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PLANT AS FACTORIES FOR ANTIOXIDANT PRODUCTION

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ABSTRACT

Plants have long be been a source of exogenous antioxidant. It is believed that two-third of the world plant species has medicinal importance, and almost all of these have excellent antioxidant potential. Plant's antioxidant can act as a radical scavenger, promote health and produce anticancer activity. But the question is why they need antioxidant, actually higher plants has facing a variety of stress condition and the antioxidant enzymes helps them to cope with these. A variety of plant materials are known to be natural sources of antioxidant, such as herbs, spices, seed fruit and vegetables. Various approaches of in vitro and in vivo methods are commonly applied for of assessing evaluation of the antioxidant activity of plant samples. There are numerous approaches such as micropropagation is used to produce antioxidant producing plant and recently looking forward to apply omics approaches to produce and control antioxidant. Recently in COVID-19 antioxidant such as Vitamin C and Zinc (Zn) are widely as immune booster and their remedies. The recent scientific studies revealed the antioxidant properties of plant, which have been examined, because of their effective pharmacological activities, economic viability and their low toxicity.

Keywords: Antioxidant; Plant; Seed; Stress; Enzyme; Toxicity; COVID-19

INTRODUCTION

Free radicals have been claimed to play a key role, affecting human health by causing severe diseases, such as cancer and cardiovascular diseases by cell degeneration. There are increasing suggestions by considerable evidence that these compounds induce oxidative damage to biomolecules (lipids, proteins and nucleic acids)[1]. Free radicals are mainly produced by oxidation processes and they have an important role in the processes of food spoilage and chemical materials degradation. They also contribute to human disorders such as aging associated diseases, cardiovascular diseases, cancer and inflammatory diseases. Free radicals may also cause a depletion of the immune system antioxidants, a change in the gene expression and may induce the synthesis of abnormal proteins. About 5% or more of the inhaled oxygen is converted to reactive oxygen species such as O_2- H_2O_2, and OH radicals[2]. Reactive oxygen species represents the major type of free radicals in any biological system. They are produced through the mitochondrial electrons transport chain. Antioxidants are used to neutralize the effects of free radicals. Thus, they protect humans against infection and degenerative diseases[3]. An antioxidant is a molecule capable of inhibiting 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. In turn, these radicals can start chain reactions that damage the cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenol. Diets high in vegetables and fruits, which are good sources of antioxidants, have been found to be healthy however, research has not shown antioxidant supplements to be beneficial in preventing diseases. Examples of antioxidants include vitamins C and E, selenium, and carotenoids, such as beta-carotene, lycopene, lutein, and zeaxanthin[4].

There is great attention towards natural antioxidants from plants. Antioxidants can act as a radical scavenger, promote health, and produce anticancer activity. Antioxidants had a growing interest owing to their protective roles in food and pharmaceutical products against oxidative deterioration and in the body and against oxidative stress-mediated pathological processes. Screening of antioxidant properties of plants and plant-derived compounds requires appropriate methods, which address the mechanism of antioxidant activity and focus on the kinetics of the reactions including the antioxidants. Many studies evaluating the antioxidant activity of various samples of research interest using different methods in food and human health have been conducted.

The physiological role of antioxidants, as this definition suggests, is to prevent damage to cellular components arising as a consequence of chemical reactions involving free radicals. In recent years, a substantial body of evidence has developed supporting a key role for free radicals in many fundamental cellular reactions and suggesting that oxidative stress might be important in the pathophysiology of common diseases including atherosclerosis, chronic renal failure, and diabetes mellitus[5]. Antioxidants are substances that when present at low concentrations, compared to those of the oxidisable substrate significantly delays or inhibits the oxidation of the substrate. An important role of antioxidants is to suppress free radicalmediated oxidation by inhibiting the formation of free radicals by scavenging radicals. The body possesses defence mechanisms against free radical-induced oxidative stress, which involve preventative mechanisms, repair mechanisms, physical defence and antioxidant defence. Enzymatic antioxidant defences include superoxide dismutase, glutathione peroxidase, catalase etc. Non-enzymatic antioxidants are ascorbic acid (vitamin C), tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, etc. All these acts by one or more of the mechanisms like reducing activity, free radical-scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body 's natural antioxidant defence or by supplementing with proven dietary antioxidants.

Free radicals & their chemical reactions

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital.The presence of an unpaired electron results in certain common properties that are shared by most radicals. Radicals are weakly attracted to a magnetic field and are said to be paramagnetic[6]. Many radicals are highly reactive and can either donate an electron to or extract an electron from other molecules, therefore behaving as oxidants or reductants. As a result of this high reactivity, most radicals have a very short half-life (10−6 seconds or less) in biological systems, although some species may survive for much longer.The most important free radicals in many disease states are oxygen derivatives, particularly superoxide and the hydroxyl radical.

