

The Plant Natural Products: Their Antioxidants, Free Radical Scavengers, DNA Protection and Antimicrobial Activities

Khalid S. Abdel-lateif^{1,4*}, Ibrahim A. Maghrabi^{2,4} and Hany A. Eldeab^{3,4}

¹Department of Genetics, Faculty of Agriculture, Menoufia University, Egypt.

²Department of Clinical Pharmacy, College of Pharmacy, Taif University, Kingdom of Saudi Arabia.

³Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, Kingdom of Saudi Arabia.

⁴High Altitude Research Center, Taif University, Kingdom of Saudi Arabia.

Abstract

The plant natural products are chemical compounds or substances produced by plants and have different activities or functions. These products exhibited several important biological activities such as antimicrobial, anti-allergic, anti-inflammatory, antioxidant and free radical scavenging due to their rich content of compounds as polyphenols. The highly reactive oxygen species (ROS) generated during the metabolism can cause oxidative damage for biomolecules as RNA and DNA, resulting in cell cellular damage and death. It was shown that the antioxidant activities found in plant natural products could provide protective effects via inhibiting DNA oxidative damage. This review highlights some of important roles of the plant natural products as antioxidants and as antimicrobials.

Keywords: Antioxidants; Antimicrobials; DNA damage; Plant natural products; ROS species

Abbreviations: ROS: Reactive oxygen species; DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid; CAT: Catalase; RA: Rosmarinic acid; UV: Ultraviolet radiation; GSH: Glutathione; AsA: Ascorbic acid; GS: Glutathione synthetase; SOD: Superoxide dismutase; GR: Glutathione reductase; GPX: Glutathione peroxidase; POX: Guaiacol peroxidase; Prxs: Peroxiredoxins; APX: Ascorbate peroxidase; MDHAR: Mono dehydro ascorbate reductase; DHAR: Dehydroascorbate reductase.

Introduction

Natural products in general are chemical compounds or substances produced by living organisms. This may be includes biotic materials (e.g., wood, silk), bio-based materials (e.g., bio plastics, cornstarch) and bodily fluids (e.g., milk, plant exudates). The natural products can be divided into two major classes, the primary and secondary metabolites. The Primary metabolites are substances with essential functions to the survival of the organism that produces them. In contrast, the secondary are not essential to survival but to increase the competitiveness of the organism within its environment [1,2].

Plant kingdom produce many types of secondary metabolites that have important ecological functions for plants, providing protection against attack by herbivores, microbes and serving as attractants for pollinators and seed-dispersing agents. They may also contribute to competition and invasiveness by suppressing the growth of neighbouring plant species [3]. The secondary metabolites extracted from plants are subdivided in three major classes; terpenoids, alkaloids and phenolics and the most common pathways taken for biosynthesis are performed through the pentose for glycosides, polysaccharides; shikimic acid for phenols, tannins, aromatic alkaloids; acetate-malonate for phenols, alkaloids and mevalonic acid for terpenes, steroids and alkaloids [4].

The essential oils (EOs) are one of plant natural products that are produced by the secondary metabolism of herbs and/or spices. These oils are used in human food (as nutraceuticals, flavourings, antioxidants and antimicrobials), in medicines (as pharmaceuticals and therapeutic products) and in manufacturing of cosmetics (perfume/fragrances, aromatherapy, hair and skin care) [5].

The human use of plants as medicines may be traced back at least 60,000 years [6]. It was reported that in the early 1900s, 80% of all medicines were obtained from roots, barks and leaves. Moreover, approximately 60% of anticancer compounds and 75% of drugs for infectious diseases are derivatives from natural products [7]. In Africa, the most of the population uses traditional medicine for primary health care according to World Health Organization report in 2003 and there are more than 20,000 plant species used in traditional medicine [8]. With the development of modern technology, more and more plant extracts have been found to be useful to medical practice [9]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, terpenoids and flavonoids that exhibit several important biological activities as antimicrobial, anti-allergic, anti-inflammatory, antioxidant and free radical scavenging [10-12].

