FREE RADICALS, ANTIOXIDANTS, DISEASES AND PHYTOMEDICINES: CURRENT STATUS AND FUTURE PROSPECT

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ABSTRACT

Free radicals are well documented for playing a dual role in our body as both deleterious and beneficial species. In low/moderate concentrations free radicals are involved in normal physiological functions but excess production of free radicals or decrease in antioxidant level leads to oxidative stress. It is a harmful process that can be mediates damage to cell structures, including lipids, proteins, RNA and DNA which leads to number of diseases. A variety of synthetic medicine employed in the treatment of different diseases also capable to generate free radicals in body which may causes another disease. The plant sources are rich of antioxidants, phyto-constituents are capable to terminate free radical reactions and prevent our body from oxidative damage. Vegetables and fruits are also important sources of antioxidant substances. Different phytoconstituents and herbal product which are safer then synthetic medicine and beneficial in the treatment of diseases caused by free radicals, it also protect the body by prevent the free radicals to cause tissue injury. Phytoconstituents are conferring less side effect and compatible to body physiology. Therefore it is demand of modern era to use such phytoconstituents or phytomedicines.

Keywords: Free radicals, Antioxidants, Oxidative stress, Diseases, Phytoconstituents.

INTRODUCTION

Oxygen is an element obligatory for life, living systems have evolved to survive in the presence of molecular oxygen and for most biological systems. Oxidative properties of oxygen play a vital role in diverse biological phenomena. Oxygen has double-edged properties, being essential for life; it can also aggravate the damage within the cell by oxidative events[1].

Free radicals and its adverse effects were discovered in the last decade. These are dangerous substances produced in the body along with toxins and wastes which are formed during the normal metabolic process of the body. The body obtained energy by the oxidation of carbohydrates, fats and proteins through both aerobic and anaerobic process leads the generation of free radicals. Overproduction of the free radicals can responsible for tissue injury. Cell membranes are made of unsaturated lipids and these unsaturated lipid molecules of cell membranes are particularly susceptible to free radicals. Oxidative damage can direct to a breakdown or even hardening of lipids, which composition of all cell walls. Breakdown or hardening is due to lipid peroxidation leads to death of cell or it becomes unfeasible for the cell to properly get its nutrients or get signals to achieve another. In addition, other biological molecules including RNA, DNA and protein enzymes are also susceptible to oxidative damage. Environmental agents also initiate free radical generation leads different complication in body. The toxicity of lead, pesticides, cadmium, ionizing radiation, alcohol, cigarette smoke, UV light and pollution may all be due to their free radical initiating capability[2-4].

Anti-oxidants are substances capable to mop up free radicals and prevent them from causing cell damage. Free radicals are responsible for causing a wide number of health problems which include cancer, aging, heart diseases and gastric problems etc. Antioxidants cause protective effect by neutralizing free radicals, which are toxic byproducts of natural cell metabolism. The human body naturally produces antioxidants but the process is not 100 percent effective in case of overwhelming production of free radicals and that effectiveness also declines with age[5,6].

Increasing the antioxidant intake can prevent diseases and lower the health problems. Research is increasingly showing that antioxidant rich foods, herbs reap health benefits. Foods may possibly enhance antioxidant levels because foods contain a lot of antioxidant substances. Fruits and vegetables are loaded with key antioxidants such as vitamin A, C, E, betacarotene and important minerals, including selenium and zinc. Fruits, vegetables and medicinal herbs are the richest sources of antioxidant compounds[7]. Herbs are staging a comeback and herbal 'renaissance' is happening all over the world. The herbal products today symbolize safety also compatible with human normal physiology. Natural products, mainly obtained from dietary sources provide a large number of antioxidants. Phytoconstituents are also important source of antioxidant and capable to terminate the free radical chain reactions[8,9].

FREE RADICALS, REACTIVE OXYGEN AND NITROGEN SPECIES

A free radical may defined as a molecule or molecular fragments containing one or more unpaired electrons in its outermost atomic or molecular orbital and are capable of independent existence[10]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are describes free radicals and other non-radical reactive derivatives. The reactivity of radicals is generally stronger than non-radical species though radicals are less stable[11]. Free radicals are formed from molecules by the homolytic cleavage of a chemical bond and via redox reactions, once formed these highly reactive radicals can start a chain reaction[12,13].