Radical formation in the body occurs by several mechanisms, involving both endogenous and environmental factors. Superoxide is produced by the addition of a single electron to oxygen, and several mechanisms exist by which superoxide can be produced in vivo[7]. Several molecules, including adrenaline, Flavin nucleotides, thiol compounds, and glucose, can oxidise in the presence of oxygen to produce superoxide, and these reactions are greatly accelerated by the presence of transition metals such as iron or copper. The electron transport chain in the inner mitochondrial membrane performs the reduction of oxygen to water. During this process free radical intermediates are generated, which are generally tightly bound to the components of the transport chain. However, there is a constant leak of a few electrons into the mitochondrial matrix and this result in the formation of superoxide.

The activity of several other enzymes, such as cytochrome p450 oxidase in the liver and enzymes involved in the synthesis of adrenal hormones, also results in the leakage of a few electrons into the surrounding cytoplasm and hence superoxide formation. There might also be continuous production of superoxide by vascular endothelium to neutralise nitric oxide, production of superoxide by other cells to regulate cell growth and differentiations and the production of superoxide by phagocytic cells during the respiratory burst[8].

Without antioxidants, free radicals would cause serious harm very quickly, eventually resulting in death. However, free radicals also serve important functions that are essential for health for example, our immune cells use free radicals to fight infections, as a result our body needs to maintain a certain balance of free radicals and antioxidant. When free radicals outnumber antioxidants, it can lead to a state called oxidative stress which can damage our DNA and other important molecules in our body. Sometimes it even leads to cell death. Prolonged oxidative stress can damage our DNA and other important molecules in our body[9].

SOURCES & ORIGIN OF ANTIOXIDANT

Antioxidants are abundant in fruits and vegetables, as well as in other foods including nuts, grains and some meats, poultry and fish. The list below describes food sources of common antioxidants. Beta-carotene is found in many foods that are orange in colour, including sweet potatoes, carrots, cantaloupe, squash, apricots, pumpkin and mangoes. Some green, leafy vegetables, including collard greens, spinach and kale, are also rich in beta-carotene .Lutein, best known for its association with healthy eyes, is abundant in green leafy vegetables such as collard greens, spinach, and kale. Lycopene is a potent antioxidant found in tomatoes, watermelon, guava, papaya, apricots, pink grapefruit, blood oranges and other foods. Estimates suggest 85% of American dietary intake of lycopene comes from tomatoes and tomato products. Selenium is a mineral, not an antioxidant nutrient. However, it is a component of antioxidant enzymes[10].

Plant foods like rice and wheat are the major dietary sources of selenium in most countries. The amount of selenium in soil, which varies by region, determines the amount of selenium in the foods grown in that soil. Animals that eat grains or plants grown in seleniumrich soil have higher levels of selenium in their muscle. In the United States, meats and bread are common sources of dietary selenium. Brazil nuts also contain large quantities of selenium. Vitamin A is found in three main forms: retinol (Vitamin A1), 3,4-didehydroretinol (Vitamin A2), and 3-hydroxyretinol (Vitamin A3). Foods rich in vitamin A include liver, sweet potatoes, carrots, milk, egg yolks and mozzarella cheese.

Vitamin C is also called ascorbic acid and can be found in high abundance in many fruits and vegetables and is also found in cereals, beef, poultry, and fish. Vitamin E, also known as alpha-tocopherol, is found in almonds in many oils including wheat germ, safflower, corn and soybean oils, and is also found in mangoes, nuts, broccoli, and other foods. Antioxidants are classified into two broad divisions, based upon their solubility in a solvent namely water-soluble antioxidants like ascorbic acid, glutathione, lipoic Acid and uric Acid and lipid soluble antioxidants like carotene and ubiquinol. In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid per oxidation[11].

PLANT AS ANTIOXIDANT

Plants have long been a source of exogenous (i.e., dietary) antioxidants. It is believed that two-thirds of the world's plant species have medicinal importance, and almost all of these have excellent antioxidant potential. The interest in the exogenous plant antioxidants was first evoked by the discovery and subsequent isolation of ascorbic acid from plants. Since then, the antioxidant potential of plants has received a great deal of attention because increased oxidative stress has been identified as a major causative factor in the development and progression of several life-threatening diseases, including neurodeg-enerative and cardiovascular disease. In addition, supplementation with exogenous antioxidants or boosting of endogenous antioxidant defences of the body has been found to be a promising method of countering the undesirable effects of oxidative stress [12].

There are currently approximately 19 in vitro and 10 in vivo methods of assessing antioxidant activity that are commonly applied for evaluation of the antioxidant activity of plant samples . In most of these in vitro assays plant samples showed potent antioxidant activity. This is likely due to their innate ability to synthesize non-enzymatic antioxidants such asascorbic acid and glutathione, as well as secondary metabolites such as phenolic compounds.

