Exploring In Vitro Antioxidant Activity: Techniques And Therapeutic Potential

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Abstract
The main roles of antioxidants are the protection of cells from oxidative damage caused by free radicals. Free radicals are extremely reactive molecules, which causes cell damage to structural components, including proteins, lipids, and DNA. There exists an imbalance in free radical formation over the body's capacity to neutralize free radicals. Several chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders, are implicated in such an imbalance. In vitro antioxidant activity assays can be considered inevitable tools in evaluating the potential of natural and synthetic compounds against oxidative damage and in the scope of their therapeutic use. This review addresses the methods of in vitro studies for evaluating the antioxidant activity of compounds with relevance to human health. Among these, some common assays include DPPH, which is 2,2-diphenyl-1-picrylhydrazyl, measures the radical scavenging ability, ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), FRAP, ferric-reducing antioxidant power, ORAC, oxygen radical absorbance capacity, among others. Different assays may focus on different aspects of antioxidant activity in a compound. Some will focus on free radicals scavenging, reducing power, and even lipid peroxidation inhibition for better insight into the potential activity of the antioxidant in the compound.

Introduction

Oxidative stress, therefore, refers to a means of describing the imbalance of ROS or free radicals with the body's defense mechanisms of its antioxidant. Because of enhancing the balance of free radicals, there is resultant cellular damage through inflammation and results in the gradual development of most chronic diseases; among them is cancer, cardiovascular disorders, neurodegenerative diseases like Alzheimer and Parkinson's, diabetes, and aging conditions. For the condition to worsen, oxidative stress causes damage to such important biomolecules like lipids, proteins, and DNA.[1]
The molecules are very reactive as they possess one unpaired electron that acts like a stimulant and triggers chain reactions resulting in damage inside the body, tissues, and organs. However, these free radicals possess antioxidants that may easily neutralize them by giving electrons or donating hydrogen atoms with no oxidative damage. These can be broadly classified into enzymic and non-enzymic antioxidants. The former ones, in the form of enzymes, are assumed to act directly upon the body, neutralizing the free radicals within it, that is, superoxide dismutase, catalase, glutathione peroxidase. The latter group, that is, the non-enzymic antioxidants include: vitamins, minerals, polyphenols, flavonoids, carotenoids, and other secondary plant metabolites.[2]
With this ever-growing knowledge of the relationship between oxidative stress and disease, it is very evident that interest in antioxidants has grown. Of all the natural antioxidants those derived from plants and other food items have been studied extensively by scientists due to their health benefits. The antioxidant activity has been of great importance in the discovery of new drugs, food constituents, and dietary supplements that can control oxidative damage and for prevention of the diseases associated with oxidative stress.[3]
The richest tools for judgment of an antioxidant potential substance would be the in vitro tests on antioxidant activity. Such assays are conducted entirely ex vivo under highly controlled laboratory conditions and informative regarding free radical scavenging, attenuation of oxidative damage, and protective effects. This will enable screening of thousands of natural and synthetic compounds for their antioxidant activities before the step forward into intricate in vivo experiments where physiological conditions can sometimes override the antioxidant action. Applications of in vitro antioxidant assays have increased in research, and several techniques have been developed to evaluate the activity of antioxidants in a wide range of biological environments. The most commonly used in vitro method is the assessment of free radical scavenging or reduction of oxidative damage in a simplified system. These are highly inexpensive tests, besides being very easy to perform with quick results and the activity of many compounds at different concentrations can be investigated. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay, FRAP (ferric-reducing antioxidant power) assay, ORAC (oxygen radical absorbance capacity) assay, TBA (thiobarbituric acid) test for lipid peroxidation inhibition, etc.[4], [5]
The DPPH radical scavenging assay remains one of the widely used methods of determining antioxidant activity. DPPH is an extremely stable free radical with an intense deep-violet color when in solution and the reduction, or decolorization, observed upon interaction with an antioxidant corresponds to a linear function of its activity. The other highly used technique is the ABTS assay, wherein the ABTS radical cation is formed, and the degree of decolorization on reaction with the antioxidants is determined. FRAP stands for a ferrous

reducing/antioxidant power assay. This assay measures the ability of a compound to reduce another chemical. The principle behind this is the fact that antioxidants reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), and the latter ferrous ions change the color of the reaction mixture blue. ORAC is the short form for oxygen radical absorbance capacity. It evaluates the ability of a compound in protecting a probe from oxidation. Thus, it provides an estimation of the capacity for the potential of free radicals' neutralization within the scale of given time.[6], [7] The antioxidant assays measure the parameters associated with the activity of antioxidants, such as radical scavenging ability, reducing power, and inhibition of lipid peroxidation. However, each one has their own strengths and weaknesses. While in vitro assays can be run separately or together to permit an understanding about the overall antioxidant activity of a compound, it does require a much better understanding of mechanisms and chemical natures of antioxidants.[8], [9] The chemical structure of an antioxidant determines its activity. Functional groups, for instance, hydroxyl (-OH), can donate an electron or proton to a free radical, thus neutralizing the reactivity. The effectiveness of antioxidants, therefore, is determined by the number and position of the functional groups, ability to delocalize the unpaired electron, and solubility of the antioxidant. Among the naturally occurring antioxidants, the most important ones include polyphenols, flavonoids, carotenoids, and vitamins C, E, and A. These compounds occur in a very diverse range of plant products, including fruits and vegetables, herbs and spices, and also in tea. Consumption of such products has been linked to low rates of oxidative stress-related diseases; in the laboratory, substantial evidence suggests antioxidant activity comes from plant sources.[7], [8] The more traditional synthetic antioxidants, in addition to plant-based sources, include butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). It has been proved that these molecules can be of high efficacy as antioxidants; however, potential toxicities and long-term safety have expedited the pursuit of more natural sources. Of late, scientific research has built momentum as researchers look for newer, safer, and effective natural antioxidants from lesser-known underutilized plants or lesser-known natural products.[9], [10] Although the data derived from in vitro studies may be useful, the assays themselves are intrinsically limited by the fact that the complexity of a biological environment of an organism cannot be simulated well. In an organism, bioavailability, metabolism, and interactions can potentially modify the in vivo antioxidant activities of these compounds. With increased awareness about the role of antioxidants in health and disease, more effort should be given to the quest of identifying new antioxidant compounds. Since numerous vitro assays exist and are applied to the estimation of antioxidant activity, challenges also arise so that efforts might be standardized to result in similar findings in multiple laboratories with various conditions. Methods already known continue to improve since newer assays become far more sensitive and specific than biological conditions might describe. Therefore, antioxidant compounds can be characterized as much better for the ultimate goal of achieving tangible benefits.[10], [11]

Types of Antioxidants

Antioxidants are compounds that protect cells from oxidative damage by neutralizing free radicals and ROS. Free radicals and ROS are harmful molecules that cause cellular damage and are linked with various diseases, aging, and inflammation. Antioxidants are classified based on their source into natural or synthetic solubility as water-soluble or fat-soluble, or chemical structure. The main classes of antioxidants are as follows:

1. Enzymatic Antioxidants

The body employs enzymatic antioxidants, which core function of which is the neutralization of free radicals and the repairing of oxidative damage. Some of these key enzymes catalyze specific reactions that help minimize oxidative stress, such as Superoxide Dismutase, SOD which catalyzes one reaction in which superoxide radicals reduce to hydrogen peroxide; Hydrogen peroxide is less active than superoxide radicals, therefore Catalase.[12]

Glutathione Peroxidase (GPx): These will catalyze the reduction of hydrogen peroxide and lipid peroxides using glutathione GSH as the reducing agent.

