# An Antioxidant: An Overview

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**Abstract:**
Various stresses causes production of free radicals which are responsible for the chain reactions that causes cellular damage. Antioxidants protects the cells from the damage caused by the unstable free radicals by interacting with and stabilize them. Examples include beta-carotene, lycopene, vitamins C, E, A etc Antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Oxidation reactions are vital for life but can be damaging sometimes; therefore, plants and animals maintain intricate systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Oxidative stress may be caused by lower antioxidant levels due to inhibition of the antioxidant enzymes, which may damage or kill cells.

**Keywords:** free radicle, polyphenols, peroxidase,

## ANTIOXIDANT

A chemical reaction incorporating the transfer of electrons from a substance to an oxidizing agent is termed as oxidation. It produces free radicals responsible for the chain reactions that causes cellular damage. A molecule capable of decelerating or inhibiting the oxidation by being oxidized themselves and terminating the chain reactions due to eradication of free radical Phytochemical investigation &intermediates and inhibition of other oxidation reactions is known as an antioxidant. Antioxidants protects the cells from the damage caused by the unstable free radicals by interacting with and stabilize them. Examples include beta-carotene, lycopene, vitamins C, E, A etc[1].

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Oxidative stress may lay down a pathway for many human ailments, thus use of antioxidants has been widely incorporated in pharmacological fields such as management for stroke, cardiovascular and neurodegenerative diseases.

Antioxidants are commonly used as dietary supplements for maintaining health and prophylactic measure for diseases such as cancer and coronary heart disease. They can be extensively used as food preservatives and in cosmetics along with the prevention of the degradation of rubber and gasoline. It has been stated that free radicals cause oxidation which can be prohibited by a variety of antioxidants substances. Small quantities of antioxidants like phenol or amine derivatives are added to oils and fats for their stability. Plastics are formed by free radical action, they also require the antioxidants like phenols or naphthol. Low density polythene is protected by carbon black which absorbs the ultraviolet light responsible for radical production**.**

### Reactive Oxygen Species

Reactive oxygen species (ROS) covers all extremely reactive, oxygen-containing molecules, including free radicals. Categories of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. These react with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules thus resulting in cellular damage.

ROS are generated by various pathways. Most of the oxidants produced by cells occur as:

• A result of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is expended in the mitochondrial electron transport system.

• Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed, and by which foreign proteins (antigens) are denatured.

• Xenobiotic metabolism, i.e., detoxification of toxic substances.

### Classification of Antioxidants

Antioxidants are categorized into two;

(1) Primary or natural antioxidants.

(2) Secondary or synthetic antioxidants.

**Primary or natural antioxidants**

They react with lipid radicals and are chain breaking antioxidants. They are mainly phenolic in structures.

(1) Antioxidants minerals - These are co-factors of antioxidants enzymes. Their absence affects metabolism of macromolecules such as carbohydrates. Examples- selenium, copper, iron, zinc and manganese.

(2) Antioxidants vitamins- They are needed for the metabolic functions of the body. Example-vitamin C, vitamin E, vitamin B.

(3) Phytochemicals - Phenolic compounds which are neither vitamins nor minerals. These include flavonoids which are polyphenolic compounds that are ubiquitously found in nature. Flavonoids are the pigments responsible for the specific colours of vegetables fruits, grains, seeds leaves, flowers and bark. Example-

- Catechins found in green tea, black tea and sesamol.
- Carotenoids are fat soluble colour in fruits and vegetables.
- Beta carotene, present in carrot is converted to vitamin A
- Lycopene, present in tomatoes
- Zeaxantin is rich in spinach and other dark greens.
- Herbs and spices-source include Diterpene, rosmariquinone, thyme, nutmeg, clove, black pepper, ginger, garlic and curcumin and derivatives.

**Secondary or synthetic antioxidants**

These are phenolic compounds that capture free radicals and inhibit the chain reactions. Example

- Butylated hydroxyl anisole (BHA).
- Butylated hydroxytoluene (BHT).
- Propyl gallate and metal chelating agent (EDTA).
- Tertiary butyl hydroquinone (TBHQ).
- Nordihydro guaretic acid (NDGA).