Despite many plants being reported to have antioxidant potential by in vitro assays, only a few of these antioxidant activities have been confirmed or investigated in vivo[13]. In vitro assays are generally used to confirm the antioxidant activity of plant samples within particular reaction systems; accordingly, the relevance of the findings of these assays to in vivo systems is uncertain . Moreover, several phytochemicals have been found to possess antioxidant activity within in vitro assays. However, only a few of these have been shown to be therapeutically useful under in vivo conditions due to their interference with physio-pharmacological processes such as absorption, distribution, metabolism, storage and excretion. Nevertheless, phytochemicals are being screened for their in vitro antioxidant activity, and the results of these studies are then directly extrapolated to their therapeutic usefulness.

WHY DO PLANTS NEED ANTIOXIDANT?

During their ontogenesis, plants face a dynamically changing environment defined by abiotic factors (e.g., light/dark, temperature, nutrient and water availability, and toxic compounds such as heavy metals) and biotic interactions (e.g., beneficial and pathogenic microbes, fungi, insects, other herbivores)[14]. Environmental perturbations which significantly disturb metabolism, development and yield, are considered as stress situations and cause stress responses in biological system. Such imposed stress is commonly accompanied by an increase in the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that lead to an imbalance between their production and scavenging. Despite their reactive and thus toxic nature, ROS and RNS are also key components of signal transduction pathways that trigger stress responses. Furthermore, ROS and RNS are involved in plant developmental processes and plant-microbe interactions . However, excessive ROS and RNS production must be counteracted by the antioxidant system to prevent damage development and cell death.

Drought stress severely impacts plant development, growth and fertility. Drought triggers water loss and a decrease in water potential, which concomitantly leads to a reduction in cell turgor . Among the fastest processes induced by drought is the abscisic acid (ABA)-mediated closure of stomata. Prolonged drought stress and increased stress intensity lead to further acclimation reactions. These responses include osmotic adjustment, decreased shoot-root ratio ,cell wall modifications reprogramming of metabolism and activation of the antioxidant system[15]. Many of these modifications are measurable and are used to characterize the severity of drought stress. Measurable traits are, for example, the stomatal and mesophyll conductance, net photosynthesis, photorespiration, abundance of osmoprotectants, tissue water potential, ABA content and membrane integrity. Drought avoidance includes morphological adaptations, like leaf curling and increased wax deposition on the leaf surface.

PLANTS WHICH HAVE LARGE AMOUNT OF ANTIOXIDANT

In recent years great interest has been focused on using natural antioxidants in food products due to studies indicating possible effects. A variety of plant materials are known to be natural sources of antioxidant , such as herbs ,spices ,seed fruit and vegetables[16].

Table 1:The common name, scientific name, family of the plant species and common use of the selected plants[17]

Sl. No	Plant	Family	Binomial Name	Parts Used for Study	Common Uses
1	Betel Leaf	Piperaceae	Piper betle	Leaves	Primarily used as a flavour wrapper for chewing of areca nut or tobacco as a mouth freshener.
2	Turmeric	Zingiberaceae	Curcuma longa	Rhizome	Widely used as a spice in various cuisines. -Used in Indian traditional medicine (also called Siddha or Ayurveda).
3	Laal Sag	Amaranthaceae	Amaranthus gangeticu	Shoots or foliage	Used in various cuisines specially in Bengali cuisine. Or Used as ornamental plants
4	Nisindha	Lamiaceae	Vitex negundo	Leaves	Used as a cough remedy. Also used for treating stored garlic against pests.
5	Brahmi	Plantaginaceae / Scrophulariaceae eae	Bacopa monnieri	Leaves with small steam	Used in traditional Ayurvedic treatment for epilepsy and asthma. -Also used in Ayurveda for ulcers, tumors, ascites, enlarged spleen, inflammations, leprosy, anaemia, gastroenteritis etc.
6	Basaka	Acanthaceae	Justicia adhatoda	Leaves	Has a number of medicinal uses in Siddha Medicine, Ayurvedic and Urani systems of medicine
7	Sajne	Moringaceae	Moringa oleifera	Leaves and soft foliage	The bark, sap, roots, leaves, seeds and flowers are used in traditional medicine. -Its leaf powder is an effective soap for hand washing because of anti-septic and detergent properties from phytochemicals in the leaves. -Moringa seed cakes are used to filter water using flocculation to produce potable water.
8	Green Tea	Theaceae	Camellia sinensis sinensis	Leaves	The leaves are used in traditional Chinese medicine and other medical systems to treat asthma, angina pectoris, peripheral vascular disease, and coronary artery disease. -Also consumed as beverage.
9	Black Tea	Theaceae	Camellia sinensis sinensis,	Leaves	The leaves are used in traditional Chinese medicine and other medical systems to treat asthma, angina pectoris, peripheral vascular disease, and coronary artery disease. -Also consumed as beverage
10	Coffee	Rubiaceae	Coffea arabica	Beans	Mostly consumed as beverage. It is also used to prevent Parkinson's disease.

