

A review on potential application of antioxidants

Pradeep Pateriya*, Prem Pal

VNS Group of Institutions, Faculty of Pharmacy, Vidhya Vihar, Neelbud, Bhopal, Madhya Pradesh- 462044 (India)

Abstract

All living organisms utilize oxygen to metabolize and use the dietary nutrients in order to produce energy for survival. Oxygen thus is a vital component for living. Oxygen mediates chemical reactions that metabolize fats, proteins, and carbohydrates to produce energy. While oxygen is one of the most essential components for living, it is also a double edged sword. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called free radicals. These free radicals are capable of attacking the healthy cells of the body. This may lead to damage, disease and severe disorders. Antioxidants are substances that neutralize free radicals or their actions. Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals: superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin, thiols and disulfide bonding are buffering systems in every cell. α -Tocopherol (vitamin E) is an essential nutrient which functions as a chain-breaking antioxidant which prevents the propagation of free radical reactions in all cell membranes in the human body. Ascorbic acid (vitamin C) is also part of the normal protecting mechanism. Other non-enzymatic antioxidants include carotenoids, flavonoids and related polyphenols, α -lipoic acid, glutathione etc. This review article summarizes the functional role of reactive oxygen species (ROS), reactive nitrogen species, reactive halogen species and their pathological importance.

Keywords: Antioxidant, Reactive oxygen species, Carotenoids, Flavonoids, Polyphenols

Introduction

Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Plant materials are cheap and significantly contribute to the improvement of human health in terms of cure and prevention of diseases. Nowadays, antioxidants have gained a lot of importance because of their potential as prophylactic and therapeutic agents in the diseases caused by free radicals. Oxidative stress occurs when the production of damaging free radicals exceeds the capacity of the body's antioxidant defences to detoxify them. Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals. These unpaired electrons are very reactive with adjacent molecules such as lipids, proteins and carbohydrates and can cause cellular damage [1]. The cellular injury caused by oxidative stress has been linked to over 200 clinical disorders [2]. Traditionally, natural medicines with free radical scavenging properties have been used for various purposes. Presently, the active constituents from the natural sources are tested for their free radical scavenging potential. A number of plants included in Ayurveda such as *Emblica officinalis*, *Curcuma longa*, *Momordica charantia*, *Swertia chirata* and *Withania somnifera* have been studied by various researchers for their antioxidant potential. Thus, plants are of enormous importance in the free radical and antioxidant field [3, 4]. They

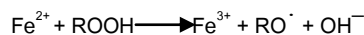
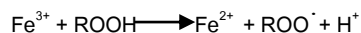
supply us with the essential biradical, O_2 . Plants expose themselves to high levels of O_2 and so are rich in antioxidant defences and repair systems against oxidative damage [5].

The chemistry of antioxidants

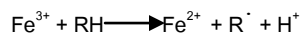
Free radicals contribute to many different diseases. Chemically, a substance is oxidized when electrons are removed and reduced when electrons are added. All chemical reactions involve the transfer of electrons. The body generates energy by gradually oxidizing its food in a controlled manner and storing it in the form of chemical potential energy called ATP (Adenosine triphosphate). Free radicals are generated largely during the production of ATP in the mitochondria. During this process, radicals coming out from the mitochondria from reactive oxygen species such as superoxide anion (O_2^-) and hydroxyl radicals (HO) and other reactive oxygen species such as singlet oxygen (O_2^1), destroy the body system especially the site where the free radicals is been generated. The ultraviolet light that penetrate the skin and the air pollutant that is high in smog which we inhale generates free radicals too.

Food, like lipid in the presence of (Fe^{3+} , Fe^{2+}) lead to the production of hydrogen peroxide from which further hydroxyl radicals are generated in a reaction that appear to depend on the presence of iron ions.

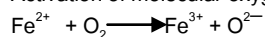
The acceleration of hydroperoxide decomposition to form peroxy radicals and alkoxy radical.



Formations of alkyl free radicals by direct reaction with fats and oils.



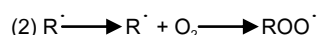
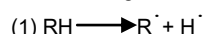
Activation of molecular oxygen for singlet oxygen formation



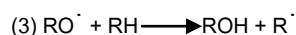
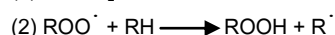
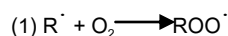
It involves the mechanism of action of antioxidant. Two principle mechanisms of action have been proposed for antioxidants. The first is a chain-breaking mechanism by which the primary antioxidants donate electrons to the free radicals present in the system, example lipid radicals. The second mechanism involves removal of ROS (reactive oxygen species) and RNS (reactive nitrogen species) initiator by quenching chain initiator catalyst.

Chain reactions of free radicals

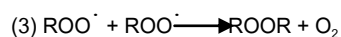
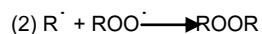
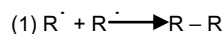
Initiation stage



Propagation stage



Termination stage



(4) Antioxidants + O₂ oxidized antioxidants

Further, in free radical chain reactions, when fats are in contact with oxygen, it forms unsaturated fatty acids which give rise to free radicals in equation (i). Also hydroperoxide which exist in trace quantities prior to oxidation reaction, break down to yield radicals in equation (iv) which abstract an hydrogen atom from another molecule and become a hydroperoxide producing further radicals. The antioxidants added to it, will neutralize the free radicals by donating one of their own electrons ending the reactions in equation (vii) and (ix). These occur generally in the body.