**Types of Antioxidants**

**Ascorbic Acid**

Ascorbic acid or “vitamin C" is a monosaccharide found both in animals and plants. It is retained in its reduced form by reaction with glutathione, catalysed by protein disulphide isomerase and glutaredoxins in cells. It is a reducing agent and can reduce and neutralize reactive oxygen species such as hydrogen peroxide.

**Glutathione**

It is a cysteine-containing peptide found in aerobic life. It is synthesized in the cells from the constituent amino acids. Glutathione has antioxidant properties due to the presence of thiol group containing cysteine moiety which acts as a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and thus reduces other metabolites and enzyme systems, like ascorbate in glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants**.** Due to its high concentration and central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants.

**Free Radicals Impairment and Maladies**

The free radical-mediated lipid peroxidation is involved in the pathogenesis of many diseases in biological membranes. All chemical reactions involve the transfer of electrons. The body generates energy by gradually oxidizing its food in a controlled manner and it is stored in the form of chemical potential energy called ATP (Adenosine triphosphate). Free radicals are generated during the production of ATP in the mitochondria. Thus, these radicals emerge from the mitochondria and form ROS like superoxide anion ($O2^{-}$ ), hydroxyl radicals (HO.) and singlet oxygen (O21), terminate the body system particularly at the site where the free radicals are generated. The ultraviolet (UV) light penetrates the skin and the air pollutants also forms free radicals. Food, like lipid in the presence of (Fe3+, Fe2+) 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.

**Antioxidants Classification in Our Body**

The body has several antioxidant systems to inhibit the cellular damage due to the free radicals generation. Two types of the antioxidant systems can be divided into enzymatic and non-enzymatic groups.

- The enzymatic antioxidants comprise of superoxide dismutase (SOD), which catalyses the conversion of O2.O to H2O2 and H2O; Catalyse, which converts H2O and O2; and glutathione peroxidase, which reduces H2O2 and H2O.
- The non-enzymatic antioxidants consist of the lipid soluble vitamins, vitamin E and vitamin A or pro-vitamin A (beta-carotene) and the water- soluble vitamin C. Vitamin E is located within the membranes in humans, where it interrupts lipid peroxidation and play an important role in modulating intracellular signalling pathways that depend on ROI. Vitamin E protects against biological systems against both lipid and protein oxidation.

- Vitamin E, even though present in very low concentration, is very competent in preventing the development of conditions such as heart disease, cancer, cataracts, neuropathies and myopathies and other related diseases.

**Therapeutic Claims of Antioxidants**

Oxidative stress to DNA, proteins, and other macromolecules is associated in the pathogenesis of a extensive range of diseases.

**Sivaramakrishnan T. *et al.* (2017)** evaluated the total phenol, flavonoid, carotenoid and antioxidant activity of four green macroalgae *Halimeda tuna, Halimeda macroloba, Enteromorpha sp.* and *Acetabularia acetabulum*. The results revealed that methanol extract of *Enteromorpha* sp. showed maximum phenolic, flavonoid and carotenoid content of 5.72±0.13 mg GAE/g, 21.15±1.05 mg RE/g and 47.78±0.46 µg/g respectively. All the analysed macroalgae species demonstrated antioxidant activities in dose dependent manner among which *Enteromorpha* sp. showed greater antioxidant potential. *A. acetabulum* exhibited greater ABTS (2, 2-azinobiz-3-ethylbenthiazoline-6-sulfonic acid) radical scavenging ability (92%) as evident by its low IC50 (6.30 mg/ml) in comparison to other species. The antioxidant potential reveals their potential for future applications in medicine, dietary supplements as well as the natural source of immunostimulants[2].