Glutathione Reductase: These are catalyzing regeneration of reduced glutathione, which is necessary for antioxidant defense.

Peroxiredoxins (Prxs): A family of enzymes, which catalyze the reduction of peroxides, especially hydrogen peroxide with thioredoxin.
These are the enzymes which can be found working in many compartments of cells including cytoplasm, mitochondria, etc., to establish redox balance and protect the organism from injury.

2. Non-Enzymic Antioxidants

These are substances which are not regarded as enzymes but would work significantly in neutralizing the activity of antioxidants through scavenging on free radicals and ROS. Non-enzymic antioxidants can be endogenously synthesized in the human body or may be acquired exogenously from diet and other environmental exposure.[13]

Endogenously Synthesized Non-Enzymatic Antioxidants
These are antioxidants which are synthesized endogenously in the human system preventing the onset of oxidative stress. A few critical antioxidants that are synthesized internally in the body are as follows:

Glutathione (GSH): Among the richest intracellular tripeptides: glutamine-cysteine-glycine. They have an important function in maintaining the redox status of a cell and eliminating deleterious peroxides.

Uric Acid: By-Product of Purine Metabolism; because of its free radical scavenger property, antioxidants protect blood, as it oxidizes ROS.

3. Natural Antioxidants
Most of the antioxidants are those that occur naturally within the plant-based foodstuffs:

Herbal antioxidants
Ginseng: it contains ginsenosides that act as antioxidants and may attenuate oxidative stress, Echinacea: this enhances the immune response and has antioxidant properties while Milk Thistle: it possesses silymarin that is a rich antioxidant composition which protects liver tissues.[14]

Spices and Herbs
Ginger: It has compounds that contain gingerol that have antioxidants and anti-inflammatory properties.
Cinnamon: It is rich in polyphenolic compounds that scavenge free radicals and thus reduce oxidative damage.
Cloves: These contain eugenols, a strong antioxidant and anti-inflammatory agent.

Fruits and Vegetables
Berries (Blueberries, Strawberries, Raspberries): These are rich in anthocyanins and other flavonoids which are antioxidants that are strongly potent.
Tomatoes: This contains lycopene, a carotenoid that has been used to reduce oxidative stress.
Spinach: It is a rich source of flavonoids and carotenoids that aid in combating oxidative damage.

4. Synthetic Antioxidants
Synthetic antioxidants are synthesized in the laboratory and find wide application in food preservation or pharmaceutical formulations.[15] Some of the most widely used synthetic antioxidants are as follows
Butylated HydroxyToluene (BHT): It is a synthetic antioxidant widely used as a preservative in processed foods and cosmetics.
Butylated HydroxyAnisole (BHA): This is another synthetic antioxidant widely used in food products to prevent rancidity.
Propyl Gallate: Synthetic antioxidant, which prevents oxidation of fats and oils in foodstuffs.
Tert-Butylhydroquinone (TBHQ): Synthetic antioxidant, which maintains the shelf life of food products and oxidative rancidity.

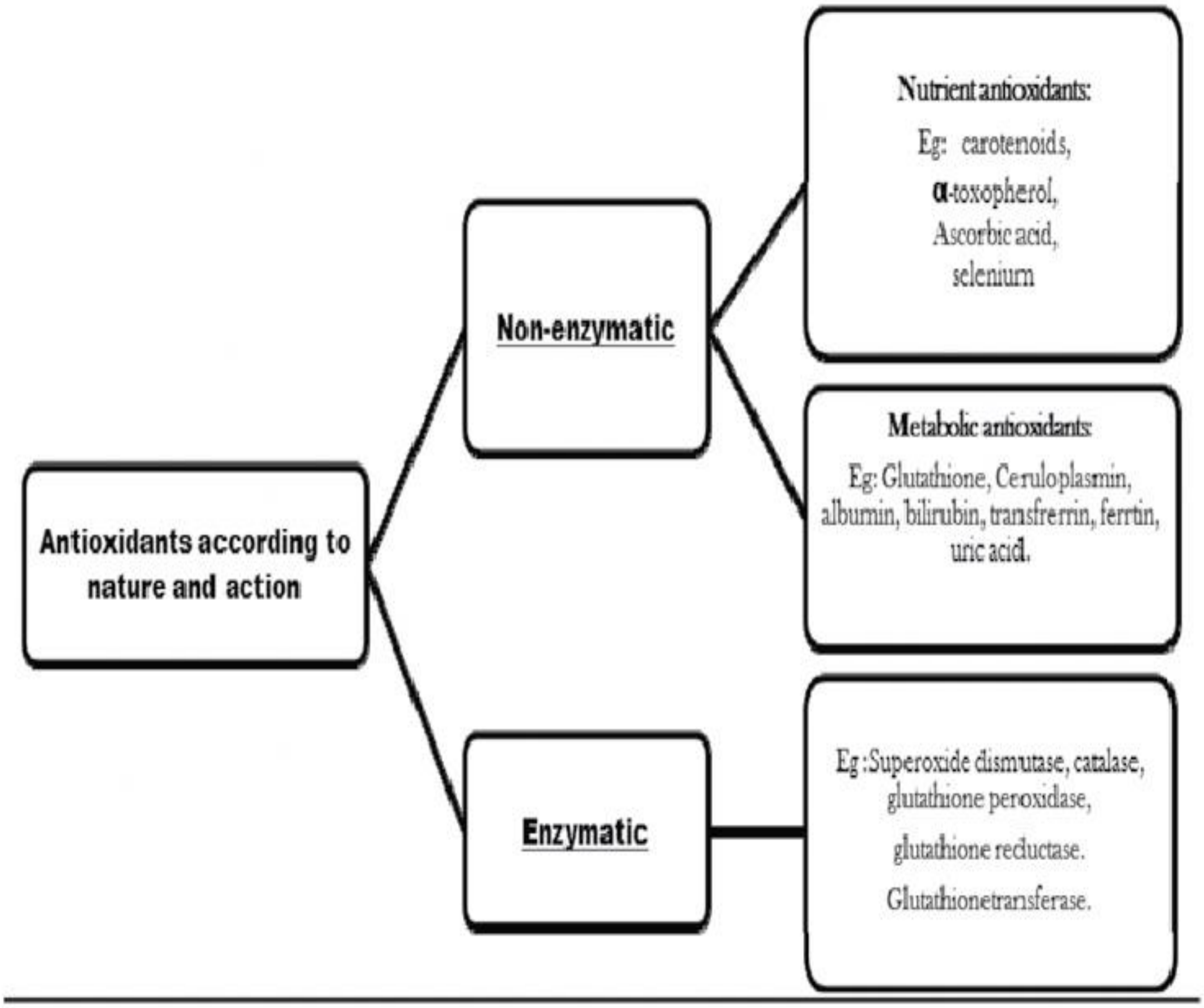


Figure: Oxygen is extremely crucial for the existence of higher organisms. As the saying goes, too much of even the best is awful. High and soaring concentration of oxygen are found to be toxic and can damage tissues. Free radicals can adversely alter lipids, proteins and DNA and have been implicated in aging and in number of human diseases. Lipids are highly prone to free radical damage resulting in lipid peroxidation that can lead to adverse alterations. Free radical damage to protein can result in loss of enzyme activity.