ROS and RNS includes radicals such as superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^\bullet$), peroxyl ($RO_2^\bullet$), hydroperoxyl ($HO_2^\bullet$), alkoxyl ($RO^\bullet$), peroxyl ($ROO^\bullet$), nitric oxide ($NO^\bullet$), nitrogen dioxide ($NO_2^\bullet$) and lipid peroxyl ($LOO^\bullet$); and non radicals like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1\Delta g$), peroxynitrate ($ONOO^-$), nitrous acid (HNO_2), dinitrogen trioxide (N_2O_3), lipid peroxide (LOOH)[11]. Non radicals are also termed as oxidants and capable to lead free radical reactions in living organisms easily. Radicals are derived from oxygen characterize as the most important class of radical species generated in living systems[13,14].

At high concentrations, ROS can be important mediators of damage to cell structures, nucleic acids, lipids and proteins[15]. $O_2^{\bullet-}$ radical is responsible for lipid peroxidation and also have the capability to decrease the activity of other antioxidant defense system enzyme such as catalase (CAT) and glutathione peroxide (GPx), it causes damage to the ribonucleotide which is required for DNA synthesis. The protonated form of $O_2^{\bullet-}$ is $HO_2^\bullet$, which is more reactive and able to cross the membrane and causes damage to tissue. $OH^\bullet$ radical is most reactive chemical species. It is a potent cytotoxic agent and able to attack and damage almost every molecule found in living tissue. H_2O_2 is not a radical but it produces toxicity to cell by causing DNA damage, membrane disruption and release calcium ions within cell, resulting in calcium dependent proteolytic enzyme to be activated. HOCl is produced by the enzyme myeloperoxidase in activated neutrophils and initiates the deactivation of antiproteases and activation of latent proteases leading to tissue damage[10]. It has ability to damage biomolecules, directly and also decomposes to liberate toxic chlorine. Metal induced generation of ROS attack DNA and other cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation[16]. Peroxyl radicals causes damage after rearranged via a cyclisation reaction to endoperoxides. Studies show that free radicals produce oxidation of the side chains of all amino acid residues of proteins, particularly cysteine and methionine[15,17].

Free radical reactions

Free radicals generally involved in chain reactions, a series of reactions leads to regenerates a radical that can begin a new cycle of reactions. Free radical reactions take three distinct identifiable steps[18].

- Initiation step: formation of radicals.
- Propagation step: in this step required free radical is regenerated repeatedly as a result of chain reaction, which would take the reaction to completion.
- Termination step: destruction of radicals

Generation and sources of free radicals

Free radicals can be formed from both endogenous and exogenous substances. They are continuously forming in cell and environment. Different sources of free radicals are as follows[13,19-22]**:**

- UV radiations, X-rays, gamma rays and microwave radiation.
- Metal-catalyzed reactions.
- Oxygen free radicals in the atmosphere considered as pollutants.
- Inflammation initiates neutrophils and macrophages to produce ROS and RNS.
- Neutrophils stimulated by exposure to microbes.
- In mitochondria-catalyzed electron transport reactions, oxygen free radicals produced as by product.
- ROS formed from several sources like mitochondrial cytochrome oxidase, xanthine oxidases, neutrophils and by lipid peroxidation.
- ROS generated by the metabolism of arachidonic acid, platelets, macrophages and smooth muscle cells.
- Interaction with chemicals, automobile exhausts fumes, smoking of cigarettes, cigars, beedie.
- Burning of organic matter during cooking, forest fires, volcanic activities.
- Industrial effluents, excess chemicals, alcoholic intake, certain drugs, asbestos, certain pesticides and herbicides, some metal ions, fungal toxins and xenobiotics.

ANTIOXIDANTS

Antioxidants are any substance that delay or inhibits oxidative damage to a target molecule. At a time one antioxidant molecule can react with single free radicals and are capable to neutralize free radicals by donating one of their own electrons, ending the carbon-stealing reaction. Antioxidants prevent cell and tissue damage as they act as scavenger. Cell produce defense against excessive free radicals by their preventative mechanisms, repair mechanisms, physical defenses and antioxidant defenses[23].

A variety of components act against free radicals to neutralize them from both endogenous and exogenous in origin[23]. These include:

- Endogenous enzymatic antioxidants.
- Non enzymatic, metabolic and nutrient antioxidants.
- Metal binding proteins like ferritin, lactoferrin, albumin and ceruloplasmin.
- Phytoconstituents and phytonutrients.

