals, which stops the chain reaction of lipid damage. Vitamin E can also regenerate other antioxidants, such as vitamin C, by donating electrons to reduce them back to their active form. Vitamin E is important for maintaining healthy skin, vision, and immune function.

Beta-carotene:

Beta-carotene is a carotenoid found in plants that acts as a scavenger of singlet oxygen and free radicals. It is a precursor of vitamin A and plays a crucial role in vision, immune function, and skin health. Beta-carotene can also act as a quencher of singlet oxygen, which is a highly reactive form of oxygen that can damage cells.

Selenium:

Selenium is a trace mineral that is an essential component of the antioxidant enzyme glutathione peroxidase, which protects cells from oxidative damage. It also has anti-inflammatory properties and is important for thyroid function and immune health. Selenium can be found in sea food, Brazil nuts, meat, and grains.

Polyphenols:

Polyphenols are a group of plant-derived compound with antioxidant properties. They include flavonoids, anthocyanins, and resveratrol, among others. Polyphenols can scavenge free radicals, chelate metal ions, and modulate cellular signalling pathways. They have anti-

inflammatory, anti-cancer, and cardio protective effects. Foods rich in polyphenols include fruits, vegetables, tea, coffee, cocoa, and red wine.

Coenzyme Q10:

Coenzyme Q10 (CoQ10) is a molecule found in mitochondria that plays a crucial role in energy production. It also acts as a free radical scavenger in cell membranes. CoQ10 can regenerate other antioxidants, such as vitamin E, and enhance immune function. It is found in small amounts in meat, fish, and whole grains, and can also be taken as a supplement.

Melatonin:

Melatonin is a hormone produced by the pineal gland that regulates sleep- wake cycles. It also acts as a free radical scavenger in the body. Melatonin can enhance immune function, reduce inflammation, and protect against oxidative damage in the brain and cardiovascular system. It is found in small amounts in foods like fruits, vegetables, grains, and nuts, and can also be taken as a supplement.

Secondary antioxidants:

Secondary antioxidants are molecules that can work in conjunction with primary antioxidants to provide additional protection against oxidative stress. They do not directly neutralize free radicals, but instead regenerate primary antioxidants or scavenge other reactive species that are generated during the antioxidant process. Here are some exams of secondary antioxidants:

Glutathione:

Glutathione is a tripeptide molecule composed of three amino acids (cysteine, glutamic acid, and glycine). It is a powerful antioxidant that can scavenge free radicals and ROS, as well as regenerate other antioxidants such as vitamin C and vitamin E. Glutathione is synthesized in liver and is found in all body cells, where it plays a crucial role in detoxification and maintaining redox balance.

NADPH:

Nicotinamide adenine dinucleotide phosphate (NADPH) is a coenzyme involved in cellular metabolism and energy production. It is also a crucial component of the antioxidant defence system, where it is used to regenerate glutathione and other antioxidants. NADPH is synthesised in cells through a series of enzymatic reactions that requires energy from glucose and other nutrients.

Phosphites:

These compounds are often used in plastic and rubber products as secondary antioxidants. They are effective at preventing the degradation of primary antioxidants such as phenolic compounds.

Thioesters: Thioesters are used as secondary antioxidants in food and cosmetic products. They work by reacting with free radicals and preventing them from causing oxidative damage.

Amines:

Amines are commonly used as secondary antioxidants in lubricants and oil. They react with free radicals to neutralize them and prevent them from causing oxidation.

Hydroxylamines:

Hydroxylamines are used as secondary antioxidants in the production of rubber and plastic products. They are effective at preventing the oxidation of primary antioxidants such as phenolic compounds

According to Ratnam, et al. (2006), antioxidants can be divided into two classes. They are :

- Enzymatic antioxidant
- Non-enzymatic antioxidants

Role of antioxidants:

Antioxidants play a vital role in maintaining health and preventing diseases by protecting the body from the damaging effects of free radicals. Free radicals are unstable molecules that can damage cells, DNA, and other important molecules in the body, leading to various health problems and diseases such as cancer, heart diseases, and aging. Antioxidants work to neutralize free radicals and prevent them from causing damage to cells and tissues.