11	Licorice	Fabaceae	Glycyrrhiza glabra	Bark or steam	Used as a flavouring agent for tobacco. -Used in a wide variety of candies or sweets. -Used by brewers to flavour and colour porter classes of beers
12	Pineapple	Bromeliaceae	Ananas comosus	Fruit	Its flesh and juice are edible around the world.
13	Ashoka tree	Fabaceae	Saraca indica	Leaf, stem , bark or root	Usedasspasmogenic, oxytocic,uterotonic ,antibacterial, anti-implantation, anti-tumour etc.
14	Bay leaf	Lauraceae	Laurus nobilis	Leaf	It is commonly used in cooking. Also used in making medicine for diabetics , stomach problem, cancer etc
15	Wood sorrel	Oxalidaceae	Oxalis acetosella	Leaf	It is a traditional herbal remedy for treating umpteen health problems including, indigestion, gastritis, irritable bowel, constipation and loss of appetite.

MAJOR ANTIOXIDANT PRESENT IN PLANTS

Phenols: Phenols are secondary products of plant metabolism derived from the aromatic amino acid phenylalanine, through the shikimic acid (phenylpropanoids) and the acetic acid (simple phenols) pathways; phenols contribute to the color, flavour and astringency of plants . Phenolic compounds can be classified based on their (i) plant species origin, (ii) chemical structure (the number and arrangement of hydroxyl moieties, double bonds in the carbon rings, and type and degree of alkylation and/or glycosylation) and (iii) solubility in water, which affects their nutritional, metabolic and physiological action; their common feature is a hydroxy-substituted benzene ring within their structure. Phenolic compounds act as hydrogen donors, thus quenching free radicals, or transfer single electrons to reduce chemical compounds with oxidative action.

Apart from their antioxidant capacity, phenolic compounds have an important functional and biological role in plant physiology, acting as structural polymers, UV radiation screeners, pollination and soil bacterium attractants, non-specific defence mechanisms, phytoalexins, and potentially signal compounds for systemic acquired resistance .Flavin monomers, dimers and polymers, and cinnamic acid derivatives form the major fraction of natural phenolic compounds , which, in general, are categorized into (i) phenols and benzoquinones; (ii) phenolic acids; (iii) acetophenones and phenylacetic acids; (iv) hydroxycinnamic acids, phenylpropenes, coumarins-isocoumarins and chromones; (v) naphthoquinones; (vi) xanthones(vii) stilbenes and anthraquinones; (viii) flavonoids; (ix) lignans and neolignans; and (x) lignins[18].

Table2: Classification of phenolic compounds based on their carbon chains[19]

Class	Basic Skeleton	Plant Sources
Phenols and benzoquinones	C6	Primula obconica, sorghum , berries, fruit wines, olive oil
Phenolic acids	C6–C2	blueberries, blackberries, persimmon, apple juice, cider, cherry laurels, canola meal, oranges, rye
Hydroxycinnamic acids, phenylpropenes, coumarins-isocoumarins and chromones	C6–C3	berries, pomes, herbs, seeds, cereal grains, leafy greens, asparagus, cinnamon, cloves and potatoes
Naphthoquinones	C6–C4	oranges, Plumbaginaceae, Droseraceae, Ebenaceae
Xanthones	C6–C2–C6	grapes, pine, peanuts, sorghum, rheum , Rubiaceae , black pepper
Flavonoids (flavones, flavonols, flavanones, flavan-3-ols, isoflavones, anthocyanidin compounds)	C6–C3–C6	celery, parsley, red prickly pears, olives, acerola, litchis, avocadoes, green and black tea, cherries, raspberries
Lignans and neolignans	C6–C3	wheat, oats, rye, barley , berries
Lignin	(C6–C3)n	Arabidopsis thaliana, Pinus radiata , sugar cane , spruce, wattle, birch, rice, eucalyptus, pine

Tannins: Tannins are water-soluble, astringent, polyphenolic substances, produced by the polymerization of phenylpropanoid compounds. Traditionally, tannins were used for protein precipitation due to their ability to interact with at least two protein molecules and form insoluble, cross-linked tannin–protein complexes. Tannins are classified into (i) condensed tannins, which are polymers of catechin, epicatechin, prodelphinidins, profisetinidins and prorobinetidins, and (ii) hydrolysable tannins, which can be hydrolysed by weak acids or bases, with the latter being mixtures of carbohydrates with gallic and ellagic acid. Proanthocyanidins donate hydrogen and electrons (primary antioxidant action), chelate forum and inhibit the activity of cyclooxygenase (secondary antioxidant action). The consumption of small quantities of tannins has a positive effect on lipid metabolism and the regulation of immune responses, reduces blood pressure and presents anticarcinogenic and antimutagenic activity[20].