Oxygen is an indispensable element for the survival of higher organisms, including humans. It plays a central role in cellular respiration, a vital process that facilitates the production of ATP (adenosine triphosphate), which powers cellular functions. Oxygen is essential for aerobic metabolism, a far more efficient energy production system compared to anaerobic metabolism. This key role of oxygen is why it is crucial for the proper functioning of complex organisms. However, despite being fundamental to life, oxygen can also be harmful when present in excessive amounts or poorly regulated within the body. This review will explore both the beneficial roles of oxygen in higher organisms as well as the detrimental effects that excess oxygen and oxidative stress can cause, contributing to a variety of diseases and aging.[16]

The Vital Role of Oxygen in Higher Organisms

Oxygen is necessary for nearly all aerobic organisms to survive. The primary biological process that depends on oxygen is cellular respiration. In the mitochondria, oxygen participates in a series of reactions that convert glucose into ATP, carbon dioxide, and water. This process, known as oxidative phosphorylation, is the most efficient form of ATP production in the body, providing the energy required for cellular activities such as growth, repair, and reproduction. In the absence of oxygen, most higher organisms would not be able to generate enough energy to maintain their biological functions. Without oxygen, cells would have to rely on anaerobic metabolism, which is significantly less efficient and can only support life for short periods. Some organisms, such as certain bacteria and yeast, can survive without oxygen, but their energy production is much lower than that achieved through oxidative respiration. Therefore, oxygen is essential to meet the high metabolic demands of complex, multicellular organisms.[16]

Oxidative Stress: The Harmful Side of Oxygen

While oxygen is vital for energy production, it becomes dangerous when present in excessive concentrations or when its metabolism is not properly controlled. Under normal conditions, oxygen molecules are stable and not directly harmful. However, during cellular metabolism, oxygen can generate highly reactive molecules known as reactive oxygen species (ROS), which include free radicals like superoxide (O2-) and hydroxyl radicals (OH•).

ROS are byproducts of cellular respiration, primarily produced in the mitochondria. While ROS serve important functions in cellular signalling and immune responses, their excessive production can lead to oxidative stress. This occurs when the accumulation of ROS exceeds the capacity of the body's antioxidant defenses, resulting in the damage of cellular components such as lipids, proteins, and DNA. This damage can disrupt normal cellular functions and lead to the development of a range of diseases.[17], [18]

Lipid Peroxidation: The Impact of ROS on Lipids

One of the main targets of ROS is lipids, particularly those in cellular membranes. When ROS interact with lipids, they undergo a process called lipid peroxidation. Free radicals attack the fatty acid chains in phospholipids, forming lipid peroxides. These lipid peroxides are highly reactive and can further damage other cellular molecules, creating a harmful cascade of damage Lipid peroxidation weakens the structural integrity of cellular membranes, affecting their fluidity, structure, and overall function. This can result in leakage of cellular contents, impaired signalling, and dysfunctional membrane receptors. In severe cases, lipid peroxidation can lead to cell death, which contributes to tissue damage and inflammation. Additionally, the byproducts of lipid peroxidation, such as aldehydes, are toxic and have been linked to the development of several diseases, including cardiovascular diseases, neurodegenerative disorders like Alzheimer's, and even cancer.[1], [19], [20]

Protein and Enzyme Damage Due to Free Radicals

Proteins, particularly enzymes, are also highly vulnerable to oxidative damage. Free radicals can alter protein structure by oxidizing amino acid residues, causing cross-linking, or promoting the aggregation of proteins. These modifications can severely impair protein function, particularly enzymes that are crucial for various biochemical reactions within cells. Oxidative damage to enzymes involved in cellular respiration or DNA repair can reduce metabolic efficiency and increase the risk of genetic mutations. This loss of enzyme function can disrupt essential cellular processes, contributing to aging and the onset of diseases such as Parkinson's disease, diabetes, and cancer.[8]

Oxidative Stress, Aging, and Disease Development

The adverse effects of oxidative stress extend beyond individual cells, impacting the organism. As organisms age, their ability to counteract ROS declines, and the accumulation of oxidative damage increases. This gradual buildup is believed to contribute to the aging process, as cells lose their ability to maintain homeostasis and repair damage. The decline in mitochondrial function, which plays a central role in oxidative stress, accelerates aging and increases susceptibility to age-related diseases.[21], [22]

Oxidative stress has been implicated in a wide variety of diseases, including cardiovascular conditions, cancer, and neurodegenerative disorders. In cardiovascular diseases, for example, ROS can oxidize low-density lipoproteins (LDL), which then accumulate in the walls of arteries, promoting inflammation and the development of atherosclerosis. In neurodegenerative diseases like Alzheimer's and Parkinson's, oxidative damage to neurons impairs their function, leading to cognitive decline and motor dysfunction.