**Jenifer P and Balakrishnan C. P. (2015)** evaluated the antioxidant activity by hydroxyl and DPPH radical scavenging methods for different organic solvent extracts of marine red algae *Gracilaria fergusonii* J. Agardh. The scavenging activity was detected in different concentration (100, 250, 500,750, 1000 µg /ml) of three different solvent extracts like methanol, chloroform and water. The performances of scavenging activity of all the listed extracts were compared with standard ascorbic acid. An IC50 value of methanol, chloroform and aqueous extract of hydroxyl radical were recorded at 940.28 µg/ml, 490.24 µg/ml and 924.65 µg/ml respectively. Also, the IC50 values of DPPH radical were recorded at 755.14 µg/ ml, 852.5µg/ml and 878.84µg/ml respectively. The IC50 value of standard ascorbic acid of hydroxyl and DPPH radical were recorded at 68.24µg/ml and 486.99µg/ml[3].

***Parthibanet al.* (2014)** investigated the antioxidant potential of the acetone and ethanol extract of three seaweeds (*Enteromorpha compressa, Turbinaria conoides* and *Gelidiella acerosa*) by total antioxidant activity assay, DPPH radical scavenging assay, hydrogen peroxide radical scavenging assay and ferric reduction assay. Among all the three seaweeds, the acetone extract of *T. conoides* showed the maximum antioxidant activity in the following assays such as total antioxidant activity assay, DPPH radical scavenging assay, hydrogen peroxide radical scavenging assay and ferric reduction assay. The higher phenolic was also recorded in acetone extract of *T. conoides*. Thus, the acetone extract of *T. conoides* was established to yield better antioxidant activity than the other seaweeds and this may be attributed with the higher phenolic content of *T. conoides*[4].

***Parthiban. et al.* (2014)** determined the potential of microalgae as new source of safe antioxidants, antioxidant capacity of *Chaetoceros calcitrans*, *Chlorella salina* and *Isochrysis galbana* using five antioxidant assays (Total antioxidant activity, DPPH radical scavenging activity, Nitric oxide radical scavenging activity, Hydrogen peroxide radical scavenging activity, Ferric reducing power) in three different solvents methanol, acetone and hexane. Total phenolic content and carotenoid content were also measured. The results showed that the maximum total antioxidant activity was detected in methanol extract of *Isochrysis galbana*. It also shows that the total phenolic and carotenoid content plays a direct vital role in total antioxidant activity. Increase in total phenolic content and carotenoid content shows increase in total antioxidant activity proving that they play a direct vital role in antioxidant properties[5]

***Parthiban*P. *et al.* (2014)** evaluated the antibacterial and antioxidant potential of eight seaweeds (*Enteromorpha compressa, Enteromorpha intestinalis, Ulva fasciata, Ulva lactuca, Chaetomorpha antennina, Padina gymnospora, Grateloupia lithophila,* and *Hypnea valentiae*) from Pondicherry coast. The result suggested that brown seaweed *P. gymnospora* showed maximum zone of inhibition against all the five pathogens examined with maximum activity against *K. pneumonia*. The antioxidant activities were also tested using five different assays, total phenolic content, total antioxidant activity, reducing power, Hydrogen peroxide radical scavenging assay, DPPH radical scavenging activity and maximum activity was observed in *P. gymnospora.*[6]

***Leelavathiet al.* (2014)** evaluated the antioxidant activity of ten seaweeds namely *Cymodeace rotundata, Acanthopora spicifera, Ulva lactuca, Ulva reticulate, Turbinaria conoides, Gracillaria edulis, Kappaphycus alvarezii, Gracillaria crassa, Gracillaria foliifera* and *Cymodeace serrulata*. The extracts of all these seaweeds were prepared with methanol and petroleum ether. The antioxidant properties of seaweeds were investigated by diphenyl-2-picrylhydrazyl (DPPH) assays by measuring the decrease in absorbance at 517 nm. The methanol extracts of *Cymodeace rotundata, Gracillaria crassa* and *Cymodeace serrulata* showed the highest total antioxidant activity of compared with other samples. *Ulva lactuca* exhibited the highest antioxidant and free radical scavenging activities in petroleum ether extract[7].