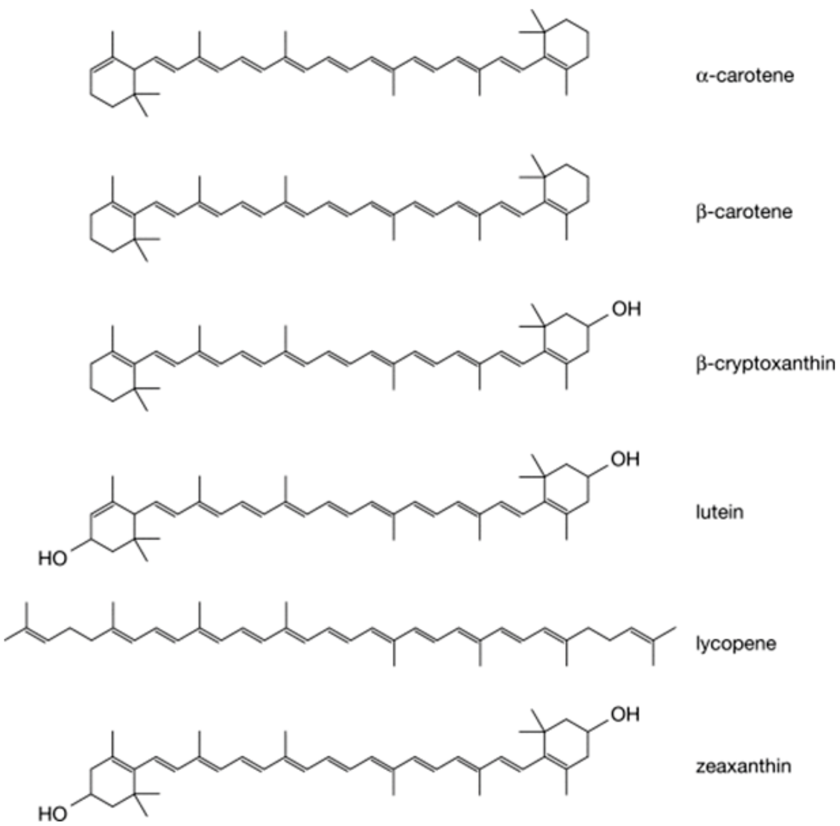
Flavonoids: Flavonoids and isoflavonoids can be found in various plants and derive from the aromatic amino acids phenylalanine and tyrosine, and from malonate. The basic structure of flavonoids is the Flavin nucleus, which consists of three rings of carbon atoms (C6-C3-C6). The level of oxidation and the pattern of carbon atom ring substitution are used to discriminate the classes of flavonoids. Flavonoids have shown significant antioxidant capacity during in vitro experiments and are considered to be associated with a decreased risk of developing cardiovascular diseases, hypertension, Alzheimer's disease and certain types of cancer.

Lignans: Lignans are diphenol compounds belonging to phytoestrogens. They derive from the amino acid phenylalanine, following the dimerization of substituted cinnamic alcohols. Lignans act as hydrogen donors and complex divalent transition metal cations, which explains their antioxidant activity. The main sources of lignans are linseed, pumpkin and sesame seeds, broccoli, soybeans and some types of berries.

Vitamin E: Vitamin E group compounds include tocopherols and tocotrienols. They are phenolic substances synthesized by plants (e.g., cabbage, oregano and paprika, etc.) and are significant components in human and animal diets. Vitamin E group compounds have a chroman head, consisting of a phenolic and a heterocyclic ring, conjugated with a phytol chain. Tocopherols occur in four homologues, α, β, γ and δ, discriminated by the number of methyl substituents of the saturated phytol chain and the substitution patterns of the phenolic ring. Tocotrienols differentiate from tocopherols by the three trans- double bonds in their hydrocarbon tails[] . The antioxidant activity of tocopherols relies on their ability to donate hydrogen to lipid free radicals, and their antioxidant capacity has been demonstrated both *in vitro* and *in vivo*. In both types of studies, the highest antioxidant action was observed for α-tocopherol, followed by the homologues β, γ and δ[21]. The highest antioxidant capacity of α-tocopherol, *in vivo*, is exhibited due to its preferential retention and distribution in animal species .