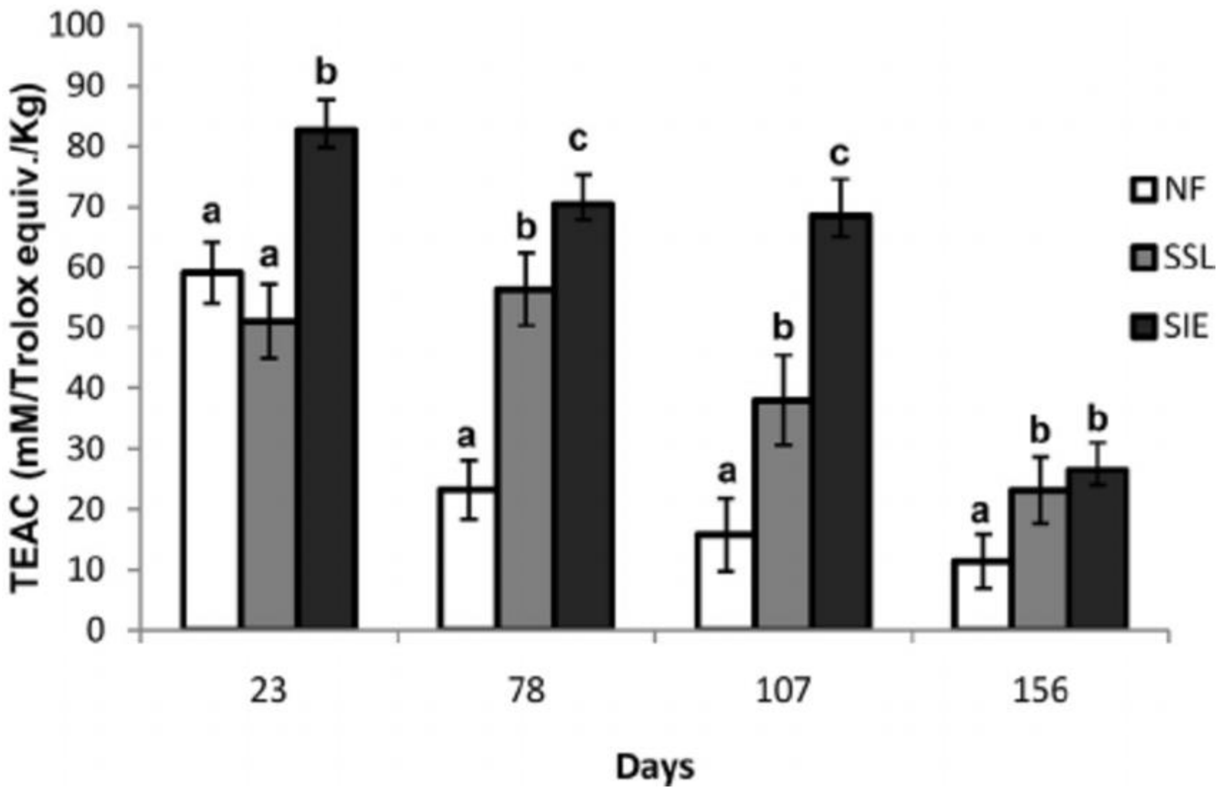


Figure : Evaluation of a single strain starter culture, a selected inoculum enrichment, and natural microflora in the processing of Tonda di Cagliari natural table olives: Impact on chemical, microbiological, sensory and texture quality.

The production of natural table olives involves fermentation processes that are critical for developing the sensory, textural, and nutritional attributes of the final product. Among the various olive cultivars, Tonda di Cagliari, a traditional Italian variety, is known for its distinctive taste and quality. This study evaluates the effects of using a single-strain starter culture, selected inoculum enrichment, and natural microflora on the fermentation process and the resulting chemical, microbiological, sensory, and texture properties of Tonda di Cagliari table olives. Experimental Design and Methods The fermentation was conducted in three distinct batches: Single-Strain Starter Culture: A single Lactiplantibacillus plantarum strain was used to initiate fermentation. Selected Inoculum Enrichment: A combination of selected bacterial and yeast strains was applied to enrich the fermentation process. Natural Microflora: The fermentation relied solely on the indigenous microbial population naturally present on the olives and in the environment. Each batch underwent fermentation under controlled conditions to ensure comparability. Periodic sampling was conducted to monitor microbial populations, chemical changes, sensory characteristics, and texture modifications throughout the fermentation process. Microbiological Dynamics The microbiological assessment revealed distinct differences among the three fermentation strategies: Single-Strain Starter Culture: The starter culture dominated the fermentation, effectively suppressing undesirable microorganisms. Lactiplantibacillus plantarum exhibited robust growth, rapidly lowering the pH and creating a stable environment for fermentation. Selected Inoculum Enrichment: The enriched inoculum demonstrated synergistic interactions between bacteria and yeast. This approach promoted diverse microbial activity, resulting in a balanced ecosystem that enhanced the metabolic profile of the olives. Natural Microflora: The natural fermentation displayed higher variability due to the uncontrolled microbial composition. While some beneficial microbes proliferated, the presence of spoilage organisms occasionally disrupted the fermentation process. Chemical Composition Chemical analyses focused on organic acids, phenolic compounds, sugars, and volatile profiles: Single-Strain Starter Culture: Fermentation led to a consistent increase in lactic acid and a significant reduction in sugar content. The phenolic profile remained stable, contributing to antioxidant activity. Selected Inoculum Enrichment: This batch exhibited a more complex chemical profile, with enhanced production of volatile compounds such as esters and alcohols. Phenolic degradation was moderated, preserving bioactive components. Natural Microflora: The chemical composition varied widely across samples. While some batches achieved desirable acidification and flavor development, others showed signs of spoilage, including off-flavors and irregular acid profiles.[23], [24]

Formation of Antioxidants

The formation of antioxidants occurs through various biochemical and chemical mechanisms. An antioxidant is a molecule that can inhibit, or delay oxidation of other molecules by neutralizing free radicals or reactive oxygen species (ROS). Some of them are produced naturally in the body, but others are derived from external sources like nutrition, plants, or synthetic formulations. There are several meanings that can be put to the phrase formation of antioxidants: endogenous synthesis (produced within the body), intake through diet, and synthetic synthesis. We continue on below with discussions on the steps involved in antioxidant formation.[25]

1. Endogenous Synthesis of Antioxidants (In the Body)

Most of the antioxidants that occur in humans are synthesized through biochemical mechanisms involving metabolism of precursors within the body. These antioxidants prevent the cells from oxidative stress and damage caused by free radicals. Some of the major synthesis pathways for endogenous antioxidants include the following.

A. Glutathione (GSH) Synthesis

Glutathione is one of the most important antioxidants that are synthesized endogenously. It is synthesized from glutamine, cysteine, and glycine by an enzyme-catalyzed process in the body in two steps.

Step 1: Synthesis of γ-glutamylcysteine

The step involves the synthesis of γ-glutamylcysteine from glutamate and cysteine using glutamate cysteine ligase as a catalyst. Because cysteine is the limiting precursor in this step, either dietary sources of cysteine or methionine must provide this amino acid for glutathione synthesis to occur.[26]

Step 2: Synthesis of Glutathione

The enzyme glutathione synthetase catalyzes the combination of glycine to γ-glutamylcysteine, which results in the formation of glutathione (GSH). GSH is necessary for the scavenging of free radicals, a decrease in hydrogen peroxide, and redox balance within cells.[4]

B. Synthesis of Coenzyme Q10 (CoQ10)

Coenzyme Q10 is also known as ubiquinone; this is an antioxidant that participates in energy production in the mitochondrion. Biosynthesis of Coenzyme Q10 begins with the amino acid tyrosine and proceeds through several steps. These steps begin from the conversion of tyrosine into 4-hydroxyphenylpyruvate and then into intermediates such as p-hydroxybenzoate, which leads to the synthesis of coenzyme Q10. The synthesis of CoQ10 is directly linked to cellular energy demand because it serves as a proton carrier in the electron transport chain within mitochondria to produce ATP.

C. Superoxide Dismutase (SOD) Biosynthesis

The superoxide dismutase is the enzyme that catalyzes the transformation of the superoxide radical (O_2^-) into hydrogen peroxide (H_2O_2), which is less reactive and may then be neutralized more easily with other antioxidants like catalase or glutathione peroxidase. Translation of SOD-specific genes leads to the synthesis of SOD, and some metal cofactors—copper, zinc, and manganese—are necessary to allow the enzyme to work optimally. There are three primary types of SOD: cytosolic SOD (CuZnSOD), mitochondrial SOD (MnSOD), and extracellular SOD (EC-SOD), which are all produced in various cellular compartments.[27]

D. Catalase and Glutathione Peroxidase Biosynthesis

Both catalase and glutathione peroxidase are enzymes involved in detoxifying hydrogen peroxide (H_2O_2) in cells.