Since synthetic antimicrobial drugs can cause a number of negative side effects, medicinal plants are proved to be effective, cheap and safe source of natural antimicrobial agents [13]. In addition, plants are also rich with flavonoids which are considered excellent source of antioxidants due to their advantages as hydrogen donor, reducing agent, and singlet oxygen scavenger. Furthermore, flavonoids are able to sequester the oxidative damage of unsaturated fatty acids and are used as an ideal preservative alternative to synthetic antioxidants in food industry [14].

This review focus on the antioxidant and the antimicrobial activities of some plant natural products and their roles in DNA protection from oxidative damage occurred by the highly ROS.

***Corresponding author:** Khalid S Abdel-lateif, High Altitude Research Center, Taif University, Kingdom of Saudi Arabia, Tel: 00201004802576; E-mail: k_dein2001@yahoo.com

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The ROS and Oxidative Damage of DNA

Oxidation is essential process for all living organisms for the production of energy needed for their biological activities [15]. The highly ROS are a group of free radicals and reactive molecules derived from O₂ as normal metabolic products including ¹O₂, O₂⁻, H₂O₂ and [•]OH. O₂ itself is a harmless molecule because it has two unpaired electrons with parallel spin which makes it paramagnetic and can participate in reactions with organic molecules [16]. The normal levels of ROS play important roles in physiological mechanisms such as cellular signaling pathways and responses to infectious agents at low and moderate concentrations [14]. On the contrast, the over production of ROS in human body can cause oxidative damage for biomolecules as RNA and DNA, resulting in cell cellular damage and death [17]. Any damage to the DNA can cause changes in the encoded proteins, which may lead to malfunctions or inhibition of proteins production [18]. Furthermore, DNA oxidative damage is considered a major source of the mutation in living organisms with estimated frequency of 10⁴ lesions/cell/day in human cells. ROS can be generated from endogenous sources such as mitochondria and peroxisomes or from exogenous sources including pharmaceuticals, environmental agents, and industrial chemicals [19]. The forms of DNA oxidative damage can include deoxy ribose oxidation, strand breakage, removal of nucleotides, modifications of the nitrogen bases and therefore mismatches between the nucleotides on the two strands of the DNA (Figure 1) [18].

The hydroxyl radical ([•]OH) is one of highly ROS that can be resulted from the radiolysis of water molecules and can reacts efficiently with the active molecules [20-22]. It was shown that the OH react with purine, pyrimidine bases and the deoxyribose backbone and generate various products from the DNA bases which include C-8 hydroxylation of guanine to form 8-oxo-7,8 dehydro-2- deoxyguanosine, hydroxymethyl urea, thymine glycol, thymine and adenine ring-opened [23,24]. If the oxidative DNA damage is not repaired, DNA mutation, replication errors and Cell death can occur.

Previous evidences indicated that living in high altitude area is linked with hypobaric hypoxia with its consequent negative effects via the generation of ROS [25]. Generation of ROS may depress myocardial contraction through interaction with membrane lipids, proteins causing DNA damage and cell death [26]. Moreover, under hypoxic conditions, cellular defense system of enzymatic and non-enzymatic antioxidants are disturbed [27]. The human body can react with these dangerous molecules by producing oxidative enzymes as catalase (CAT) or by using the antioxidant substances in blood as polyphenols to neutralize this oxidative damages [15,28]. It was shown that the antioxidant activity of phenolic compounds found in dietary oils could provide protective effects via inhibiting DNA oxidative damage [29]. Moreover, the mutagenic activity of food mutagens can be modulated by vegetable oils [30,31]. Previous studies showed that increased nutrition based on olive oil is linked with a small decreased risk of cancer.

Plant Products as Antioxidants and Free Radical Scavengers

An antioxidant is any substances that can prevents or reduce the oxidation of the cell components as DNA, proteins and lipids [32]. Plant natural products are being extensively used as antioxidants for their capacity to protect organisms and cells from oxidative damage. For example, although *Mentha spicata* L. (spearmint) is commonly used as a crop for their essential oil for food products and cosmetics;, Spearmint also produces rosmarinic acid (RA), an antioxidant which

important in modulating inflammatory diseases such as asthma, allergies and atherosclerosis [33]. Recently, a potent antioxidant named canolol was isolated from crude canola oil and was found to be more active than other-common antioxidants such as tocopherol [34].