Free radicals oxidize many biological structures, damaging them. This is known as oxidative damage, a major cause of aging, cancer, atherosclerosis, chronic inflammatory processes and cataracts, which are the most characteristic. In certain circumstances, production of free radicals can increase uncontrollably, a situation known as oxidative stress. This means an imbalance between the speeds of production and destruction of toxic molecules, leading to an increase in cellular concentration of free radicals. Cells have mechanisms to protect against the harmful effects of free radicals based on

a complex defense mechanism consisting of the antioxidants. Oxidative stress has been implicated in over one hundred human disease conditions, such as cancer, cardiovascular disease, aging and neurodegenerative diseases. However, the innate defense in the human body may not be enough for severe oxidative stress. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS. As an example, epidemiological evidence indicates that the consumption of grapes reduces the incidence of coronary heart disease (CHD), atherosclerosis and platelet aggregation. This greater protection may be due to the phenolic components of grapes, which are particularly abundant since they behave as reactive oxygen species-scavengers and metal-chelators. Polyphenolic substances in grapes and other red fruits are usually subdivided into two groups: flavonoids and nonflavonoids. The most common flavonoids are flavonols (quercetin, kaempferol, and myricetin), flavan-3-ols (catechin, epicatechin, and tannins), and anthocyanins (cyanin). Nonflavonoids comprise stilbenes, hydroxycinnamic acids and benzoic acids. Numerous papers have been published red fruits and their antioxidant properties have been correlated with their polyphenol contents.

Oxidative stress and disease

It is thought that oxidative stress contributes to the development of a wide range of diseases including Alzheimer's disease, Parkinson's disease, the pathologies caused by diabetes, rheumatoid arthritis, and neurodegeneration in motor neuron diseases. In many cases, it is unclear if oxidants trigger the disease, or occur as a result of this and cause the symptoms of the disease as a plausible alternative, a neurodegenerative disease may result from defective axonal transport of mitochondria that perform oxidation reactions. A case in which it fits is particularly well understood in the role of oxidative stress in cardiovascular disease. Here, the oxidation of low density lipoprotein (LDL) seems to trigger the process of atherogenesis, which leads to atherosclerosis, and ultimately to cardiovascular disease. In diseases that have a high impact on the health sector Diabetes Mellitus is one of the most known. The World Health Organization (WHO) estimates that there are just over 180 million diabetics worldwide and likely to double this number for 2030 is quite high. Countries like China, India, United States of America and Mexico are at the top of this pathology. In India, this condition is a major cause of mortality and morbidity are estimated to be approximately 10 million individuals with diabetes, of whom 22.7% did not know they are sick, while 55% do not have good control netheir condition. This pathology is multifactorial, presenting various metabolic problems (polyuria, polyphagia, polydipsia, weight changes). The disorder is characterized by the inadequate

use of glucose, due to insufficient production, insulin resistance and some without production of the hormone, resulting in unfavorable a high index of this monosaccharide in the blood. This causes abnormal function of some organs, tissues and systems that can cause kidney failure, vision loss, and amputation of a limb, diabetic coma and even death. Different factors increase the likelihood of the individual to develop diabetes as are smoking, sedentary lifestyle, lack of exercise coupled with unbalanced diet causes both overweight and obesity. Naturally the body causes the formation of free radicals (highly unstable molecules), these chemical species are responsible for cellular aging, but when there is a greater concentration of these molecules may contribute to the development of various diseases and chronic degenerative neuro Parkinson's, Alzheimer's and diabetes. Obesity increases oxygen consumption and thus the production of free radicals, thus creating the phenomenon known as oxidative stress. Excess fat naturally stored in fat cells, causes the more than normal synthesis of substances called adiposines IL6 or leptines. These substances in higher concentrations also cause insulin resistance.

Alternative medicine

Due to the current problem in the health issue we propose the use of herbs as an option to improve the style of living of the people, not only for the adjuvant treatment, but because the use of plants offers great nutritional benefits somehow reducing the incidence of such chronic degenerative diseases. This is not intended to impair the option of preventive diagnosis by the health sector does not provide such benefits, but rather the use of plants known to have medicinal activity coupled with the clinical - pharmacology, could present better results, for the treatment of the various degenerative chronic diseases. Given the increasing scientific evidence that the etiology of several chronic degenerative diseases such as diabetes is influenced by factors such as metabolic redox imbalance. Is currently booming studying the formation of metabolites against free radicals that diverse plant species presents. An example of this has been widely documented, is the cranberry, a plant used for treating various diseases and, as has been discovered, is due to its potential antioxidant that has these properties beneficial to health.

Similarly, India has focused attention on other plants with potential antioxidant properties and for some years and was used in the treatment of diabetes. In this regard, since 2014, our research work focused on the task of describing the effects of plants such as Noni (*Morinda citrifolia*), Moringa (*Moringa oleifera*), the Guarumbo (*Cecropia obtusifolia* Bertolt), the Musaro diabetes with streptozotocine. More recently, we began to evaluate the antioxidant properties of some of these plants through *in vitro* techniques.