Carotenoids: Carotenoids are lipophilic, pigmented compounds naturally synthesized by microorganisms and plants (e.g., acerola, cabbage, carrots and paprika) but not animals. Carotenoids share a polyisoprene structure, consisting of a carbon chain of conjugated carbon bonds, and possess a near-bilateral symmetry around the central double bond. Cyclic end-groups are attached at the central chain and can be substituted with functional groups that contain oxygen . Based on the type of substitution, carotenoids are further classified into carotenes (e.g., β-carotene and lycopene, which contain only carbon and hydrogen atoms) and oxycarotenoids, which contain at least one oxygen atom. The most studied carotenoids, are lycopene and β-carotene.

Chemical structures of the six most prevalent carotenoids in the humandiet[22,23]



Vitamin C: Vitamin C or L-ascorbic acid is a six-carbon lactone. It is synthesized from glucose and is considered as a vitamin for only a few vertebrate species (humans, primates and guinea pigs) due to the deficiency of l-gluconolactone oxidase caused by mutations in the enzyme's gene . Vitamin C functions as an electron donor, thus scavenging free radicals and preventing the oxidation of other molecules; it is capable of donating two electrons from a double bond located between C2 and C3 of the six-carbon chain. Vitamin C is well-known for the prevention and treatment of scurvy. Moreover, it has a positive effect on the innate and adaptive immune systems by supporting the cellular functions of neutrophils and epithelial barrier cells and the differentiation and production of B- and T-cells [24].

MECHANISM & ACTION OF MAJOR ANTIOXIDANT

Antioxidants exhibit various courses of action and bio-kinetics, which are determined by their chemical structures and interaction modes, as well as by the physiological factors of animals and physicochemical traits of meat. The courses of action of antioxidants include (i) the inhibition of chain-reaction initiation by scavenging oxidation-initiating radicals, (ii) the breaking of chain reactions that abstract hydrogen for prolonged times, (iii) the decreasing of localized oxygen concentrations, (iv) the decomposition of peroxides and the prevention of their conversion to initiation radicals, and (v) the chelation of chain-reaction initiating catalysts, such as metal ions.

Antioxidants are classified into five categories based on their courses of action. (i) Primary antioxidants terminate the free-radical chain reactions by donating electrons or hydrogen. Primary antioxidants include phenolic compounds, tocopherols and synthetic antioxidants such as alkyl gallates, BHA, BHQ and TBHQ. (ii) Oxygen scavengers react with oxygen and reduce it in closed systems; they include vitamin C, ascorbyl palmitate, erythorbic acid and its sodium salt. (iii) Secondary antioxidants decompose lipid hydroperoxides into stable end-products such as dilauryl thiopropionate and thiodipropionic acid. (iv) Enzymatic antioxidants remove oxygen (e.g., glucose oxidase) or ROS (e.g., superoxide dismutase, catalase etc.). (v) Chelating agents capture metallic ions such as copper and iron that act as catalysts of lipid oxidation. Some common chelating agents are citric acid, ethylenediaminetetraacetic acid (EDTA) and amino acids, which may also exhibit synergistic action with phenolic antioxidants [25].

The complexity of metabolism and the oxidative pathways in animal tissues, the scarcity of wide-scale studies on the effects of antioxidants on human health, the contradictory literature e.g., the case of β-carotene and the variety of antioxidants contained in food systems do not allow. The efficient extrapolation of the results from *in vitro* studies to fit into in situ applications on an evidentiary basis is needed.

BROAD SPECTRUM ANTIOXIDANTS AND THEIR APPLICATION IN DISEASE REMEDIES

Free radicals are responsible for causing pathogenesis of healthy cells to lose their structure and functions to develop various degenerative diseases that is caused due to aging such as cancer, cardiovascular disease, cataracts, immune system decline, brain dysfunction as well as illness, stress and so many more diseases.Antioxidant has become scientifically interesting compounds due to their many benefits such as anti-inflammatory. Today, it is widely used in many areaslikein food technology ,antioxidants are added to many foodstuffs in order to enrich the foods and eliminate the problem. Many results have been found for the elimination of diseases by either *in vivo* or *in vitro* studies regarding antioxidants. Thus, the importance of antioxidantsis increased with the use in pharmacology, cosmetics and medicine .[26]

Antioxidant in prevention of cancer

Evidence from basic research and observational epidemiologic studies suggest that individuals with high intakes of fruits and vegetables experience lower risks of developing cancer. Although there are many compounds in fruits and vegetables that may potentially influence cancer risk, it is generally assumed that certain antioxidants such as vitamin E, Vitamin C and beta-carotene may be responsible for the lower cancer rates. A number of phytochemicals such as genistein, tea polyphenols, the soy isoflavone that are present in edible plants, have anticarcinogenic and antimutagenic effects and can interfere with a particular stage in the development of cancer for lowering the risk of developing some cancers particularly of the digestive and respiratory tracts. The ability of lycopene to scavenge free radicals that can be considered to prevent oxidative damage to DNA, which partly explains its anticarcinogenic activity.4- Omega-3 fatty acids can also be helpful in reducing inflammatory mediators and leukotrienes by producing superoxide anions [27].