Catalase: This enzyme is synthesized from CAT genes and requires iron as a cofactor. It catalyzes the decomposition of hydrogen peroxide into water and oxygen.

Glutathione Peroxidase: This enzyme is synthesized from GPx genes and utilizes reduced glutathione (GSH) as a cofactor to reduce hydrogen peroxide to water, thereby protecting cells from oxidative damage.

2. Dietary Formation of Antioxidants (External Sources)

Most of these antioxidants are not produced by the body and must be obtained from exogenous sources. Rich sources of antioxidants include plants, fruits, vegetables, nuts, seeds, and rich metabolic synthesis routes in plants.

A. Flavonoids and Polyphenols (Plant-Derived Antioxidants)
Flavonoids and polyphenols are two of the most prevalent plant-derived antioxidants. These compounds are synthesized within the plant through a complex network of biosynthetic routes known by various names, such as the phenylpropanoid pathway or flavonoid biosynthesis.[28]

Phénolypropanoide: using an amino acid like phenylalanine which is ammoniated with the enzyme called phenylalanine ammonia lyase-known by the abbreviated form of PAL and can eventually be modified or bring into some phénol-like compounds for the secondary metabolisms relevant for a plant cell in its defenses and antioxidant capacity.

Flavonoid Biosynthesis: The terminal chalcones appear in the presence of chalcone synthase, whose action causes the p-coumaroyl-CoA to be converted so that it produces flavonoids like quercetin and kaempferol, catechins, and anthocyanins. It has the perfect blend of the strongest antioxidants.

Beta-Carotene, Beta-Xanthin, Cryptoxanthin and other carotenoids end Synthesis
Biosynthesis of carotenoids in plants is through the isoprenoid pathway. Some of these include beta-carotene, lycopene, lutein, and zeaxanthin. Beta-carotene is a vitamin A precursor that is synthesized from phytoene. Several enzymatic steps convert IPP to phytoene. Carotenoids can act as antioxidants because they can remove free radicals to reduce oxidative damage.[29]

C. Vitamins and Minerals
Vitamin C (ascorbic acid), and vitamin E (tocopherols) are important dietary-acquired antioxidants
Vitamin C (Ascorbic Acid): Vitamin C is endogenously synthesized by most plants; however, man lacks the enzymatic machinery necessary to produce vitamin C, making us a dietary dependent species for its supply. It is highly potent and soluble antioxidant that scavenges ROS besides reactivates other antioxidants.
Vitamin E (Tocopherols): Vitamin E is a fat-soluble antioxidant which protects cell membrane from lipid peroxidation. It is endogenously produced in plants but can be available via many foods and includes vegetable oils, nuts and seeds.
Selenium and Zinc: These are very important minerals. They act as very important cofactors to the antioxidant enzymes glutathione peroxidase for selenium and superoxide dismutase for zinc, which neutralize the free radicals. Selenium is food-based and gets obtained from Brazil nuts and seafood, whereas zinc is obtained from meat, shellfish, and legumes.

3. Chemical Synthesis of Antioxidants
In addition to that, synthetic antioxidants are also prepared chemically in the laboratory. These antioxidants have been used a lot in several aspects that encompass food preservation, cosmetics, and pharmaceuticals as it protects these food, cosmetics, and drugs from oxidative damage.[30]

A. Synthetic Preparation of Synthetic Antioxidants
Butylated HydroxyToluene (BHT): BHT is prepared by alkylation in which butyl groups are attached to toluene. BHT is one of the preservative antioxidants mainly used in food and cosmetics.
Butylated HydroxyAnisole (BHA): Like BHT, BHA is prepared by reacting 4-methylphenol, or anisole, with isobutene. It is mainly used as an inhibitor to protect fats and oils in food against oxidation.
Propyl Gallate: Propyl gallate is synthesized by esterifying propyl alcohol with gallic acid. It prevents the rancidification of oils and fats in foods caused by oxidation.
Tert-Butylhydroquinone (TBHQ): TBHQ is synthesized by alkylation of hydroquinone using tert-butyl groups. This antioxidant is added to foodstuffs to protect them from becoming rancid.

How does it work
That's done by neutralization of free radicals, which are very reactive molecules, after their formation causes damage to your cells, proteins, and DNA. Free radicals may also originate as by-products of metabolism or in the process of food breakdown to generate energy. Others are exposed to them from particular environmental stressors like cigarette smoke, air pollution, or exposure to UV radiations. Additionally, there is a connection of free radicals with certain infections and inflammations.[31]

What Antioxidants Do

Oxidative stress is a disturbed state of redox, which causes cell damage. Free radicals are those molecules that have an unpaired electron. To get stable, free radicals seek out and "steal" electrons from other molecules, known as oxidation. Oxidation damages the cells, hence these phenomena are known as oxidative stress.

Antioxidant Action: An antioxidant is a molecule that has free electrons which it can donate without becoming unstable. When an antioxidant encounters a free radical, it donates an electron to neutralize the free radical so that the free radical cannot cause damage. This helps reduce oxidative stress in the body.[32]
The free radicals can be balanced through interaction by antioxidants, thereby protecting the cells, proteins, and DNA from oxidative damage, which leads to chronic diseases like heart disease and cancer and neurodegenerative diseases.
Besides preventing oxidative damage, antioxidants support the overall health. They boost the body's immune function, enhance healthy aging, and reduce inflammation. All these contribute to ensuring better overall health.

Monitoring Antioxidants therapies
This makes monitoring antioxidant therapy extremely important toward its effectiveness and safety in order for optimum use to be achieved. Since antioxidants contribute positive effects towards health, impact on different patients will vary by type of antioxidant being used or applied and disease that is intended to be either treated or prevented. The important elements of antioxidant therapies monitoring would be as shown below:[33]

1. Effectiveness monitoring
Clinical Outcomes: Antioxidant therapy aims to reduce oxidative stress and damage to cells with a concomitant improvement in health outcomes. These can be measured as improvements in such conditions as heart disease, eye health, or skin health via reductions in inflammation, prevention of chronic disease, and support of immune function.

Markers of Oxidative Stress: Blood tests may be used to quantitate markers of oxidative stress, which indicate the body's ability to fight free radicals. These include but are not limited to:
Malondialdehyde (MDA): It is a marker of lipid peroxidation and oxidative damage to cell membranes.
F2-Isoprostanes: These are specific markers of oxidative damage to lipids.
8-OHdG (8-hydroxy-2'-deoxyguanosine): It is a marker for oxidative DNA damage.
Cytokine Levels: The level of inflammatory markers, such as C-reactive protein (CRP), can be measured to observe the inflammation level, which antioxidants may reduce.