Antioxidant effects in Indian plants

The use of traditional medicine is widespread in India and plants are indeed the first source for preparing remedies in this form of alternative medicine. Among the various compounds found in plants, antioxidants are of particular importance because they might serve as leads for the development of novel drugs. Several plants used as anti-inflammatory, digestive, antinecrotic, neuroprotective, and hepatoprotective properties have recently been shown to have and antioxidant and/or antiradical scavenging mechanism as part of their activity. The search for natural sources of medicinal products that also have antioxidant and radical scavenging activity is on the rise. Among the medicinal properties associated with them are the following: the fruit and bark of *Licania arborea* is used as a soap for hair infections, the latex from *Ficus obtusifolia* is employed as an anti parasitic and also for reducing fever, *Bunchosia cannesens* is prescribed as an antidiarrhoeic, *Sideroxylon capiri* is used for hiccups, as an antiseptic for cleaning wounds, and women use its leaves in a water bath after giving birth. The latex of *Sapium macrocarpum* is used against scorpion stings, fever and some skin problems such as warts; its use as an anti-coagulant is also widespread. The latex of *Ficus cotinifolia* is used in the treatments of urinary infections, vomiting, malaria and against inflammatory pathologies of the spleen. The leaves of *Annona squamosa* are used in cicatrisation of wounds, diarrhoea, ulcers, menstrual disorders and also to help weight loss. The seeds of this plant are also employed as an insecticide. The leaves of *Vitex molli* are used to treat stomach ache, digestion disorders, nervous alterations and also scorpion stings. *Piper leucophyllum* is employed for reducing fever and its dried leaves are used for cleaning eyes and as spice in cooking. The leaves and bark of *Gliricidia sepium* are used against high fever, skin infections, urine disorders, malaria, and headache. However, its seeds are reported to be toxic. *Hamelia paten* is used to accelerate wound cicatrisation. The Indian and Central India species of *Astianthus viminialis* is used for the curing of diabetes and malaria and to reduce hair loss. *Swietenia humilis* is used as anti parasitic, and it is also utilized for hair care as a shampoo. It is also used with other plants in mixed herbal teas, and used as home remedies. *Stemmandenia bella* is employed for curing wounds; *Rupechtia fusca* is used in some stomach disorders; *Bursera grandifolia* is used as a tooth paste and against digestive disorders; *Ziziphus amole* is prepared as infusion and it is applied for washing wounds and to treat gastric ulcers. The fruit and the latex of *Jacaratia mexicana* are used against ulcers in the mouth and digestive disorders. *Gyrocarpus jathrophifolius* leaves and bark are used as an analgesic. *Pseudobombax ellipticum* is used in respiratory disorders

such as cough, and also against fever and as an anti microbial. The stems and flowers of *Comocladia engleriana* are toxic because they produce dermatitis. The flowers and the latex of *Plumeria rubra* can be used for stopping vaginal blood shed, and toothache, and the latex of the plant is used against earache. Infusions are used as an eye-cleaning liquid. Polyphenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Herbs are used in many domains, including medicine, nutrition, flavouring, beverages, dyeing, repellents, fragrances, cosmetics. Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, antiinflammatory, antimicrobial, hypolipidemic, antimutagenic effects and anticarcinogenic potential. Crude extracts of herbs and spices, and other plant materials rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. Plant species belong to several botanical families, such as Labiatae, Compositae, Umbelliferae, Asteraceae, Polygonaceae and Myrtaceae. Many spices have been investigated for their antioxidant properties for at least 50 years.

Review of literature

Sabu MC *et al.*, 2004 found that methanolic extract of *A. marmelos* reduces oxidative stress induced by alloxan in rats at a dose of 120 mg/kg body weight i.p. Catalase and glutathione peroxidase activity in blood and liver were found to be increased from 9th day onwards after drug administration. Superoxide dismutase and glutathione levels were found to be increased only on 12th day. These results indicated that *A. marmelos* extract effectively reduced the oxidative stress induced by alloxan and produced a reduction in blood sugar level [6].

Golichha M *et al.*, 2010 evaluated the antioxidant activity using hydroalcoholic extract of *E. officinalis* (HAEO) at different doses of 300, 500 and 700 mg/kg for seven days on rats. HAEO administered i.p. to rats was evaluated on Pentylene tetrazole (PTZ) induced seizures, cognitive deficit and oxidative stress markers via malondialdehyde (MDA) and glutathione. The 500 and 700 mg/kg i.p. doses of HAEO completely abolished generalised tonic seizures. HAEO dose dependently also ameliorated the oxidative stress induced by PTZ [7].

Rizvi MM *et al.*, 2009 studied the antioxidant properties of different extracts of *C. fistula*, both *in vitro* and *in vivo*. The *in vitro* 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and deoxyribose damage protection properties were reported using aqueous extract of *C. fistula* root and the results showed 50% effective concentration

(EC₅₀) of 59±2.7 mg/ml and 30% protection against deoxyribose damage at a concentration of 125 mg/ml. The elevated DPPH radical scavenging ability of the stem bark and leaves extract might be due to the presence of high concentration of tannins, proanthocyanidins, flavonols and xanthenes. The DPPH scavenging activities indicated the ability of *C. fistula* extracts to act as radical scavenger and metal quencher thereby, protecting free radical mediated damage [8].