Role of antioxidant in diabetes

The antioxidants therapy defends the beta-cell against oxidative stress induced apoptosis and preserves the function of the beta-cell. Data from earlier studies show that antioxidants diminish diabetic-related complication and recover insulin sensitivity. Epidemiological studies revealed a strong association between the dietary antioxidant's intake and protection against diabetes.

Role of antioxidant in prevention of skin aging

Human skin is equipped with an array of antioxidant enzymes to protect the cells from damaging effects of free radicals. Enzymes such as superoxide dismutase (SOD), catalase, and glutathione (GSH) biosynthesizing enzymes protect the tissues from free radicals. In addition, antioxidant molecules such as vitamins A, C, and E slow the process of aging either by preventing free radicals from oxidizing sensitive biological molecules or by reducing the formation of free radicals and quenching the already formed ROS. The levels of these antioxidants, as well as antioxidant enzymes, are reduced by age and various environmental stressors, such as Ultraviolet exposure. Replenishing these antioxidants either by topical application or by dietary ingestion can protect skin from aging. The level of dietary or topical antioxidants achieved in the skin varies with the individual antioxidant and also with absorption and other factors. Different antioxidant systems relevant for skin biology and anti-aging benefits have been described [28].

Role of antioxidant in Covid 19

Coronaviruses are single-stranded RNA viruses, infecting animals and humans, and causing respiratory, gastrointestinal, hepatic, and neurologic diseases. Nowadays, this pathogen has become the Center of global attention, due to the recent spread of a new strain, named SARS-CoV-2 (previously 2019-nCoV), the pathogenic agent of COVID-19 disease, which started to expand from Wuhan, China, on December 2019. Further, there is no specific drug for use against COVID-19 as well as substantial data both at the national or international level on the effects of nutritional supplements on risk or severity of COVID-19. The development of new antivirals for COVID-19 is a great challenge and needs a considerable length of time and effort for designing and validation. Several shreds of evidence indicate that many nutritional supplements from various spices, herbs, fruits, roots, and vegetables can reduce the risk or severity of a wide range of viral infections by boosting the immune response, particularly among people with inadequate dietary sources and also by their anti-inflammatory, free radical scavenging, and viricidal functions. These nutrients can be repurposed in mitigating the pathological effects induced by the SARS-CoV-2 infection.

Therefore, the use of natural compounds may provide alternative prophylactic and therapeutic support along with the therapy for COVID-19. The beneficial effects of some of the nutrients have been described[29].The food-derived antioxidants and metal-chelating agents with treatment and prevention of oxidative stress and inflammation play a key role in the progression of COVID-19.

Zinc

Zinc (Zn) is an essential metal involved in a variety of biological processes due to its function as a cofactor, signalling molecule, and a structural element. It regulates inflammatory activity and has antiviral and antioxidant functions . Studies in the rat model show that deficiency of Zn increases oxidative stress, pro-inflammatory TNF-α and vascular cell adhesion molecule (VCAM)-1 expression and causes lung tissue remodelling which was partially reversed by the Zn supplementation. Studies have shown that oral supplementation of Zn reduces the occurrence of acute respiratory infections by 35%. Zn also shortens the duration of flu-like symptoms by 2 days as well as improves the rate of recovery. The recommended dose from various studies ranges from 20 to 92 mg/week . Zinc is considered as the potential supportive treatment against COVID-19 infection due to its anti-inflammatory, antioxidant as well as direct antiviral effects.

Vitamin C

Vitamin C (VC) can potentially protect against infection due to its essential role on immune health. This vitamin supports the function of various immune cells and enhances their ability to protect against infection. Supplementing with VC has been shown to reduce the duration and severity of upper respiratory infections (most of which are assumed to be due to viral infections), including the common cold . The recommended dose of VC varied from 1 to 3 g/day. The total recommended daily allowance (RDA) for VC is 60 mg.VC is also a potent antioxidant. As an antioxidant, it scavenges ROS, prevents lipid peroxidation, and protein alkylation and thus protects cells from oxidative stress induced cellular damage. Studies have also revealed that administration of VC in combination with quercetin provides synergistic antiviral, antioxidant and immunomodulatory effects. Recently, based on the clinical trial it is proposed that the oral administration of 250–500 mg quercetin, 500 mg VC for high risk and mild symptomatic subjects twice a day for 7 days and up to 3 g VC and 500 mg quercetin twice a day for 7 days in ARDS patients (assisted ventilation/ intubation) improves the overall recovery in SARS-CoV-2 subjects. Therefore, having the food supplement incorporated with sources of VC can help in alleviating and providing immune boosting as well as an anti-inflammatory, antioxidant effect against SARS-CoV-2 infection[30].