**Pandithurai M. and Murugesan S. (2014)** evaluated the methanolic extract of *Spatoglossum asperum* for its radical scavenging activity against DPPH, ABTS, superoxide and nitric oxide radicals. It was suggested from the results of antioxidant activities, that the methanolic extracts showed an increase with increasing concentration (between 100 and

900 µg/ml) indicating the dose dependency of these algal extract. The significant free radical scavenging activity of *Spatoglossum asperum* specified that it could be a potential source of natural antioxidant lead molecules[8].

**Kokabi *et al.* (2013)** investigated the in vitro antioxidant activity of *Ulva lactuca* (Chlorophyta) and three Phaeophyta seaweeds. The species *Padina pavonica, Colpomenia sinuosa, Cystoseira myrica* and *U. lactuca* were collected during low tide between March and June 2012 from the coastline of the Persian Gulf, Iran. Ethyl acetate, n-Hexane and methanol extracts were prepared. The ethyl acetate extract of *Ulva lactuca* showed the highest radical scavenging activity (IC50 62.13 µg/ml) in comparison with brown seaweeds, when tested by the DPPH (1-diphenyl-2-picrylhydrazyl). Butylated hydroxytoluene (BHT) was used as reference. The results indicated that the ethyl acetate extract of seaweeds had better antioxidant effect in comparison with methanol and n-Hexane extracts. Moreover, the antioxidant activities of ethyl acetate extracts of all the four seaweeds exhibited dose dependency and increased with increasing concentration of the extract.[9]

**Rhimou *et al.* (2013)** evaluated the antioxidant activity of ten aqueous and methanol extracts of the red seaweeds, *Pterosiphonia complanata, Boergeseniella thuyoides, Sphaerococcus coronopifolius, Asparagopsis armata, Halopitys incurvus, Hypnea musciformis, Gelidium spinulosum, Plocamium cartilagineum, Gelidium pulchellum* and *Ceramium rubrum* through three different tests. Using the DPPH (2,2-diphényl-1-picrylhydrazyl) test, four methanol extracts allowed the transformation of DPPH radical in reduced form with an EC50 between 96 and 862 µg.mL-1. With respect to the β-carotene test, 7 methanol extracts showed activity against peroxide radicals with an EC50 between 9 and 176 µg.mL-1. In the deoxyribose test, the inhibition percentage of hydroxyl radicals varies between 25 and 68% for five aqueous extracts; the most important being the extract of *A. armata.*[10]

***Gouda et al.* (2013)** evaluated the possible free radical scavenging potential of *Gracilaria verrucosa* of the acetone and chloroform extracts via total antioxidant capacity, Phosphate Molybdenum method, scavenging of DPPH free radical, total phenol content by Folin–Ciocalteau Phenol reagent (FCP) method and scavenging of Metal chelating activity. It was concluded from the results that strong antioxidant capacity of 9.82±1.64 and 12.13±1.63 mg catechol equivalent/gm dry weight (DW) was observed in both acetone and chloroform extracts of *Gracilaria verrucosa* respectively. The total phenol content was found out to be 2.011±0.035 and 1.31±0.028 % catechol equivalent/ gm DW respectively in acetone extract and chloroform extract. The DPPH scavenging potential of the organic extracts was found to be more active in comparison to the standard commercial Butylated hydroxyl toluene (BHT). The Metal chelating activity of the algal extracts was found to be concentration dependent that increases with the increase in concentration of the sample. It was concluded that both the extracts are successful in extracting the active antioxidant compounds from the alga species.[11]