Ramesh *et al.*, 2009 determined free radical scavenging activity by DPPH. The highest antioxidant capacity was exhibited by extracts of lyophilized latex (IC₅₀ = 0.060 mg.ml⁻¹) and the lowest (IC₅₀ = 0.27mg.ml⁻¹) was in root extracts of field grown plants [9].

Desai VR *et al.*, 2002 examined the antioxidant effect of PPI (partially purified immunomodulator) from the plant against reactive oxygen and nitrogen species (ROS/RNS), generated by photosensitization/peroxynitrite. Levels of lipid peroxidation products, superoxide dismutase (SOD) and catalase in liver/spleen homogenate from mouse were monitored. Photosensitization induced significant increase in thiobarbituric acid reactive substances (TBARS) in liver. The activities of SOD and catalase were reduced considerably. PPI, present during photosensitisation, prevented lipid peroxidation and restored the activities of both the enzymes. Likewise, oxidative damage induced by peroxynitrite was inhibited by PPI [10].

Ahmad MF *et al.*, 2008 investigated the antioxidant activity in rats with ethanol-induced erythrocyte damage. Chronic administration of ethanol (20% w/v, 2 g/kg p.o. daily for four weeks) increased the level of lipid peroxidation (LPO), decreased the activity of superoxide dismutase (SOD) and catalase and reduced the content of glutathione (GSH). The concurrent treatment of ethanol-administered rats with *M. azadirach* (500 mg/kg, p.o.) prevented the ethanol-induced changes and the effect was compared with combination of vitamin E and C, thus suggested its antioxidant potential [11]. Kath RH *et al.*, 2006 evaluated the antioxidant activity by estimating plasma malondialdehyde (MDA) in ethanol treated rats and histamine treated guinea pigs and estimating superoxide dismutase (SOD) in pyloric ligated rats and histamine treated guinea pigs. In ethanol treated rats, *O. sanctum* leaf extract (100 mg/kg & 200 mg/kg) significantly decreased the levels of MDA [12].

Nazeema TH *et al.*, 2009 investigated the extent of lipid peroxidation (LPO) and ROS elimination and its defense mechanisms by the enzymic and non enzymic antioxidants in liver and serum. Hepatotoxicity was manifested by significantly decreased (p<0.05) levels in the activities of the enzymic antioxidants such as Superoxide dismutase (SOD) Catalase

(CAT), Glutathione peroxidase and the non enzymic antioxidants such as glutathione and Vitamin C in rats induced hepatic damage by ethanol. The study revealed that the co-administration of *M. pudica* aqueous extract significantly lowered the level of lipid peroxidation in alcohol fed mice [13].

Bakshi *et al.*, 2010 conducted the study to elucidate the effect of extract of *C. longa* (CL) on blood and liver glutathione, Na⁺ K⁺ ATPase activity and thiobarbituric acid and reactive substances (TBARS) against carbon tetrachloride induced hepatic damage in rats had been studied. In addition antioxidant enzymes like superoxide dismutase (SOD) catalase (CAT), glutathione peroxidase (GPX), glutathione transferase (GST) and glutathione reductase (GSH-R) were also studied. It was observed that the ethanolic extract of *C. longa* leaves had reversal effects on the above mentioned parameters in carbon tetrachloride hepatotoxicity. Based on these findings, it was concluded that the liver protective and antioxidant effect of CL possibly involves mechanism related to free radical scavenging effects [14].

Kumaraswamy MV *et al.*, 2008 evaluated antioxidant activities and anti-inflammatory activity of the extracts by a 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH), 2, 2'-Azinobis (3- ethylbenzothiazoline-6-sulfonic acid) (ABTS) and lipoxygenase inhibition assay. The methanol and water extract of *B. utilis* was showed DPPH and ABTS scavenging activity (8.4, 35.08 µg/ml IC₅₀ for DPPH, and 83.18, 37.14 µg/ml IC₅₀ for ABTS assay) but very mild activity against lipoxygenase inhibition activity (18.74 and 28.78% inhibition at 1.0 mg/ml) [15].

Kumaraswamy MV *et al.*, 2008 evaluated the antioxidant activities and anti-inflammatory activity of the extracts by a 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH), 2, 2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and lipoxygenase inhibition assay. In both DPPH and ABTS scavenging activities, methanol and water extract of *W. fruticosa* had showed significant scavenging activity (4.96, 5.08 µg/ml IC₅₀ for DPPH, and 6.4, 7.15 µg /ml IC₅₀ for ABTS assay). In addition to antioxidant activity methanol and water extract of *W. fruticosa* showed lipoxygenase inhibition activity (45.22 and 74.24% inhibition at 1.0 mg/ml). The results obtained in the present study indicated the potential of *W. fruticosa* as anti-inflammatory and antioxidant plant [15].