2. Dosage and Compliance Monitoring
Adherence: The knowledge of whether the patient is taking the recommended dose on a daily basis is important for one to be certain that they are receiving adequate levels of the antioxidant treatment.
Proper Dosage: There are some antioxidants, for example, vitamin C and E, which in large amounts are dangerous and can cause bleeding or interfere with prescription drugs. Follow-up prevents the overuse of the drug and reduces the dosage in a manner that suits every patient's requirement.[34]

Equilibrium with Diet: The antioxidant treatment must complement the diet of the patient. An enriched diet consisting of more fruits and vegetables and all those foods containing a high quotient of antioxidants can benefit the health status of the patient and may also help to reduce the supplementation at such high doses.

3. Monitoring of Side Effects and Toxicity
Vitamin Overload Some antioxidants, fat-solubility in particular, such as vitamins E and A, can accumulate in body tissues and subsequently reach toxic concentration depending on how supplement levels are dosed. Therefore, they will cause a series of side effects including nausea and fatigue, further causing damage through liver destruction thus ensuring that side effect monitoring is indeed crucial.[35]
Interaction with drugs: Since antioxidants interact with other medications like blood thinners (vitamin E), chemotherapy drugs (high-dose vitamin C), or immunosuppressants, check-ups regularly are important, and a good discussion with the healthcare providers is very essential.
Changes in Health: If a patient presents with new, unpredictable symptoms or changes in health, such as some kind of gut discomfort, rashes on the skin, or changes in blood pressure, then one must find out if these symptoms are associated with antioxidant supplementation.[36]

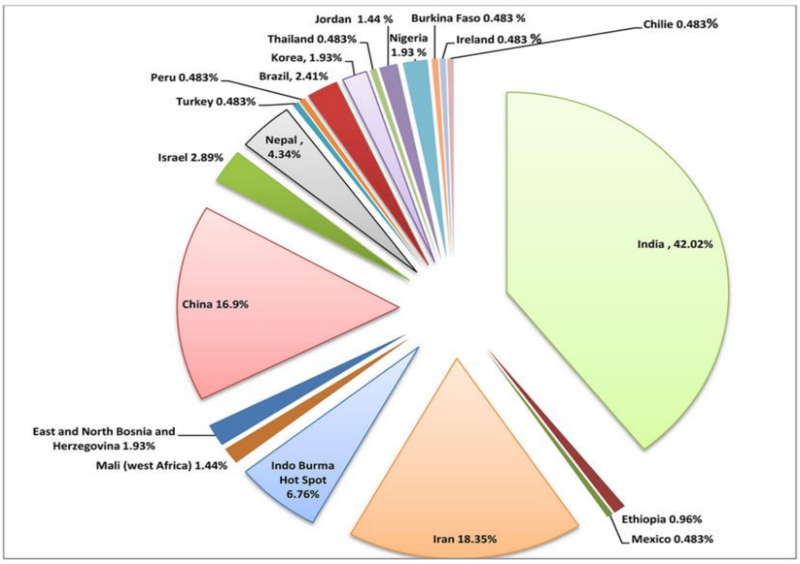
4. Long-Term Follow-Up

It would then require routine checks on the health status of the patient, for example through monitoring of blood pressure, cholesterol levels, and imaging studies on patients taking antioxidants, primarily aimed at preventing chronic diseases like cardiovascular disease, cancer, or neurodegenerative disorders.
Eye Health: If antioxidant supplements containing lutein, zeaxanthin, and vitamin E, for example, are used to protect against the eyes and AMD, an ophthalmologic examination should be conducted with retinal imaging when vision or eye health change.[37]
Skin Health: A dermatologist will add to their assessment tests that include skin exams or wrinkle assessment to show the effectiveness of antioxidants in preventing and/or reversing any visible signs of aging or by showing that these events of oxidation would not develop.

5. Genetic and Personalized Monitoring
Genetic testing: Perhaps somebody has some other genes which can alter the way a body functions to oxidative stress or the metabolic ways that make antioxidants. Genetically therefore will determine who to take specific sorts of antioxidants as well as quantity one may want to use more of the breaking products.[38]
Personalized Interventions: The antioxidant treatment can be planned according to the health status, lifestyle, and genetic profile of an individual. This can include personalized testing, such as oxidative stress biomarkers and antioxidant enzyme levels, to find out the best antioxidant treatment.[39]

6. Monitoring in Special Populations
Pregnancy and Lactation: Pregnant or lactating women should exercise special care in using antioxidant supplements as some, like vitamin A, may be dangerous to the fetus or infant. Such patients must be under close observation and informed by a professional.[40]
Chronic Conditions For patients with chronic conditions, like diabetes, heart diseases, or cancers, antioxidant treatment may be under close surveillance, because at sometimes antioxidants can interfere with medications or therapeutic interventions, as in chemotherapy which needs to be readjusted.

7. Routine Laboratory Tests
Antioxidant levels: Blood tests can measure the levels of particular antioxidants like vitamin C, vitamin E, or selenium in a person to find out whether one is receiving sufficient or excessive from the diet or supplements.
Liver and Kidney Function: As some antioxidants are metabolized through the liver and then excreted through the kidneys, routine tests of liver and kidney functions might be necessary in case one is on antioxidant therapy for a prolonged period or with high dosages.[41]



Pie chart showing the distribution of the ethnopharmacologically important plants for the treatment of worldwide.[42]

Ethnopharmacology explores the use of plants in traditional medicine across various cultures. Many plants are known for their medicinal properties and have been used for centuries. For instance, Echinacea is used to boost the immune system, especially for colds, while Ginseng is valued for its energy-boosting and stress-reducing qualities. Turmeric is widely used in Ayurveda for its anti-inflammatory and antioxidant effects. Willow bark, known for pain relief, was used by ancient cultures, while Aloe vera is favored for healing burns and aiding digestion. Ginger is a common remedy for nausea and inflammation, and St. John's Wort is well-known for treating depression and anxiety.

Neem is a powerful antimicrobial plant used for skin conditions, and Garlic supports cardiovascular health. Lavender is widely used in aromatherapy for relaxation and sleep, while Ayahuasca, a powerful plant from the Amazon, is used for spiritual healing. Moringa is a nutritional powerhouse with anti-inflammatory properties, and Cinchona has historically been used to treat malaria. Ashwagandha is a popular adaptogen for stress relief, and Coca leaves have been used for energy and altitude sickness in the Andes. These plants contribute to both traditional and modern medicine.

How to maintain a balance healthy life including balance diet

This is a marking of a diet that sustains a healthy and well-balanced life. It is the time when one is very cautious of what is going to get him closer to a healthy balance in every aspect of wellness in a physical, mental, and emotional way. Healthy behavior therefore is part of how such a balance may be achieved, and nutrition is above most things that bring general wellness about. A total guide, therefore, the ways in which one might lead a fit and balanced life congruent with healthful eating habits.

1. Basic Needs of Balanced Diet. A healthy diet is a summation of all nutritional requirements of one's body for energization, growth, and survival; rather, amounts of diverse types of food coming from various different food groups correct proportions.

Essential Components of Healthy Diet.

Fruits and Vegetables: A portion of colorful fruits and vegetables in the daily diet. They provide extremely vital nutrients, vitamins, minerals, antioxidants, and fiber for a good healthy body and immunity.