Although, the artificial antioxidants such as butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) are commonly used in food industry as neutralizing agents of free radicals, their uses is linked with toxicity or mutations problems [14]. Hence, it's very important to find normal alternative antioxidants with high safety. Antioxidants originated from natural plant sources are more potent and safe due to their harmless nature [35].

Phenolic compounds

Phenolics are diverse secondary metabolites including flavonoids, tannins and lignin that are ubiquitous in the plant kingdom. Flavonoids are secondary metabolites derived from the phenylpropanoid pathway and more than 9,000 flavonoids have been characterized in plants. Major subgroups of flavonoids that are found in most higher plants include chalcones, flavones, flavonols, anthocyanins, proanthocyanidins and aurones. These compounds play important and diverse functions include UV protection, sexual reproduction process, defense, flower coloring and as signal molecules in the establishment of plant roots endosymbiosis with Fungi and bacteria [36]. Moreover, it was shown that the polyphenols have antioxidant activities because of their strong capacity to donate electrons or hydrogen atoms and can directly scavenge ROS [37,38]. Lukaszewicz et al. [39] reported that the transgenic potato plant with increased concentration of flavonoid exhibited improved antioxidant capacity. Moreover, it was shown that the root bark of *Diospyros abyssinica* is rich with polyphenols and exhibited the greatest radical scavenging activity [40]. In the same side, the extracts of *Pistacia lentiscus* leaves were found to be very rich in phenolic content and exhibited good radical scavenging activity against H₂O₂ [41,42]. Furthermore, The extracts from *Geranium sanguineum* L and *Acacia* species plants were also rich with polyphenol compounds and were found to exhibit a strong antioxidant, anti-mutagenic and radical scavenging capacities [43-45]. The antioxidant activities for each the Moldavian balm (*Dracocephalum moldavica* L), olive oil and *A. chamaepitys* essential oil were found to be as result of its contents of flavonoids and can be used in medicine and pharmacology for treatment of several diseases [46-48].

Essential oils

Since the synthetic antioxidants have possible roles as promoters of carcinogenesis. Hence, the need exists for safe, economical and effective antioxidants to replace synthetic ones. Essential oils (EOs) are volatile, complex compounds characterized by a strong odor and are formed by aromatic plants as secondary metabolites. They have been widely used as antibacterial, antifungal, anticancer, antioxidant and in cosmetic and food industries [49]. Priya et al. [35] studied the chemical composition, antimicrobial and antioxidant activity of the hydro-distilled essential oil of *Murraya koenigii* leaves from the south region of Tamilnadu, India. The major compounds detected in the oil were Linalool (32.83%), Elemol (7.44%), Geranyl acetate (6.18%), Myrcene (6.12%), Allo-Ocimene (5.02), α -Terpinene (4.9%), (E)- β -Ocimene (3.68%) and Neryl acetate (3.45%). It was found that *Murraya koenigii* essential oil inhibited xanthine oxidase activity, which caused a decrease of the generation of superoxide radicals. In addition, *M. koenigii* essential oil was capable of an effective inhibition of both the conjugated diene formation and the generation of secondary products from lipid peroxidation. Goze et al. [50] examined the antioxidant activity of the