Rach PR *et al.*, 2009 studied the antioxidant activity in *in vitro* antioxidant models like DPPH radical scavenging activity, superoxide radical scavenging activity, ferric reducing power and hydrogen peroxide scavenging activity. Total antioxidant capacity was also determined. *G. sylvestre* alcoholic leaf extract showed antioxidant activity by inhibiting DPPH,

scavenging superoxide and hydrogen peroxide. It also showed reducing power ability in ferric reducing model. Total antioxidant capacity was found to be 17.54 mg/g expressed as ascorbic acid. Significant antioxidant activity of alcoholic extract of *G. sylvestre* R. Br. was found which might be due to the presence of acidic compounds, flavonoids, phenols, saponins, tannins (phenolic compounds) and triterpenoids [16].

Wauthoz N *et al.*, 2007 investigated the protective antioxidant were investigated *in vivo* in mice using 12-Otetradecanoylphorbol- 13-acetate (TPA), an inductor of oxidative damage in serum, liver and brain and a stimulator of ROS production by peritoneal macrophages, was administered (0.1 µg, i.p.). Mangiferin (50 mg/kg), vitamin C (100 mg/kg), vitamin E (100 mg/kg), vitamin E plus vitamin C (100 mg/kg each) and β-carotene (50 mg/kg) were orally administered once. Results were evaluated using a series of biomarkers like antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx); a marker for protein oxidation, total sulfhydryl group protein content (TSH); markers for lipid peroxidation (LP), malondialdehyde (MDA) and 4-hydroxyalkenals (4-HA); fragmentation of nuclear DNA; and (v) cytochrome c reduction and H₂O₂ levels). Results showed a significant antioxidant activity of extract [17].

Krishnamoorthy P *et al.*, 2006 evaluated the antioxidant activity of *S. cumini* was evaluated on alloxan (150 mg/kg) induced diabetic rats. A 1.0 ml of seed extract showed a remarkable increase in antioxidant enzymes like catalase (CAT), glutathione, glutathione peroxidases, superoxide dismutase. Level of lipid peroxidation decline gradually on administration of extract, indicating the potential of *S. cumini* as an antioxidant [18].

Wu SJ *et al.*, 2008 evaluated the antioxidant and free radical scavenging activities of bitter melon (BM) aqueous (BM-H₂O) and ethanol (BMEtOH) extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH), metal chelation, cytochrome C and xanthine oxidase inhibition (XOI) assays, as well as FeCl₂- ascorbic acid induced lipid peroxidation (thiobarbituric acid reactive substances, TBARS) assay in rat liver homogenates *in vitro*. Results showed that both BMH₂O (IC₅₀ ¼ 129.94 mg/ml) and BMEtOH (IC₅₀ ¼ 156.78 mg/ml) possessed potent DPPH radical scavenging activity. Both BM extracts showed a weak anti-lipid peroxidation activity in plasma [19].

Arise RO *et al.*, 2009 studied the effects of repeated administration of varying concentrations of the aqueous extract (80 mg/kg, 100 mg/kg and 120 mg/kg body weight respectively) of *E. globulus* leaves on some biochemical parameters of rat liver. The activities of acid phosphatase

(ACP), alkaline phosphatase (ALP), superoxide dismutase (SOD) and the level of malondialdehyde (MDA) were determined in the liver and serum. ACP and ALP activities were significantly increased ($P < 0.05$) in the liver with no significant difference ($P > 0.05$) in their serum activities while the activity of SOD was significantly increased ($P < 0.05$) in the liver at concentrations of 100 and 120 mg/kg body weight (b.w) of extract. There existed a significant increase ($P < 0.05$) was found in the level of MDA in the liver of all the treatment groups and at 120 mg/kg b.w of extract in the serum. Over all, the results indicated antioxidant potential of *E. globulus* leaves extract [20].

Kiran H *et al.*, 2009 found that aqueous extract at a dose of 100 and 200 mg/kg orally in type 2 diabetic rats had the enzyme induction effect with respect to catalase (CAT) and glutathione peroxidase (GSH-Px) activity, However decreased the exaggerated activity of super-oxide dismutase (SOD). *F. religiosa* modulated the enzymes of antioxidant defence system to combat oxidative stress [21].

Wang ZS *et al.*, 2009 evaluated DPPH radical scavenging ability, reducing power and Fe^{2+} chelating ability. Higher DPPH radical scavenging (IC_{50} , 2.3–2.4 mg/mL) and Fe^{2+} chelating (IC_{50} , 1.7–1.8 mg/mL) abilities were observed for the hemp protein hydrolysates while the high reducing power was only observed for the hydrolysate. The DPPH radical scavenging and Fe^{2+} chelating abilities were closely correlated with the peptide profiles and its hydrolysates. The peptide profiles of the hydrolysates with higher hydrophobic amino acids exhibited higher DPPH radical scavenging and Fe^{2+} chelating abilities [22].

Kaleem M *et al.*, 2006 found that oral administration of ethanolic extract of *N. sativa* seeds (300 mg/kg body weight/day) to streptozotocin induced diabetic rats significantly reduced levels of blood glucose, lipids, plasma insulin, and improved altered levels of lipid peroxidation products (TBARS and hydroperoxide) and antioxidant enzymes like superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase in liver and kidney [23].