Quantity Suggestion: Have at least 5 servings in a day, both of fruits and vegetables.

Whole Grains: Whole grains are the best source of much fiber, B vitamins, and minerals. They support digestion but conserve energy for a longer period.

Examples include brown rice, quinoa, oats, whole wheat bread, and pasta. Half of the total daily grain is recommended for whole grains.

Protein: It is used in repairing muscles, the immune function, and in the production of enzymes. There are animal sources and plant sources.

Examples: Lean meats: chicken, turkey, fish, eggs, beans, lentils, tofu, nuts

2. Physical Activity

The regularity in physical activities keeps it away from chronic diseases and renders a good mood with elevated energy levels. Typical body and mind require the regularity in the activities, which require at least 150 minutes of moderate intensity aerobic activity per week or 75 minutes of vigorous intensity aerobic activity such as brisk walking, cycling or swimming, and running or high intensity HIIT per week.[43]

Strength Training: Add strength training to your program two times a week in order to build and maintain muscle mass. This can be weightlifting, bodyweight exercises like squats and push-ups, or resistance band exercises.

Flexibility and Balance: Add some stretching and balance exercises to keep one flexible and avoid injury. Yoga or Pilates will do the trick.

3. Mental and Emotional Well-being

Life, good living is having mental and emotional well-being. All this constitutes relieving stress, having proper sleep, and all self-care techniques to ensure that your mind stays in the right condition

Mental Well-being Techniques

Relaxation techniques would range from deep breathing, meditation to even mindfulness through which the anxiety is taken out. A good routine of sports and physical exercise will also ensure a reduction of stress.

Sleep: An effort to secure 7 to 9 hours of sleep every night would be the most significant factor for sound sleeping. Scheduling times to sleep without being exposed to any gadget before that, along with a quiet sleep environment, is still good sleeping hygiene.[44]

The most important of them is to be good with the family, friends, and people one loves. These positive relationships bring a great amount of positive value to the emotional well-being and work as a good supporting system.

Self-Care: Do hobbies, recreation, and games that you love or which will make you satisfied. The better you enjoy whatever you do, the lesser chance you have for burnout and your well-being will also be fine.

4. Healthy Habits for Long-term Wellness
Reduce smoking and abstain from alcohol and other drugs permanently. All these habit formations are fatal to the human body and facilitate chronic diseases.
Health Check up: Attend regular visits and screening by a health physician. In reality, every disease is better found in comparison to any health-threatening conditions.
Mindful Eating: Be attentive of your hunger cue, eat slowly, and savour food. This will increase digestion, reduce overeating, and even healthier choices of foods.

5. Environmental Factors
Your surrounding is also talking for your health and well-being. Bring a healthy environment at home and at workplace for better physical and mental health.
Create Healthy Environment
Healthy Workspaces: Your workspace should be ergonomically friendly so as not to put a strain on your body. Take breaks and stretch and move around during the day.
Clean and Organized Living Spaces: A clutter-free home reduces stress, which is a key factor towards creating that calmness and sense of organization in living spaces.
Limit Screen Time: The reduction of screen time on a phone, computer, or even TV improves mental focus, sleep, and overall wellness.

Diagnosis
Diagnosis is the determination of a disease, condition, or disorder that a patient suffers from, determined by symptoms, clinical signs, medical history, and diagnostic test or procedure results. This is an important part of the medical field because it determines the right treatment plan and management strategies for the patient. Diagnosis is generally performed by medical experts, which include doctors, nurses, and medical specialists, and there are various techniques according to the disease to be examined.[5]

Types of Diagnosis
There are several types of diagnosis. They can be classified as follows, taking into account the stage of the disease, the tools used, or even the purpose of the diagnostic procedure:[45]

1. Clinical Diagnosis
Clinical diagnosis is essentially based on the patient's symptoms reported, physical examination, and a relevant medical history. Doctors diagnose from what they see: recognized patterns of symptoms that characterize specific diseases or conditions. For example:
Symptoms: The patient may complain of being tired, having aches, or shortness of breath.
Signs: The physician might notice alterations in the vital signs such as blood pressure, temperature, pulse rate, or other body signs during the examination.
Clinical diagnosis often forms the first step in many cases and can be confirmed later with additional tests.[46]

2. Laboratory Diagnosis
Laboratory findings are used to study physical fluids like blood, urine, saliva etc and tissues or any other specimens extracted from the patient. These tests help confirm or rule out any suspected reason behind the symptoms. Some examples of laboratory diagnostic tests include the following:
Blood tests, including complete blood count, liver function tests, kidney function tests
Urine analysis, such as urine culture to check infections
Biopsy, such as tissue samples to diagnose cancer and infections
Culture studies (e.g., bacterial, viral, or fungal cultures to isolate pathogens)
These tests produce objective information that assists in determining the clinical diagnosis.

3. Radiological Diagnosis
Radiological diagnostic tools, such as imaging procedures, aid in diagnosing the inner deformities of a body. Images are provided by imaging of the organs and other formations within the human body.[47] Some of the usual radiological diagnostic tests are the following:
X-rays: help diagnose the bone fractures, pneumonia, and tumors.
CT scan: It provides a detailed image of the soft tissues, organs, and bones. It is primarily used in the diagnosis of cancers, internal bleeding, and infections.
MRI (magnetic resonance imaging): In diagnosis, the MRI helps in soft tissue injury, neurological condition, and musculoskeletal disease.

Ultrasound: It depends on sound waves in producing images in the body; this diagnostic method is commonly used in pregnancy checkup and other abdominal-related disorders.

4. Molecular Diagnosis
Molecular diagnostic methods rely on the study of genetic material, DNA, RNA, or proteins in an organism to look for specific diseases, gene mutations, and other molecular changes. It is very helpful:
For a genetic test that identifies genetic disorders, like cystic fibrosis or sickle cell anemia, or being a carrier for specific genetic disease.
Polymerase chain reaction (PCR): It is really a very potent tool for virus and bacteria identification, like the diagnosis of COVID-19 or any other disease.
Next-generation sequencing (NGS): It would be used when genetic mutation or the complete genome sequencing would be useful to personalized medicine, cancer diagnosis etc.

5. Differential Diagnosis
It is the process of distinguishing between two or more conditions that have similar symptoms. For example, chest pain can be caused by several conditions, including heart attack, pulmonary embolism, or gastroesophageal reflux disease (GERD). With systematic evaluation of symptoms, risk factors, and diagnostic test results, healthcare providers rule out some conditions to come up with the most likely diagnosis.

6. Radiological Diagnosis
This is the scanning of the inside of the human body using some imaging techniques. The imaging can allow the diagnosis of many diseases: tumors, fractures, infections, or malformations. Most common imaging include:
X-ray
CT scan (Computed tomography)
MRI (Magnetic resonance imaging)
Ultrasound
These provide useful information to the clinician and are also used together with clinical examination and laboratory tests.