The body produces different antioxidants (endogenous antioxidants) to neutralize free radicals and protect the body from different disease leads by the tissue injury. Exogenous antioxidants are externally supply to the body through food also plays important role to protect the body. The body has developed several endogenous antioxidant defense systems classified into two groups such as enzymatic and non enzymatic. The enzymatic defense system includes different endogenous enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and non enzymatic defense system included vitamin E, vitamin C and reduced glutathione (GSH)[23,24].

SOD is an important endogenous antioxidant enzyme act as the first line defense system against ROS which scavenges superoxide radicals to H_2O_2. GPx present in the cytoplasm of the cells removes H_2O_2 by coupling its reduction to H_2O with oxidation of GSH. GR is a flavoprotein enzyme, regenerates GSH from oxidized glutathione in the presence of NADPH. GSH is a tripeptide and a powerful antioxidant present within the cytosol of cells and is the major intracellular nonprotein thiol compound (NPSH). SH groups present in GSH to react with H_2O_2 and the $OH^{\bullet}$ radical and prevent tissue damage and GSH is also capable of scavenging ROS directly or enzymatically via GPx. Vitamins C and E are non-enzymatic endogenous antioxidant also exists within normal cells and react with free radicals to form radicals themselves which are less reactive than the radicals. They break radical chain reactions by trapping peroxyl and other reactive radicals[16,20,25].

Non-enzymatic antioxidants also can be divided into metabolic antioxidants and nutrient antioxidants. Metabolic antioxidants are the endogenous antioxidants, which produced by metabolism in the body like lipoid acid, glutathione, L-ariginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin etc[125,26]. While nutrient antioxidants belonging to exogenous antioxidants, which cannot be produced in the body but provided through diet or supplements viz. trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids etc[11]. Vitamin E and C are the non enzymatic antioxidants exist within normal cells as well as they can be supplied through diet[27].

Antioxidants may exert their activity by several mechanisms, like by suppressing the production of active species by reducing hydroperoxides and H_2O_2, by sequestering metal ions, termination of chain reaction by scavenging active free radicals and also caused repairing and/or clearing damage of cell. Biosynthesis of other antioxidants or defense enzymes also induced by some antioxidants[27,28]. Therefore antioxidant synthesized in body or supplied from outside like phytoconstituents plays important role to protect the body from free radical induced injury.

OXIDATIVE STRESS AND HUMAN HEALTH

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism. They are continuously produced by the body via enzymatic and non-enzymatic reactions like respiratory chain reaction, the phagocytosis, prostaglandin synthesis, cytochrome P450 system and oxidative phosphorylation (i.e. aerobic respiration) in the mitochondria[28,29,30].

ROS and RNS are the products of normal cellular metabolism, having both deleterious and beneficial effect in the body[31]. At low or moderate concentration some of the free radicals plays beneficial physiological role *in vivo* this include defense against infectious agents by phagocytosis, energy production, cell growth, function in different cellular signaling systems and the induction of a mitogenic response at low concentrations[3,32].

Free radicals occur continuously in all cells as part of normal function. Oxygen free radicals are detrimental to the integrity of biological tissue and mediate their injury. The mechanism of damage involves lipid peroxidation, which destroys cell structures, lipids, proteins and nucleic acids. They causes damage to cell membranes with the release of intracellular components, leading to further tissue damage[32,33]. Antioxidant enzymes and non-enzymatic defense system minimizes the harmful effect of ROS by various antioxidant mechanism.

Oxidative stress is a harmful condition that occurs when there is an excess of ROS and/or a decrease in antioxidant levels, this may caused tissue damage by physical, chemical, psychological factors that lead to tissue injury in human and causes different diseases[34]. Living creatures have evolved a highly complicated defense system and body act against free radical-induced oxidative stress involve by different defense mechanism like preventative mechanisms, repair mechanisms, physical defenses and antioxidant defenses[15].

Oxygen derived free radical reactions have been implicated in the pathogenesis of many human diseases including[11,15,35-41]:

- Neurodegenerative disorder like alzheimer’s disease, parkinson’s disease, multiple sclerosis, amyotrophic lateral sclerosis, memory loss and depression.
- Cardiovascular disease like atherosclerosis, ischemic heart disease, cardiac hypertrophy, hypertension, shock and trauma.
- Pulmonary disorders like inflammatory lung diseases such as asthma and chronic obstructive pulmonary disease.
- Diseases associated with premature infants, including bronchopulmonary, dysplasia, periventricular leukomalacia, intraventricular hemorrhage, retinopathy of prematurity and necrotizing enterocolitis.
- Autoimmune disease like rheumatoid arthritis.
- Renal disorders like glomerulonephritis and tubulointerstitial nephritis, chronic renal failure, proteinuria, uremia.
- Gastrointestinal diseases like peptic ulcer, inflammatory bowel disease and colitis.
- Tumors and cancer like lung cancer, leukemia, breast, ovary, rectum cancers etc.