Stoilova *et al.*, 2006 studied the antioxidant effect and the total phenols of ginger extract. 2, 2-Diphenyl-1-picryl hydrazyl radical (DPPH) scavenging reached 90.1% and exceeded that of butylated hydroxytoluene (BHT), the IC_{50} concentration for inhibition of DPPH was 0.64 μ g/ml [24].

Miladi S *et al.*, 2008 assayed antioxidant activity through some *in vitro* models such as the antioxidant capacity by phosphomolybdenum method, β -carotene bleaching method, radical scavenging activity using 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay and reducing power assay. The highest scavenging activity and the greatest reducing power

followed by ethyl acetate, butanol and hexane extracts. However, the hexane fraction showed the highest antioxidant capacity (471.300 ± 0.013) and the highest antioxidant activity coefficient (AAC) by the β - carotene bleaching method [25].

Mahesh R *et al.*, 2007 evaluated antioxidant activity of aqueous extract against age-related oxidative stress in heart tissues of young and aged rats. Young and aged rats were treated with *T. chebula* aqueous extract at a dose of 200 mg/kg body weight in 1.5 ml sterile water orally for 4 weeks. Control young and aged rats were received sterile water only. In aged rats, the increased content of malondialdehyde (MDA) was observed. The antioxidants, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) activities, reduced glutathione (GSH), vitamin C and E levels were decreased in heart tissues of aged control rats. Administration of *T. chebula* to aged rats prevented the depletion of SOD, CAT, GPx activities and GSH, vitamin C and E contents. Also, the levels of MDA content were decreased in heart tissues [26].

Kiran B *et al.*, 2010 evaluated the antioxidant properties of seeds extract *in vitro* employing different standard assays. All the extracts tested were effective in quenching superoxide anion. Maximum superoxide scavenging activity was observed in the alcohol and water (1:1) extract (AWEP) at 200 μ g/ml. Lipid peroxidation was assessed by production of thiobarbituric acid reactive substances (TBARS) in RBC membrane and highest antioxidant activity (71.0%) was observed in the alcohol water extract at 50 μ g/ml. Maximum hydroxyl radical scavenging activity of 87.0 percent was observed at 20 μ g/ml. Similarly maximum 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of 89.0 percent was observed at 25 μ g/ml in alcohol water extract when compared with standard %-tocopherol and BHA. The results suggested strong antioxidant potential of alcohol and water (1:1) extract of seeds of *P. corylifolia* [27].

Ghosh T *et al.*, 2008 found that the extract (300 mg/kg, 600 μ g/kg) prevented significant elevation of glycosylated hemoglobin *in vitro*, with IC_{50} value being 11.25 μ g/ml that is comparable with the reference drug α -tocopherol. Administration of the extract and glibenclamide significantly decreased the levels of TBARS, increased the content of GSH and increased the activity of SOD and CAT in liver of diabetic rats. The extract increased peripheral glucose utilisation in the diaphragm of diabetic rats *in vitro*, which is comparable with the action of insulin. Thus, the extract might have insulin like activity and the antihyperglycemic effect of the extract might be due to an increase in peripheral glucose consumption as well as protection against oxidative damage in alloxanised diabetes [28].

Me DY *et al.*, 2007 evaluated the antioxidant activities of the extracts by a 2, 2'-diphenylpicrylhydrazyl (DPPH) assay and a β -carotene bleaching assay, and compared with that of butylated hydroxyanisole (BHA) and ascorbic acid. Methanol showed the highest extract yield among all of solvents. Extract of either methanol or acetone demonstrated the highest DPPH scavenging activity at both 66.7 mg/L and 133.3 mg/L. All extracts exhibited higher antioxidant activity coefficient (AAC) than that of ascorbic acid, furthermore, dichloromethane and petroleum extracts had comparable AAC with BHA by the β -carotene bleaching assay. The properties of the extracting solvents significantly affected the yield, total phenolics content and antioxidant activity of *N. nucifera* rhizome extracts [29].

Kaviarasan S *et al.*, 2007 evaluated antioxidant activity using various *in vitro* assay systems. The seed extract exhibited scavenging of hydroxyl radicals (OH) and inhibition of hydrogen peroxide-induced lipid peroxidation in rat liver mitochondria. The OH scavenging activity of the extract was evaluated by pulse radiolysis and the deoxyribose system. The extract at high concentrations acted as a scavenger of 2, 2-diphenyl-1-picryl hydrazyl hydrate (DPPH) and 2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS-) radicals. The results indicated antioxidant potential of fenugreek extract [30].

Devi PR *et al.*, 2007 studied the effect of the oral administration of leaf extract on the levels of enzymic and nonenzymic antioxidants in the adjuvant induced arthritic (AIA) rats. The levels of antioxidant enzymes such as SOD, CAT, GPx, G6PD, GSH and Vit-C were estimated in various groups of the experimental rats. It was observed that the antioxidant enzyme levels in the AIA were significantly low when compared to normal rats. A significant decrease in enzymic antioxidant SOD, CAT, GPx, G6PD and non-enzymic antioxidant GSH, Vit-C were observed in the liver of AIA rats compared to the normal rats. These results suggested that the leaf extract of *Vitex negundo* possesses antioxidant activity [31].