CLONAL PROPAGATION OF ANTIOXIDANT PLANT

The berry crops in genus *Vacciniun L*. are the richest sources of antioxidant metabolites which have high potential to reduce the incidence of several degenerative diseases. *In vitro* propagation or micro propagation has been attractive to researchers for its incredible potential for mass production of a selected genotype in a short time, all year round. Propagation techniques affect the antioxidant activity in fruits and leaves. Total antioxidant activity was higher in the fruit of *in vitro* propagated plants compared to the plants grown *in vivo*. This review provides critical information for better understanding the micropropagation and conventional propagation methods, and their effects on antioxidant properties and morphological differentiation in Vaccinium species, and fills an existing gap in the literature.

Micropropagation influenced the synthesis of phenolic and flavonoid compounds, and their antioxidant activities in blueberries and lingonberries. However, tissue culture effects were specific to genotype, tissue or organ, development and maturity stages and seasonal variations. Tissue culture enhances synthesis of phenolics in one cultivar which may not occurred in another cultivar of same species. Blueberry and lingonberry leaves contain substantially higher levels of polyphenolics, flavonoids and proanthocyanidins than those in the fruits. Leaf tissues respond to the tissue culture in diverged way than the fruits of same plants do for their phytochemical contents. The leaves of conventionally propagated blueberry plants contained phytochemicals in higher level and performed greater antioxidant activity than the leaves of micro propagated plants did. Whereas tissue culture fruits have higher level secondary metabolites compared to SC fruits. Maturity stage plays an important role in phenolic synthesis. The berries at early developmental stages contained higher level of phenolics than matured stages. In case of leaf tissue, matured leaves had higher bioactive phytochemicals and antioxidant potential than the green leaves. Green fruits had significantly higher phenolic and flavonoid content and antioxidant activity compared to semi-ripe and fully ripe berries and those were gradually decreased with the progression of ripening. In contrary, anthocyanin content increased with the advancement of fruit maturity. Growing seasons exhibited significant effect on the total phenolic, flavonoid, anthocyanin & Proanthocyanidins contents and antioxidant activity[31].

OMICS APPROACH-BASED INVESTIGATION FOR THE OXIDANTS & EFFECTS OF ANTIOXIDANTS

During the last decade, a number of new techniques havebeen developed and optimised which enable the simultaneous detection of a large number of alterations of biological functions. The use of these approaches in nutritional sciences has led to the formation of a new discipline termed “Nutrigenomics”. The overall aim of this new field of research is tofind out how dietary factors alter gene transcription, protein expression and metabolism in regard to health effects. The area covers three interrelated main areas namely transcription (gene expression analyses), proteomics and metabolomic.

A number of currently used biochemical measurements aimed of determining the total antioxidant capacity and oxidised lipids and proteins are carried out under unphysiological conditions and are prone to artefact formation. Probably the most reliable approaches are measurements of isoprostanes as a parameter of lipid peroxidation and determination of oxidative DNA damage. Also, the design of the experimental models has a strong impact on the reliability of antioxidant studies: the common strategy is the identification of antioxidant by *in vitro* screening with cell lines. This approach is based on the assumption that protection towards ROS is due to scavenging, but recent findings indicate that activation of transcription factors which regulate genes involved in antioxidant defence plays a key role in the mode of action of antioxidant. These processes are not adequately represented in cell lines. Another shortcoming of *in vitro* experiments is that antioxidant is metabolised *in vivo* and that most cell lines are lacking enzymes which catalyse these reactions. Compounds with large molecular configurations (chlorophylls, anthocyans and polyphenolics) are potent antioxidant*in vitro*, but weak or no effects were observed in animal/human studies with realistic doses as they are poorly absorbed. The development of omics approaches will improve the scientific basis for health claims. The evaluation of results from microarray and proteomics studies shows that it is not possible to establish a general signature of alterations of transcription and protein patterns by antioxidant. However, it was shown that alterations of gene expression and protein levels caused by experimentally induced oxidative stress and ROS related diseases can be normalised by dietary antioxidant[32].

CONCLUSION

As far have confirmed the importance of anti-oxidant for scientific studies and hence the search for natural resources has continued. The popularity of anti-oxidants, besides it's therapeutic properties, is because of their usage in foods as enrichments, stabilization, and to bring about pleasant taste and in the technical field as, encapsulation, edible film and packaging, and *in vivo* studies in plants and animals. Thus, the impression of anti-oxidants in the field of food, cosmetics, pharmacology, and medicine is widespread. The positive effects of anti-oxidant on our health, such as reducing oxidative stress, makes it essential that anti-oxidants are taken daily. Current manuscript discussed antioxidant, different type of antioxidant, source and application. Next we discussed micropropagation and omics approaches to produce more antioxidants.

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