Herbal approach Drugs
Many drugs or compounds' efficacy as free radical scavengers and as reducers of oxidative stress to prevent cellular damage is tested and determined by in vitro antioxidant activity tests. Many synthetic and natural drugs have been studied about their in vitro antioxidant properties. These include those derived from plant products, vitamins, and other natural substances or chemical synthetic agents. Some of the drugs so far investigated and showed in vitro antioxidant activities are:[48]

1. Natural Plant-Based Antioxidants
From these experiments, it was noted that most compounds of plant origins possessed potential in vitro antioxidant activity. These extracts were known to possess an ample supply of polyphenol and flavonoid chemicals, which captured the free radical resulting in no additional formation of the oxidative stress compound.

Curcumin
Curcumin is a well-known natural antioxidant from the Curcuma longa family that has strong anti-inflammatory and antioxidant properties. Several studies have established that curcumin can scavenge free radicals, including hydroxyl radicals (OH•) and superoxide radicals ($O_2{\bullet}^-$), which further upregulate endogenous antioxidant enzymes. In vitro research findings indicate that curcumin protects cells from oxidative damage caused by ROS.

B. Resveratrol
Resveratrol is a polyphenolic compound present in red wine, grapes, berries, and other vegetation. This compound was exhaustively researched for its antioxidant and anti-inflammatory properties. In vitro studies show that the resveratrol possesses free radical-scavenging activity, which enhances the activity of antioxidant enzymes like SOD and catalase and provides protection against oxidative stress.

C. Epigallocatechin Gallate (EGCG)

EGCG is one of the major polyphenols present in green tea. It is reported to have antioxidant activity. Free radicals are scavenged and ROS generation is inhibited. The reduction of oxidative stress in various studies has been reported by scavenging hydroxyl radicals, superoxide anions, and peroxyl radicals in vitro.

D. Quercetin

Quercetin is one of the flavonoids present in many fruits and vegetables, such as apples, onions, and citrus fruits. Quercetin has been demonstrated to have intense antioxidant activities in vitro through scavenging free radicals and modulation of antioxidant enzyme's expression levels. It has also been shown to inhibit lipid peroxidation in biological systems.

E. Ginkgo Biloba Extract

Ginkgo biloba from the Ginkgo tree has been used as an active ingredient in its medicine for hundreds of years due to its antioxidant property. Flavonoids and terpenoids of the Ginkgo biloba extract scavenge ROS, preserve cellular constituents against oxidation, and also improves circulation. Ginkgo biloba in cell line experiments conducted in a laboratory without the presence of a living being was found to reduce oxidative stress in various cells.

2. Antioxidants from among Vitamins

Vitamins, especially those whose antioxidant properties have been established, have been widely studied for their in vitro antioxidant activity. Among these are vitamins C and E, which have been shown to neutralize ROS and thereby prevent oxidative damage.

A. Vitamin C (Ascorbic Acid)

Vitamin C is one of the most soluble antioxidants. It scavenges ROS directly: superoxide, hydrogen peroxide, and hydroxyl radicals. Studies from in-vitro established that vitamin C would neutralize oxidative stress and thus can be a preventor for cellular damage resulting from free radicals. Obvious areas to note are the cells of the skin and the immune system.

B. Vitamin E (Tocopherol)

Vitamin E is a lipid-soluble antioxidant that primarily acts by protecting the cell membrane against lipid peroxidation. In vitro studies of vitamins E were able to prove that it was capable of inhibiting the oxidation of polyunsaturated fatty acids present in the cell membrane, and removal of free radicals such as the peroxyl radicals (ROO•).

3. Synthetic Antioxidants

In addition to natural antioxidants, various synthetic compounds have been synthesized as well and studied for their potential in scavenging free radicals and reducing oxidative stress. Among the synthetic antioxidants are the following:

A. Butylated HydroxyToluene (BHT)

BHT is a synthetic antioxidant, commonly used in preserving food products. The in vitro study shows that BHT is potent to scavenge free radicals such as DPPH radicals, and offers protection against lipid peroxidation. It's also widely used both in food and pharmaceutical formulations because of its antioxidant property.

B. Butylated HydroxyAnisole (BHA)

As such, BHA is another synthetic antioxidant that also protects food. Its working is through hindering the oxidation of fats and oils. Several in vitro investigations indicate that it may prevent damage to cells due to oxidation present in a culture and even it may prevent the lipid peroxidation; thereby, BHA happens to be the antioxidant most commonly utilized in foods and cosmetics.

D. Trolox

Trolox is a water-soluble derivative of vitamin E, which is a pretty popular compound in antioxidant assays as a reference standard. A very common compound in in vitro antioxidant testing to assess the potential potency of another compound, Trolox is indeed quite a potent antioxidant; it has been shown to have an in vitro ability to scavenge ROS.

NAC is an antioxidant precursor and possesses in vitro antioxidant activities. It neutralizes free radicals and is also used to replete intracellular glutathione stores. It is employed for the treatment of various oxidative stress-related disorders, such as chronic respiratory diseases and hepatotoxicity.

4. Herbal and Natural Extracts

Quite many plant extracts are tested for its ability to potentially inhibit oxidation processes; a number have shown to have abundant bioactive antioxidant moieties whose potential has only been demonstrated through invitro processes. These comprise:

A. Aloe Vera

Aloe vera is also a medicinal extract and has recently exhibited antioxidant action; extracts were used in different experiments in the laboratory, thus confirming antioxidant ability which scavenged free radicals besides enhancing antioxidation activity.

B. Ashwagandha (Withania somnifera)

Ashwagandha is an adaptogenic herb, that is, used in Ayurvedic medicines. This plant has shown, in vitro antioxidant activity. Some researches established that extracts from Ashwagandha help protect cells against oxidative damage via increased antioxidant enzyme activity and scavenging free radicals.

C. Cinnamon Extract

Other naturally occurring compounds, which can be antioxidants, occur in Cinnamon. One of the bioactive constituents is the chemical cinnamaldehyde showing excellent in-vitro radical-scavenging activity. The extract is inhibitory to lipid peroxidation but reduces oxidative stress.

POMEGRANATE EXTRACT

Rich with polyphenolic compounds such as ellagic acid and anthocyanins, Pomegranate extract has recently demonstrated significant in vitro antioxidant activities. The in vitro studies thus far show pomegranate extract is effectively an oxidative stress-neutralizing free radical.

F. Ginger Extract

Ginger is one of the recognized spices with a wide range of health benefits and is also antioxidant in nature. In vitro experiments had shown that ginger extracts scavenge free radicals, hence preventing oxidative damage of several cell lines.

5. Other Drugs and Compounds with Antioxidant Activity

Other than the above compounds, several drugs and other substances have also been reported to possess antioxidant activities in vitro, such as:

Melatonin: It acts as a hormone antioxidant, scavenging the free radicals and possesses the regulatory capacity towards antioxidant enzymes in cells.

Caffeic Acid: It is a phenolic compound. Most commonly, it is found in coffee, fruits, and vegetables and has been seen to possess antioxidant and anti-inflammatory properties in in vitro.

Flavonoids: The most studied flavonoids whose antioxidant activities have been included in many fruits and vegetables besides the tea beverages include Kaempferol, Luteolin, and Catechins.

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