Hosein HKM *et al.*, 2007 evaluated henna leaves extract as a natural antioxidant during 16 d storage of refined soybean oil at 63 °C. Peroxide values (PV) and 2-thiobarbituric acid values were used as criteria to assess the antioxidant activity of henna leaves extract. Water extract in comparison with the methanolic extract had been more efficient. BHA and BHT at 200ppm and methanolic extract at 800ppm and 1400ppm had equal TBA and PV value in soybean oil. Also the antioxidant activity of water and methanolic extracts was determined by using the rancimat method (90,120,150 °C) on refined

soybean oil and compared with the induction period of synthetic antioxidants (BHA, BHT, TBHQ)[32].

Zen T 2009 evaluated extracts for total antioxidant activity by ferric thiocyanate method, total reducing power by potassium ferricyanide reduction method, 1, 1-diphenyl-2-picrylhydrazyl (DPPH.) radical scavenging activities, superoxide anion radical scavenging activities *in vitro* and lipid peroxidation *in vivo*. Those various antioxidant activities were compared to standards such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and α -tocopherol. The ethanolic extract was found as the most active in total antioxidant activity, reducing power, DPPH radicals and superoxide anion radicals scavenging activities [33].

Nikkah E *et al.*, 2009 evaluated antioxidant activity of Berry (*Morus alba* var. *nigra*). For superoxide anion radical assay, the superoxide anion radicals were generated by a pyrogallol auto oxidation system, nitric oxide radical inhibition by the use of Griess Illosvoy reaction and reducing power was determined according to the Oyaizu method [34].

Jainu M *et al.*, 2004 investigated the methanolic extract of *S. nigrum* berries (SBE) on aspirin induced ulceration in rats with respect to antioxidant status in the gastric mucosa. Oxygen free radicals are considered to be important factors in the pathogenesis of gastric ulcer. The level of lipid peroxides, which was elevated highly in rats with acute gastric mucosal injury, was taken as an index of oxidative stress. The activities of antioxidant defence enzymes were also decreased considerably by oral gastric administration of aspirin. The decreased levels of antioxidant enzymes and increased mucosal injury were altered to near normal status upon pre-treatment with (SBE) when compared to the ulcer induced rats. The results indicated that (SBE) exerted its gastro protective effect by a free radical scavenging action [35].

Choi UK *et al.*, 2010 investigated antioxidant activity that possible hypolipidemic and antioxidative effects of *Taraxacum* root and leaf in rabbits fed with a high-cholesterol diet. A group of twenty eight male rabbits was divided into four subgroups; a normal diet group, a high-cholesterol diet group, a high-cholesterol diet with 1% (w/w) *Taraxacum* leaf group and a high-cholesterol diet with 1% (w/w) *Taraxacum* root group. After the treatment period, the plasma antioxidant enzymes and lipid profiles were determined. *Taraxacum* root and leaf could protected against oxidative stress linked atherosclerosis and decrease the atherogenic index [36].

Ziying T *et al.*, 2007 tested the extract fraction with the scavenging activities on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl free radicals. The results showed that ethyl acetate fraction of ethanol-extract of tea flower (EEA)

exhibited the highest quenching activity to hydroxyl radicals (SC_{50} 11.6 $\mu\text{g/ml}$), followed by ethanol-extract (EE) of tea flower (SC_{50} 19.7 $\mu\text{g/ml}$). Same tea flower extract showed big different scavenging activities on different free radicals [37].

Reddy KY *et al.*, 2010 found methanolic extracts of *O. corniculata* (MEOC) had been proven experimentally to possessed antioxidant activity in *in vitro* methods. MEOC showed effective response on 2, 2-Diphenyl-1-picryl-hydrazyl radical (DPPH) method for determining the free radical scavenging activity. The concentration of plant extract required for 50% inhibition of DPPH radical scavenging effect (IC_{50}) were recorded as 30 mg/ml and 37 mg/ml for MEOC and standard ascorbic acid. This result suggested that the MEOC possess antioxidant activity compared to ascorbic acid [38].

Dhalwal K *et al.*, 2009 studied the comparative antioxidant potential of ethanolic extracts of *S. cordifolia* leaf, stem, root, and whole plant was studied. Anti-lipid peroxidation, free radical scavenging, reducing power, nitric oxide scavenging, superoxide scavenging antioxidant assay. Various antioxidant activities were compared with standard antioxidants such as BHA, tocopherol, and ascorbic acid. Ethanol extracts were found to be a good scavenger of DPPH radical in the order roots > stem > leaves > whole plant with values 76.62%, 63.87%, 58% and 29% at a dose of 1 mg, respectively. The highest antioxidant activity was observed in the root extract [39].

Nehete JY *et al.*, 2009 evaluated the antioxidant potential of the methanolic extract of the leaves and roots of *A. aspera* Linn. was evaluated by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay. The extract showed antioxidant activity in dose dependent manner. In DPPH scavenging assay the IC_{50} value of the leaves and root extracts were found to be 241.86 $\mu\text{g/ml}$ and 129.91 $\mu\text{g/ml}$ respectively, the IC_{50} value of the reference standard ascorbic acid was 7.81 $\mu\text{g/ml}$. This study revealed that methanolic extract of root possesses potent anti-oxidant activity than methanolic extract of leaves [40].