- Eye diseases like cataract and age related of ratina, maculopathy.
- Ageing process.
- Diabetes.
- Skin lesions
- Immunodepression.
- Liver disease, pancreatitis.
- AIDS.
- Infertility.

PHYTOMEDICINE AS ANTIOXIDANT

Human body system is enriched with natural antioxidants and can prevent the onset as well as treat diseases caused and/or fostered due to free-radical mediated oxidative stress. Human also takes antioxidants through diet. In foods, antioxidants found in small quantities but capable to prevent or greatly retard the oxidation of easily oxidizable materials[27].

Recent researches have shown that the antioxidants of plant origin with free-radical scavenging properties could have great importance as therapeutic agents in several diseases caused due to oxidative stress[42]. Plant extracts and phytoconstituents found effective as radical scavengers and inhibitors of lipid peroxidation[43,44]. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, which have stimulated the interest of many investigators to search natural antioxidant[45].

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The chemical constituents present in the herbal medicine or plant are a part of the physiological functions of living flora and hence they are believed to have better compatibility with human body. Natural products from plants are a rich resource used for centuries to cure various ailments. The use of bioactive plant-derived compounds is on the rise, because the main preoccupation with the use of synthetic drugs is the side effects which can be even more dangerous than the diseases they claim to cure. In contrast, plant derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness and proved to be safe, better patient tolerance, relatively less expensive and globally competitive. So, in respect of the healing power of plants and a return to natural remedies is an absolute requirement of our time[41,42,46].

Even synthetic drugs used to treat various disorders can capable of produce free radical which leads oxidative stress and caused tissue damage. For example, non steroidal anti-inflammatory drugs (NSAIDs) are used widely in the treatment of pain, fever, inflammation, rheumatic and cardiovascular disease but chronic administration of those drugs leads the generation of free radicals which may results gastric erosions, gastric or duodenal ulceration and severe complications such as gastrointestinal hemorrhage and perforation[46].

The use of phytoconstituents as drug therapy to scavenge free radicals and to treat disorders leads due to oxidative stress has proved to be clinically effective and relatively less toxic than the existing drugs. Therefore it is demand of time to uses drugs from plant sources or phytoconstituents to prevent and/or treat oxidative stress. Table 1 listed different phytochemicals having antioxidant property and Table 2 listed some plants producing antioxidant activity *in vitro* and *in vivo*.

CONCLUSION

Currently there has been an increased interest globally to identify antioxidant compounds from plant sources which are pharmacologically potent and have low or no side effects for use in protective medicine and the food industry. Modern civilization, use of different chemicals, pesticides, pollutant, smoking and alcohol intake and even some of synthetic medicine increases the chance of disease due to free radicals. Plants produces large amount of antioxidants to prevent the oxidative stress, they represent a potential source of new compounds with antioxidant activity. More or less the free radicals plays a role in health of modern era and the diseases caused from free radical are becoming a part of normal life. Increasing knowledge in antioxidant phytoconstituents and include them in daily uses and diet can give sufficient support to human body to fight those diseases. Phytoconstituents and herbal medicine are also important to manage pathological conditions of those diseases caused by free radicals. Explore the antioxidant principles from natural resources; identification and isolation of those phytoconstituents are simultaneously presenting enormous scope for their better therapeutic application for treatment of human disease. Therefore it is time for us, to explore and identify our traditional therapeutic knowledge and plant sources and interpret it according to the recent advancements to fight against oxidative stress, in order to give it a deserving place.

Table 1: Phytoconstituents with antioxidant activity[47-66]