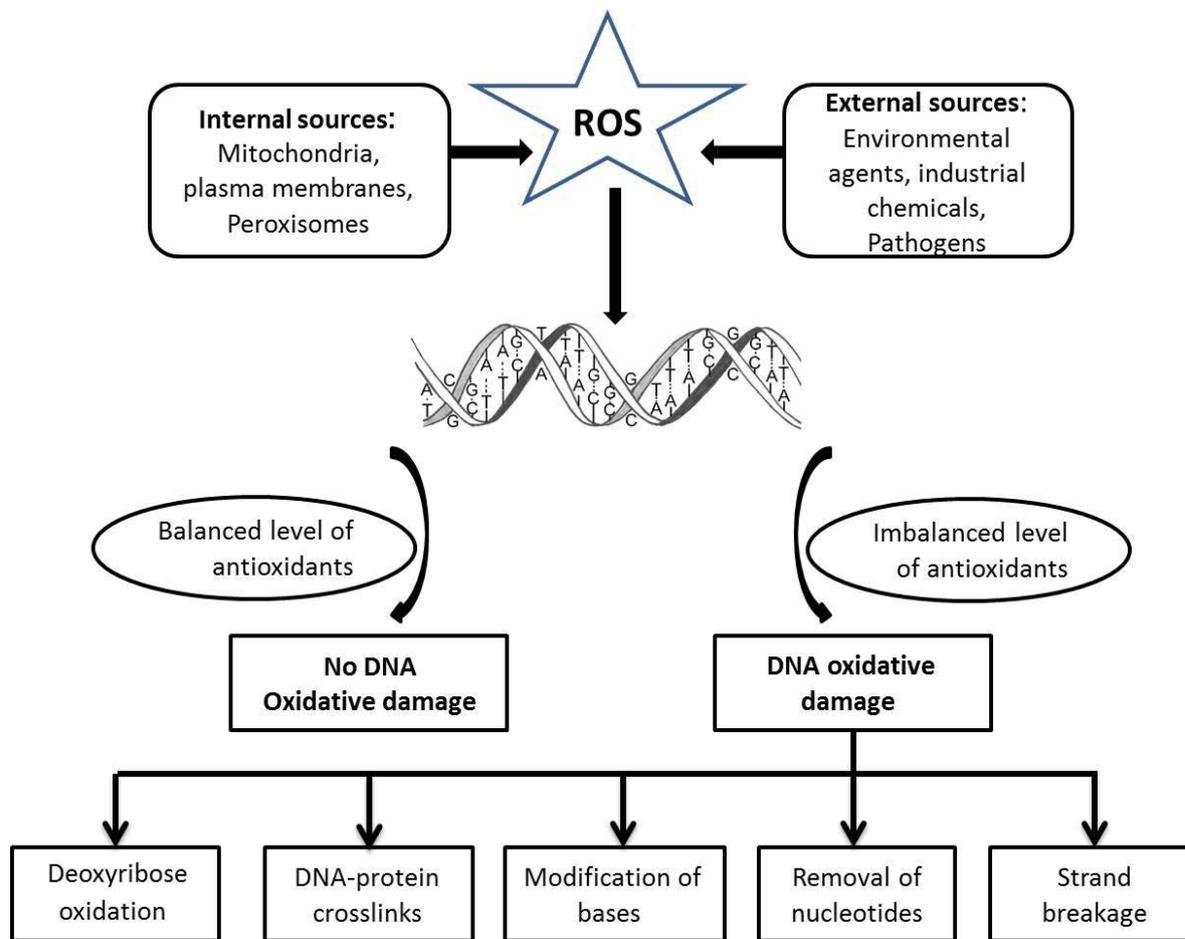


Figure 1: Production of ROS-by the cell components (mitochondria, plasma membrane and peroxisomes) and by external sources (industrial factors and pathogens). The DNA oxidative damage can be occurred when the antioxidants are not enough.

essential oil of various extracts of *Origanum rotundifolium* Boiss. from Turkey. Major components of the oil were borneol, terpinen-4-ol, and spathulenol. A strong correlation was observed between the radical scavenging capacity and polarity of the extracts. The methanol-soluble extract which contains the most polar phytochemicals showed the strongest antioxidant effect in all test systems. Moreover, Sepahvand et al. [51] indicated that essential oil extracted from the aerial parts of *Salvia sclareoides* includes rather higher proportions of non-terpenoid and sesquiterpenes compounds with high antioxidant activities. Furthermore, it was shown that each essential oil and aqueous extract of ginger can be utilized together as a source of natural antioxidants that act more efficiently against lipid oxidation compared to aqueous extract [52].

Alkaloids

Alkaloids are important class of structurally diversified compounds that are having the nitrogen atom in the heterocyclic ring and are derived from the amino acids. These compounds form about 20% of plant based secondary metabolites and have long history in medication through their diverse pharmacological effects [43]. Zulkffle et al. [53,54] identified alkaloid compounds in *Tetrastigma tuberculatum* using the phytochemical screening method and HPLC technique. It was shown that the radical scavenging activity of *T. tuberculatum* alkaloids is comparable to the standard reference, BHT but higher than

the standard alpha tocopherol. In addition, the purified alkaloids from *Amaranthus viridis* leaves have protective effects *in vitro* conditions against hydrogen peroxide induced oxidative damage in human erythrocytes through decreasing Lipid peroxidation levels [55]. On the same side, Novelli et al. [56] identified of 28 alkaloids in leaves of *Ficus benjamina* and 14 alkaloids in its barks. A significant correlation was observed between the total alkaloid content of crude extracts and 2,2-diphenyl-1-picrylhydrazyl assay (DPPH), suggesting that the level of antioxidant activity in this species is strongly correlated to the alkaloid content.