Singh R *et al.*, 2010 investigated that methanolic extract of seeds and leaves of *C. dichotoma* using *in vitro* models viz. DPPH and hydrogen peroxide model show free radical scavenging potential. These models demonstrated positive antioxidant activity in a concentration dependent manner and demonstrated that highest concentration exhibits highest (100 $\mu\text{g/ml}$) antioxidant activity. This activity was more pronounced in leaves as compared to seeds [41].

Joharapurkar AA *et al.*, 2003 studied *in vivo* antioxidant activity of alcoholic extract of the roots of *R. cordifolia* Linn. (RC) on ethanol-induced impairment of immune responses.

The ethanol-treated (2 g/kg, 20% w/v, p.o., daily for four weeks) rats concurrently received either RC or a combination of vitamin E and C (each 100 mg/kg, p.o.) daily for the same period. The parameters like phagocytosis, total leukocyte count (TLC), humoral and cell-mediated immune responses, lipid peroxidation (LPO), reduced glutathione (GSH) content, superoxide dismutase (SOD) and catalase (CAT) activities were assessed. It was concluded that the ethanol induced immunosuppression is due to oxidative stress and *R. cordifolia* can prevent the same by virtue of its *in vivo* antioxidant property [42].

Ogunlana OE *et al.*, 2008 studied *in vitro* assessment of the ability of the extract to scavenge the Reactive Oxygen Species (ROS), hydrogen peroxide, superoxide and the synthetic radical 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was determined with reference to the synthetic antioxidant Butylated hydroxyanisole (BHA). Plant extract showed concentration dependent scavenging activity on all reactive species used. Scavenging activity of plant extract on hydrogen peroxide and superoxide was more than that of BHA on same. However, BHA showed greater DPPH scavenging activity than plant extract [43].

Sun T *et al.*, 2009 determined antioxidant capacities of the hydrophilic and hydrophobic fractions by using the 2, 20-azinobis (3 ethylbenzothiazoline-6- sulfonic acid) (ABTS) and 2, 20-diphenyl-1-picrylhydrazyl (DPPH) methods. The relative antioxidant capacity index was determined. Anthocyanins were the major antioxidants in purple-yellow and purple-orange carrots and chlorogenic acid was a major antioxidant in all carrots. Both the DPPH and ABTS assays showed that the hydrophilic extract had higher antioxidant capacity than the hydrophobic extract [44].

Gupta RC *et al.*, 2008 evaluated antioxidant activity was evaluated *in vitro* by using diphenyl-picryl-hydrazyl (DPPH) assay. The scavenging effect of plant extracts and standard (L-ascorbic acid) on the DPPH radical decreases in the following order: L-ascorbic acid > ethyl acetate (I) > methanol (II) > water (III) and it was found to be 97.4%, 61.4%, 40.8% and 29.2% at concentration of 100 mg/mL, respectively. Ascorbic acid which was used as a standard showed an IC_{50} of 24.0 mg/mL, whereas, the crude ethyl acetate (I), methanolic (II) and water (III) extracts of leaves of *O. indicum* showed antioxidant activity with IC_{50} values of 49.0, 55.0 and 42.5 respectively at 100 mg/mL concentration [45].

Resmi CR *et al.*, 2006 determined the oxidative stress in alloxan-induced diabetic rats was determined by estimating the levels of thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD) and reduced glutathione (GSH) in liver and kidneys. Activities of antioxidant enzymes, such as

superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione S transferase (GST) were assessed in diabetic as well as rats co-administered with aqueous extract of *A. lebbbeck* (ALL) at dose of 75 mg/kg. Oxidative damage in the liver and kidneys of diabetic rats as evidenced by a marked increment in the levels of TBARS and CD [46].

Conclusion

Life expectancy has nearly doubled during the 20th century. This dramatic increase in the aging population presents with an enormous challenge to maintain a healthy old age. A comprehensive solution to address this important problem is compounded by a multitude of factors that contribute to the accumulation of damage to macromolecules, cells, tissues and organs during aging. The rejuvenation strategies, currently being employed with a targeted approach, to reverse or repair the damage associated with aging have only yielded partial benefits. The complexity of the aging process highlights the need for a holistic approach, as recommended by the alternative systems of medicine, to prevent the progressive deterioration of the aging cells. Herbal antioxidants have been successfully employed as rejuvenators, for several centuries in the Indian systems of alternative medicine.

Emerging studies on herbs employed as rejuvenators, for centuries in Indian system of alternative medicine, validates their clinical application by highlighting the mechanistic principle underlying the biological effects of these herbs. The abundance of phytochemicals with antioxidant properties, such as phenolics, flavonoids and carotenoids, may be held responsible for the rejuvenating activity of these medicinal herbs. The molecular targets of major phytochemicals identified in each of the herbal antioxidants suggest that NF-KB signaling pathway plays an important role in regulating the biological activity of the herbal rejuvenators. In addition, it is also evident that several other signaling pathways, which differ for each of these herbs, also mediate their biological effects. Thus, the medicinal herbs employed to promote longevity in Indian systems of alternative medicine have multiple pharmacological effects. However, the antioxidant property of these herbs seems to have a major role in determining their rejuvenating potential.

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