Phytoconstituents	Example
Alkaloids	Alkaloid extract of *Fumaria capreolata* and *Fumaria bastardii* contain protopine, cryptonine, stylopine, fumariline, phtalidiisoquinoline, fumaritine, fumarafne and dehydrobenzophenanthridine possess antioxidant activity.
Carotenes and xanthophylls	Antioxidant activity of astaxanthine, α and β carotene, lutein, lycopene, zeaxanthin, canthaxanthin were investigated.
Volatile and essential oil	Essential oil (e.g.: α-terpinene, δ-3-carene, myrcene, α-pinene, p-cymene, β-phellandrene, citronellol, trans-geraniol, α-copaene, agarospirol, globulol) isolated from *Citrus reticulate* and *Pelargonium graveolens* having antioxidant activity.
Anthocyanins	Cyanidin-3-*O*-β-glucopyranoside isolated from *Chrysophyllum cainito*, *Eugenia uniflora*, *Myrciaria cauliflora* and delphinidin-3-*O*-β-glucopyranoside was identified from *Eugenia uniflora* possess antioxidant activity.
Isoflavones	Isoflavones one of the important types of flavonoids having antioxidant activity.
Flavan-3-ols	Catechins posses antioxidant activity found in different plant like green tea.
Flavones	Apigenin having antioxidant potential found in *Thunbergia laurifolia*
Flavonols	Quercetin and isorhamnetin isolated from *Haplopappus multifolius* possess antioxidant activity.
Flavanones	Naringenin, a major flavanone constituent isolated from *Citrus junos* possess antioxidant activities.
Coumarins	Coumarins like hernianin, O-prenyl-umbelliferone, prenyletin, haplopinol isolated from *Haplopappus multifolius* possess antioxidant activity
Stilbenes	Cajaninstilbene acid from *Cajanus cajan* have similar antioxidant activity like the natural antioxidant resveratrol.
Lignans	Lignans from *Myristica fragrans* having antioxidant potential.
Lignins	Lignins are complex phenolic polymers occurring in higher plant tissues possess antioxidant activity. Example of lignins secoisolariciresinol diglycoside.
Phenolic Acids	Phenolic acid possess antioxidant activity. Example of phenolic acid gallic acid, ellagic acid, *p*-coumaric acid, ferulic acid, vanillic acid, protocatechuic acid
Triterpenoid saponins	Extract of *Salvia macrochlamys* contain terpenoids like monogynol A, 3β-acetylmonogynol A, 3β-acetyl,22β-hydroxymonogynol A, 3β-acetyl,21β,22β-dihydroxymonogynol A and extract possess antioxidant activity.
Phytosterols	Antioxidant activity of beta-sitosterol found in *Morinda citrifolia* investigated.
Tannins	Tannins like ellagitannins and propelargonidin isolated from *Syzygium cumini* fruit showed antioxidant effect.
Hydroxycinnamic acids	Hydroxycinnamic acid derivatives like caffeic acid, chlorogenic acid, sinapic acid, ferulic acid and *p*-coumaric acid are widely distributed in plants important for their antioxidants.
Flavonoids	Flavonoid glucosides like apigenin-7-*O*-β-glucopyranoside, luteolin-7-*O*-β-glucopyranoside, luteolin-3'-*O*-β-glucopyranoside and chrysoeriol-7-*O*-β-glucopyranoside are isolated aerial parts of *Verbascum salviifolium* possess antioxidant activity. Flavonoids such as myricetin, quercetin, rutin, catechin, kaempferol, fisetin and naringenin also important for their antioxidant property.

Table 2: List of some plants having antioxidant properties [35,43, 67-92]