Ascorbate, glutathione and carotenoids

Ascorbic acid (AsA) is an antioxidant that play important role in defense against oxidative stress caused by enhanced level of ROS and in several physiological processes in plants including growth, differentiation, and metabolism. Most of AsA, is localized in cytoplasm, synthesized via uronic acid intermediates such as D-galacturonic acid [57] or contributed by D-mannose/L-galactose pathway [58]. As a protects the biomolecules from oxidative damage because of its ability to donate electrons in a number of enzymatic and nonenzymatic reactions. It can react directly with $O_2^{\cdot-}$, H_2O_2 and regenerating α -tocopherol from tocopheroxyl radical and therefore protection of membranes [59].

Glutathione (GSH) is one of the low molecular weight molecules that play important roles in cell growth/division, regulation of sulfate transport, signal transduction, enzymatic regulation, conjugation of metabolites, synthesis of proteins and nucleic acids [18]. GSH is synthesized in the cytosol and chloroplasts of plant cells by enzymes like glutathione synthetase (GS) and it can be found in chloroplasts, endoplasmic reticulum, vacuoles, and mitochondria [60]. GSH functions as a free radical scavenger whether by reacting directly with free radical molecules or by acting as proton donor for protection of active molecules as DNA [18].

Carotenoids are group of lipophilic antioxidants that found in plants as well as microorganisms and are capable of able to detoxify various forms of ROS by scavenging 1O_2 for inhibiting oxidative damage [61]. Carotenoids impart orange and red colors in vegetables, the yellow-orange color of peppers is formed by α - and β -carotene, zeaxanthin, lutein and β -cryptoxanthin. Beta-carotene is a hydrocarbon carotenoid found widely in the chloroplasts of higher plants, and has powerful antioxidant activities [62]. It was shown that carotenoids, being exceptionally efficient physical and chemical quenchers of 1O_2 and other ROS and are considered protective agents against ROS-mediated disorders. The experiments with β -carotene, lycopene, lutein and zeaxanthin, have been supported the observation that the adequate intake of fruits and vegetables rich with carotenoids may significantly reduce the risk of some chronic diseases [63]. Furthermore, Lycopene is the main carotenoid present in tomatoes and other red fruits including red carrots, red bell peppers, watermelons, gac, and papayas has the highest antioxidant capacity to quench singlet oxygen and trap peroxyl radicals [64].

Enzymes as antioxidants

The enzymes such as superoxide dismutase (SOD), catalase (CAT), mono dehydro ascorbate reductase (MDHAR), glutathione reductase (GR), glutathione peroxidase (GPX), guaiacol peroxidase (POX) peroxidoxins (Prxs), ascorbate peroxidase, (APX), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) are well known enzymes that are present in Plasma and can transform ROS into stable compounds [32,65]. For example, *Catalase* is among the antioxidant enzymes that are very specific to catalyze the dismutation of two molecules of H_2O_2 into water and oxygen. Moreover, Ascorbate peroxidases catalyze the conversion of H_2O_2 into H_2O and use ascorbate as a specific electron donor [65]. Plants contain several types of H_2O_2 -degrading enzymes and these enzymes are unique as they do not require cellular reducing equivalent [18].

Plant Products as Antimicrobials

The random and the increasing uses of commercial antimicrobial drugs in the treatment of infectious diseases developed resistance against several of these drugs. In addition, antibiotics are sometimes associated with adverse side effects on the host correlated with immune suppression and allergic reactions [66].

Recently, the world attention aim to find new effective and safe antimicrobial therapeutic agents. Moreover, an increasing needs in finding natural antimicrobials for application in food products as preservatives to extend their shelf life [67].