There are different types of antioxidants, and they function in different ways.

Some antioxidants work by donating hydrogen atoms, which neutralize free radicals. Others, like vitamin E, act as chain breaking antioxidants, by stopping the free radical chain reaction. Antioxidants can also help repair damaged cells and DNA. For example, vitamin C can help repair collagen, which is an essential protein in connective tissues. Vitamin C also helps the body absorb iron, which is important for the production of red blood cells. Vitamin E has been shown to reduce inflammation and improve heart health.

In addition of these dietary antioxidants, the body can also produce its own antioxidants, such as glutathione and superoxide dismutase, to neutralize free radicals. However, the body's natural production of antioxidants may not be sufficient to protect against the harmful effects of free radicals, especially in presence of environmental toxins, such as air pollution or cigarette smoke. Antioxidants are important for maintaining overall health and preventing disease. Studies have shown that diets rich in antioxidants, such as the Mediterranean diet, can reduce the risk of chronic diseases such as heart disease, diabetes, and cancer. However, it is important to note that excessive intake of antioxidants, such as through supplements, may not provide additional health benefits and may even have harmful effects. It is best to obtain antioxidants through a balanced and varied diet.

Today it is still used in many areas. In food technology, antioxidants are added to many foodstuffs in order to enrich the foods and eliminate the problems. So, studies to determine the antioxidant activities of natural foods and their components are also continuing rapidly. Antioxidants have also been replaced in the encapsulation studies used for the preservation and stabilization of food components.

Of course, Preservations are edible films and coatings. The protective function of edible films and coatings can be improved by the addition of antioxidants. Unlike these studies on plants and animals have been investigated in vivo in terms of how the antioxidant activity changes as a result of metabolic activities.

The role of antioxidant enzymes in these studies is quite large. Many results have been found for the elimination of diseases by either in vitro or in vivo studies regarding antioxidants.[1] The use of antioxidant sources in different fields for different purposes in studies

- Studies for enrichment of food and eliminating negativity
- In vitro studies on natural foods
- Studies in encapsulation
- Studies in edible films and active packaging
- In-vivo studies in plants and animals and their importance in eliminating diseases

Mechanisms involved in antioxidant activity:

The mechanism of action of antioxidants involves several different processes, which can vary depending on the specific antioxidant molecule involved. However, in general, antioxidants work by either scavenging free radicals directly or by indirectly preventing their formation.

Direct Scavenging Free Radicals:

Many antioxidants are able to directly neutralize free radicals by donating an electron to them. This process helps to stabilize the free radical and prevent it from causing further damage to cells and tissues. Antioxidants that wok in this way include vitamin C and E, beta-carotene, and selenium. These antioxidants are typically found in foods such as fruits, vegetables, nuts, and seeds.

Indirect Prevention of Free Radical Formation: Other antioxidants work by preventing the formation of free radicals in the first place. This accomplished by several different mechanisms[8][12]

Inhibition of Enzymes:

Some antioxidants can inhibit the activity of enzymes that are involved in the generation of free radicals. For example, the antioxidant resceratrol found in grapes and red wine has been shown to inhibit the enzyme cyclooxygenase, which is involved in the production of inflammatory molecules that can generate free radicals.

Binding to metals:

Some antioxidants, such as flavonoids found in tea and cocoa, can bind to metal ions such as iron and copper. These metals are known to promote the formation of free radicals, and binding to them prevents this process from occurring.

Regulation of Gene Expression:

Some antioxidants can regulate the expression of genes that are involved in the generation of free radicals. For example, the antioxidant sulforaphane found in broccoli has been shown to up-regulate genes that promote the production of enzymes that promotes the production of enzymes that neutralize free radicals.

Free radical Scavenging activity:

Antioxidants scavenge and neutralize free radicals by donating an electron or hydrogen atom, which stabilizes the free radical and prevents it from causing further damage.

Chain Breaking:

Antioxidants interrupt the chain reaction of free radical damage by donating an electron or hydrogen atom to free radicals, which stops the propagation of oxidative stress.