Plant Name	Family	Part Used	Method used for antioxidant study
Achyranthes aspera	Amaranthaceae	Leaves	Antioxidant activity by lipid peroxidation method.
Acorus calamus	Acoraceae	Rhizome	*In vitro* DPPH, TBA, FTC method.
Adiantum capillus-veneris	Adiantaceae	Whole plant	*In vitro* DPPH free radical scavenging activity method.
Aegle marmelos	Rutaceae	Leaves	GST, GSH, MDA determination in diabetic and drug treated animals.
Albizia amara	Mimosaceae	Leaves	Antioxidant activity by lipid peroxidation method.
Albizzia lebbeck	Mimosaceae	Leaves	SOD, GPx, GST, CAT, GSH, TBARS, CD estimation in diabetic and drug treated rat.
Aphanamixis polystachya	Meliaceae	Bark	*In vitro* methods like superoxide anion scavenging activity, DPPH, ABTS, FRAP method and assay of MDA, GSH after oxidative stress was induced by Freund's Complete Adjuvant.
Aquilaria malaccensis	Thymelaeaceae	Leaves	*In vitro* DPPH method.
Bauhinia divaricata	Caesalpiniaceae	Leaf and stem	*In vitro* DPPH method.
Bougainvillea apectabilis	Nyctaginaceae	Leaf and stem	*In vitro* DPPH method.
Cassia auriculata	Caesalpiniaceae	Leaves	Antioxidant activity by lipid peroxidation method.
Cassia fistula	Caesalpinaceae	Leaves	*In vitro* DPPH, nitric oxide and hydroxyl radical scavenging activity method and CCl_4 induced lipid peroxidation.
Centella asiatica	Apiaceae	Whole plant	*In vitro* DPPH assay method.
Clerodendrum serratum	Verbenaceae	Root	*In vitro* DPPH, FRAP, hydrogen peroxide scavenging method.
Curculigo orchioides	Amaryllidaceae	Rhizome	*In vivo* estimation of TBARS, SOD, CAT, GSH, GPx, GST, CD, GR in CCl_4 induced hepatotoxicity.
Cydonia vulgaris	Rosaceae	Leaves	Antioxidant activity was determined by thiocyanate and reducing power method.
Cyperus rotundus	Cyperaceae	Rhizome	*In vitro* methods like superoxide anion scavenging, hydroxyl radical scavenging, nitric oxide scavenging, metal chelating activity, reducing power assay, lipid peroxidation inhibition assay.
Datura stramonium	Solonaceae	Leaves	Antioxidant activity by lipid peroxidation method.
Equisetum maximum	Equisetaceae	Whole plant	*In vitro* DPPH free radical scavenging activity method.
Ficus deltoidea	Moraceae	Leaves	*In vitro* method like reduction power of iron, superoxide scavenging, xanthine oxidase, nitric oxide scavenging and LPO method.
Hemidesmus indicus	Asclepiadaceae	Stem	*In vitro* DPPH, TBA, FTC method.
Holarrhena Antidysenterica	Apocynaceae	Bark	*In vitro* DPPH, TBA, FTC method.
Ichnocarpus frutesecens	Apocynaceae	Whole plant	SOD, CAT, GSH, TBARS estimation in paracetamol induced liver damage.
Inonotus obliquus	Hymenochaetaceae	Whole mushroom	Antioxidant activity by DPPH, superoxide and peroxyl radicals scavenging method.
Lippia Alba	Vebenaceae	Leaves	*In vitro* reducing power ability and DPPH method.
Mellilotus officinalis	Fabaceae	Whole plant	*In vitro* DPPH free radical scavenging activity method.
Morinda lucida	Rubiaceae	Bark	*In vitro* reducing power ability and antioxidant property determined by using β-carotene.
Phyllanthus emblica	Phyllanthaceae	Fruit	Determination of antioxidant activity by cyclic voltammetry, lipid peroxidation and SOD determination method.
Plantago major	Plantaginaceae	Whole plant	*In vitro* DPPH free radical scavenging activity method.
Plumbago zeylanica	Plumbaginaceae	Root	*In vitro* DPPH, TBA, FTC method.
Psidium guajava	Myrtoideae	Fruit	Total phenolic content and FRAP estimation were carried out.
Rhizophora mangle	Rhizophoraceae	Bark	Determination of SOD, CAT, GPx and lipid peroxidation in NSAIDs induced gastric ulcer.
Rosa canina	Rosaceae	Ripe fruit	Bleomycin iron dependent DNA damage, lipid oxidation, prorein oxidation and carbohydrate damage method.
Rubia Cordifolia	Rubiaceae	Root	Estimation of LPO, GSH, SOD, CAT in ethanol induced oxidative stress.
Sideritis raeseri	Lamiaceae	Aerial parts	Antioxidant activity by Co(II) EDTA-induced luminol chemiluminescence and DPPH scavenging activity method.
Sutherlandia frutescens	Fabaceae	Whole plant	Superoxide and hydrogen peroxide scavenging activities of the plant investigated.
Trichosanthes tricuspidata	Cucurbitaceae	Root	Estimation of SOD, CAT, GPx, LPO in sildenafil induced migraine.
Urtica dioica	Urticaceae	Whole plant	*In vitro* DPPH free radical scavenging activity method.
Utleria salicifolia	Periplocaceae	Rhizome	Determination of SOD, CAT in ulcer induced animals.

(DPPH – 1,1-diphenyl-2-picryl hydrazyl radical; GST – glutathione-S-transferase; GSH – glutathione; MDA – malondialdehyde; ABTS – Free-radical scavenging activity; FRAP – Ferric Reducing Antioxidant Power; TBARS – Thiobarbituric acid reactive substances; CD –Diene conjugates; CAT – catalase; GPx – Glutathione peroxidase; GR – Glutathione reductase; LPO – Lipid peroxidation; FTC – Ferric thiocyanate method; TBA – Thiobarbituric acid method)

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