Medicinal plants are now becoming more widely used by people all over the world [68]. Antimicrobials of plant origin are effective in the treatment of infectious diseases than the synthetic drugs that are associated with many side effects. The secondary metabolites of plants such polyphenols exhibit several benefits including antimicrobial

properties against pathogenic and spoilage microbes. It was shown that the variations in the chemical composition of these compounds lead to differences in their antimicrobial action [69]. The hydroxyl (OH) groups in phenolic compounds are able to scavenge the free radicals and can disrupt the bacterial cell membranes causing the leakage of cellular components and cell death [70]. The plant by-products or wastes which include fruit pomaces, seeds, peels and pulps that result from food processing are rich in phenolic compounds and have a wide range of antimicrobial activities [71]. The extract of grape pomace was found to inhibit the growth of microbes such as *Enterobacteriaceae*, *S. aureus*, *Salmonella*, *Aeromonas hydrophila*, *B. cereus*, *E. coli*, *Mycobacterium smegmatis*, *Proteus vulgaris* and *Pseudomonas aeruginosa* [72,73]. In addition, the wastes of olive pomace were shown to be good source of phenolic compounds that are responsible for inhibition of the foodborne pathogens *E. coli*, *L. monocytogenes*, *S. enterica*, and *S. aureus* [74,75]. Moreover, pomegranate fruit peels extracts have been widely utilized to inhibit the growth of many foodborne pathogens including *S. aureus*, *E. coli*, *Y. enterocolitica*, and *B. cereus* [76-78]. It was illustrated that Bergamot peel is effective against gram-negative foodborne pathogens (*E. coli*, *S. enterica*) and the gram-positive *Bacillus subtilis* [79]. Furthermore, extracts of mango seed kernels exhibited antimicrobial potential against *E. coli* and able to inhibit the growth of gram-positive food spoilage (*B. subtilis*, *S. aureus*), and gram-negative (*E. coli*) bacteria [80,81]. Olive leaves extracts are rich in the phenolic compounds such caffeic acid, luteolin 7-O-glucoside, rutin, apigenin 7-O-glucoside and luteolin 4'-O-glucoside and have a strong antibacterial and antifungal activities [82,83]. Tea and coffee contains also different phenolic compounds with antimicrobial activity against some gram-positive and negative bacteria [84,85].

Herbal plants and spices like cinnamon, clove, garlic, coriander, lemongrass, sage and vanillin have antimicrobial activity against gram-positive and gram-negative bacteria and are used as preservatives in food industry. The extracts of spinach, pumpkin, suran and ghuiya plants showed antimicrobial activity against different gram-positive and negative bacteria [86].

Essential oils from some plants were found to be important sources of a huge number of antimicrobial, antifungal and anticancer drugs components [87,88]. For example, the plants of *Teucrium* genus belonging to the *Lamiaceae* family that comprises about 300 species are rich in phenolic compounds and are used in food and medicine industries due to their antimicrobial, antioxidant and anti-inflammatory properties [89]. Moreover, the roots of *Jurinea dolomiaea* plants related to family *Compositae* are used as antiseptic and its oil is useful in treatments of some diseases as eye infection, rheumatism gout incense and stomachache [90,91]. Zengin and Baysal studied the antibacterial activity and antioxidant effect of the compounds α -terpineol, linalool, eucalyptol and α -pinene obtained from essential oils (EOs), against pathogenic and spoilage forming bacteria [5]. *In vitro* cell growth experiments showed the tested compounds had toxic effects on all bacterial species with different level of potency. Priya et al. found that the essential oil of *Murraya koenigii* leaves from the south region of Tamilnadu, India has a maximum zone of inhibition ability against *Corynebacterium tuberculosis*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Klebsiella pneumonia* and *Enterobacter aerogenes* [35].

Conclusion

The plant kingdom are rich with natural products such as polyphenols, alkaloids and terpenoids that have several important biological activities as antimicrobials, anti-inflammatory, antioxidants and could provide protective effects via inhibiting DNA oxidative

damage. These natural substances can be found in any part of plant or in the plant by-products which include fruit pomaces, seeds, peels and pulps. The research efforts must be reformed for obtaining new medical drugs of plant origin with high efficiency in the treatment of infectious diseases than the synthetic ones that are associated with many side effects. Moreover, healthy advices must be done toward consumption the plant foods with high component of antioxidants for reducing the highly ROS and therefore protection of our cells from oxidative damages.

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