Singlet Oxygen Quenching:

Some antioxidants, such as carotenoids and tocopherols, can quench singlet oxygen, a highly reactive form of oxygen that can cause oxidative damage to cells. Antioxidants neutralize singlet oxygen by transferring energy to it and stabilizing it.

Methods used in the evaluation of Antioxidant activity:

Antioxidants can be found in various foods and dietary supplements, and dietary supplements, and their activity can be evaluated using in vitro and in vivo assays. In vitro assays are performed outside of a living organism, usually in test tube or cell culture dishes, to assess the antioxidant activity of a substance [4]. These assays are useful for screening large numbers of compounds and can provide information on the mechanisms of antioxidant activity.

In-vitro methods:

In vitro antioxidant methods are used to evaluate the ability of a substance to scavenge or quench free radicals in a controlled laboratory environment. These methods are important for screening and comparing the antioxidant potential of various substances, including natural and synthetic compounds.

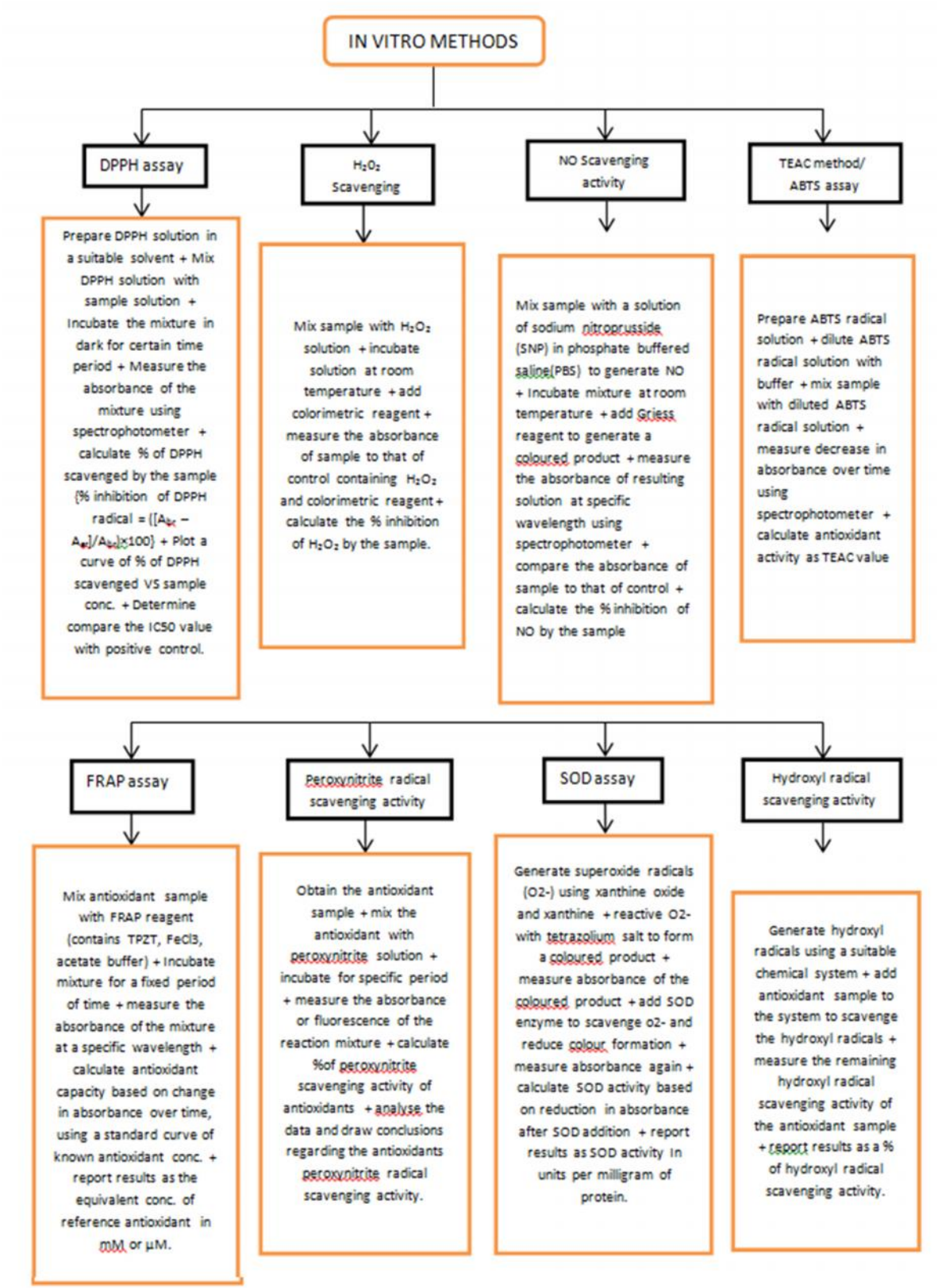
In-vivo methods:

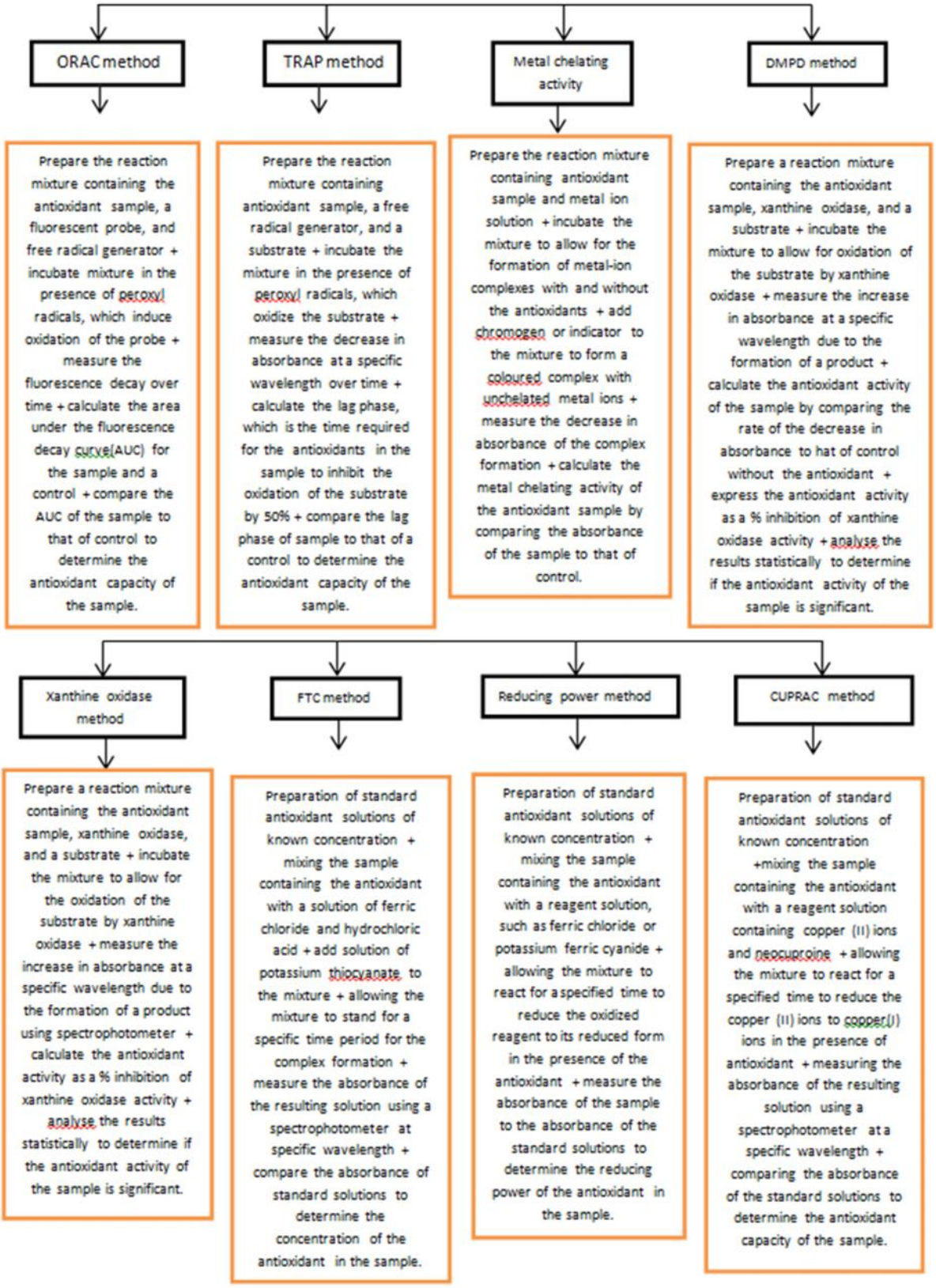
In vivo methods of antioxidants involve testing the antioxidant activity of a substance within a living organism. These methods are useful in determining the effectiveness of antioxidants in protecting the body against oxidative stress and related diseases. One common in vivo method is the use of animal models, such as mice or rats, which are exposed to oxidative stress through various means, such as chemical exposure or dietary manipulation. The animals are then given a substance or compound with potential antioxidant activity to see if it can protect against the negative effects of oxidative stress [2][6]. Another in vivo method is human clinical trials, where volunteers are given a supplement or food with potential antioxidant activity and their antioxidant status and overall health are monitored over time.

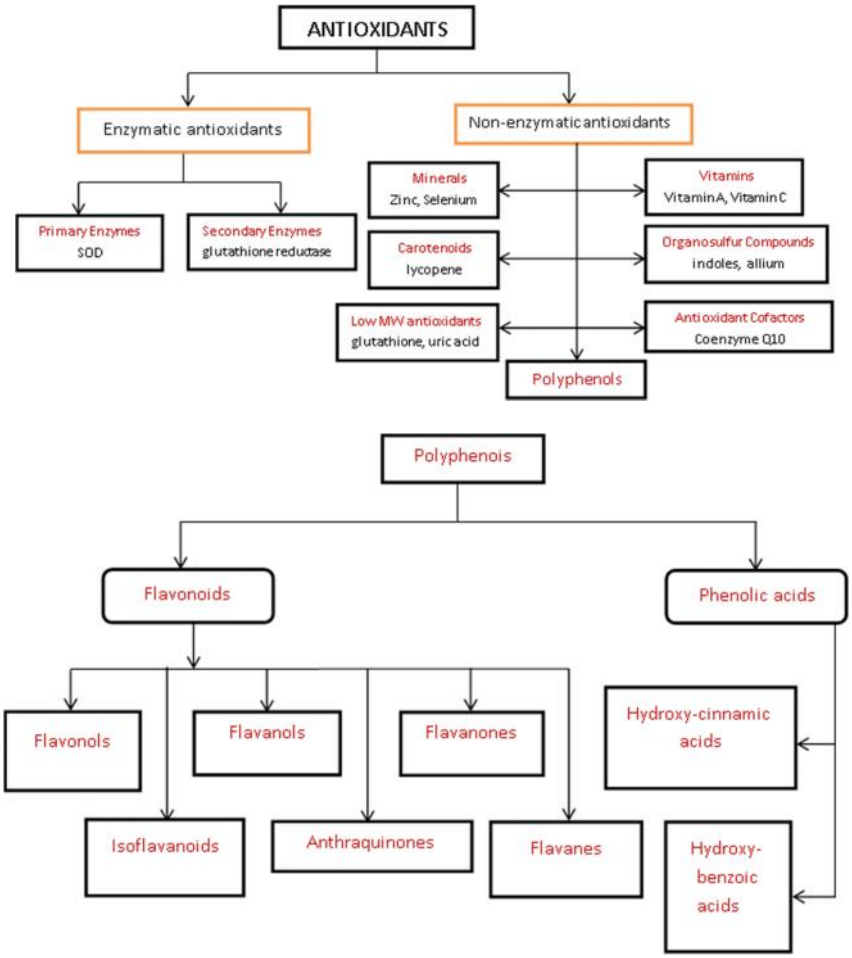
Animal Models:

- Select animal species
- Induce oxidative stress
- Administer antioxidant treatment
- Assess antioxidant activity
- Measure health outcomes
- Statistical analysis

- Interpretation and report




ORAC method
Prepare the reaction mixture containing the antioxidant sample, a fluorescent probe, and free radical generator + incubate mixture in the presence of peroxyl radicals, which induce oxidation of the probe + measure the fluorescence decay over time + calculate the area under the fluorescence decay curve(AUC) for the sample and a control + compare the AUC of the sample to that of control to determine the antioxidant capacity of the sample.
TRAP method
Prepare the reaction mixture containing antioxidant sample, a free radical generator, and a substrate + incubate the mixture in the presence of peroxyl radicals, which oxidize the substrate + measure the decrease in absorbance at a specific wavelength over time + calculate the lag phase, which is the time required for the antioxidants in the sample to inhibit the oxidation of the substrate by 50% + compare the lag phase of sample to that of a control to determine the antioxidant capacity of the sample.
Metal chelating activity
Prepare the reaction mixture containing antioxidant sample and metal ion solution + incubate the mixture to allow for the formation of metal-ion complexes with and without the antioxidants + add chromogen or indicator to the mixture to form a coloured complex with unchelated metal ions + measure the decrease in absorbance of the complex formation + calculate the metal chelating activity of the antioxidant sample by comparing the absorbance of the sample to that of control.
DMPD method
Prepare a reaction mixture containing the antioxidant sample, xanthine oxidase, and a substrate + incubate the mixture to allow for oxidation of the substrate by xanthine oxidase + measure the increase in absorbance at a specific wavelength due to the formation of a product + calculate the antioxidant activity of the sample by comparing the rate of the decrease in absorbance to hat of control without the antioxidant + express the antioxidant activity as a % inhibition of xanthine oxidase activity + analyse the results statistically to determine if the antioxidant activity of the sample is significant.
Xanthine oxidase method
Prepare a reaction mixture containing the antioxidant sample, xanthine oxidase, and a substrate + incubate the mixture to allow for the oxidation of the substrate by xanthine oxidase + measure the increase in absorbance at a specific wavelength due to the formation of a product using spectrophotometer + calculate the antioxidant activity as a % inhibition of xanthine oxidase activity + analyse the results statistically to determine if the antioxidant activity of the sample is significant.
FTC method
Preparation of standard antioxidant solutions of known concentration + mixing the sample containing the antioxidant with a solution of ferric chloride and hydrochloric acid + add solution of potassium thiocyanate to the mixture + allowing the mixture to stand for a specific time period for the complex formation + measure the absorbance of the resulting solution using a spectrophotometer at specific wavelength + compare the absorbance of standard solutions to determine the concentration of the antioxidant in the sample.
Reducing power method
Preparation of standard antioxidant solutions of known concentration + mixing the sample containing the antioxidant with a reagent solution, such as ferric chloride or potassium ferric cyanide + allowing the mixture to react for a specified time to reduce the oxidized reagent to its reduced form in the presence of the antioxidant + measure the absorbance of the sample to the absorbance of the standard solutions to determine the reducing power of the antioxidant in the sample.
CUPRAC method
Preparation of standard antioxidant solutions of known concentration +mixing the sample containing the antioxidant with a reagent solution containing copper (II) ions and neocuproine + allowing the mixture to react for a specified time to reduce the copper (II) ions to copper(I) ions in the presence of antioxidant + measuring the absorbance of the resulting solution using a spectrophotometer at a specific wavelength + comparing the absorbance of the standard solutions to determine the antioxidant capacity of the sample.



3. Conclusion

This review article mainly focused on in vitro and in vivo methods of antioxidant evaluation and also the role of antioxidants and the mechanisms involved in the antioxidant activity. It was prepared based on the plenty literatures search. The evidence presented shows that there are several different methods available for assessing antioxidant activity. Presently, 16 in vitro methods are being used for antioxidant evaluation purpose. DPPH method is the most widely used one for in vitro antioxidant activity. This article will be a comprehensive ready reference for those who are interested on antioxidant studies.

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