**Farasat M *et al.* (2013**) evaluated the antioxidant activity and the contents of total phenolics and flavonoids in the methanolic extracts of four *Chaetomorpha* species including *C. aerea, C. crassa, C. linum* and *C. brachygona*. Eight samples of *Chaetomorpha* plants were collected from five locations along the northern coasts of the Persian Gulf in south of Iran. Methanolic extracts of the seaweeds were assessed for their antioxidant activity using DPPH radical scavenging assay. results showed that *C. linum* showed highest antioxidant potential with a relatively low IC50 (1.484 ± 0.168 mg mL-1), the highest flavonoid content (18.177 ± 2.238 mg RE g-1 ) and a relatively high content of phenolics (2.895 ± 0.415 mg GAE g-1) in comparison with the other species. *C. crassa*, collected from two different areas, showed lowest antioxidant activity and lowest phenolics and flavonoid contents than other species. Thus the results revealed that IC50, total phenolics and flavonoid content were influenced by the time of collection and location. Also there were positive correlations between the phenolic and flavonoid contents with DPPH radical scavenging activity.[12]

**Anantharaman P. *et al.* (2013)** investigated the antioxidant potential of acetone and ethanol extract of six seaweeds collected from Tuticorin coast using total antioxidant activity, total phenolic activity, DPPH radical scavenging activity, hydrogen peroxide radical scavenging assay and reducing power. It was concluded from the result that the acetone and ethanolic extract of brown seaweed *Dictyota dichotoma* showed higher phenolic content than all seaweeds studied. Higher antioxidant activity was observed in acetone extract of *D. dichotoma* and *Turbinaria ornata*. Higher DPPH radical scavenging activity was also detected in the acetone extracts of *D. dichotoma* and *T. ornata*. The maximum and minimum FRAP value was observed in acetone extract of *D. dichotoma* and ethanol extract of *Enteromorpha compressa* respectively. In this study, the extract of *D. dichotoma* was found to possess strong antioxidant activity. The antioxidant mechanisms of seaweed extracts may be attributed to their free radical scavenging ability. Also, phenolic compounds appear to be responsible for the antioxidant activity of seaweed extracts[13].

**Rajauria G. *et al.* (2012)** investigated the water, methanol and mixtures (20–80%) of Irish brown seaweed *Himanthalia elongata* for the extraction of phenolic compounds along with its antimicrobial and antioxidant properties. The 60% methanolic extract exhibited significantly the highest value of yield (6.8 6 0.24%), total phenol (286.0 6 4.61 mg gallic acid equivalents/g), flavonoid (109.8 6 2.68 mg Quercetin equivalents/g) and condensed tannin content (35.6 6 1.03 mg catechin equivalents/g). Antimicrobial activity of 60% methanolic extract tested from disc diffusion and broth dilution methods was effective against various food spoilage and pathogenic bacteria studied. The same extract exhibited statistically highest reducing power and antioxidant capacity against DPPH radical, metal ions, lipid peroxides and

hydrogen peroxide radicals. The UV-visible spectroscopy showed absorption maxima at 205 and 260 nm and the presence of hydroxyl group and an aromatic ring in Fourier Transform Infrared spectroscopy, suggested the presence of phenolic compounds in the extract[14].

**Souza B.W.S. *et al.* (2012)** evaluated the hydrocolloids from seaweed *Gracilaria birdiae* for its remarkable functional properties, like antioxidant activity and gelling ability. A polysaccharide was isolated from the aqueous extract of the red seaweed *Gracilaria birdiae.* The antioxidant properties of the sulfated polysaccharide of *Gracilaria birdiae* were evaluated by measuring DPPH free radical scavenging effect. The results concluded that this polysaccharide has a moderate effect in obstructing the formation of the radicals[15].

**Gautam and Shivhare (2011)** evaluated the antioxidant effect of petroleum ether and methanolic extract of *Praecitrullus fistulosus* against free radical damage by standard method as DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical model. The results indicated that fruits possess variable degree of antioxidant activity when compared with standard ascorbic acid. The IC50 of pet-ether extract is 18µg/ml and ethanol extractis 20µg/ml[16].

**Pise*et al*. (2010)** determined the inorganic elements, organic composition and antioxidant properties of *P. indica* and *P. veiatnamensis*. Antioxidant potentials of algae was evaluated through phenolic content, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) activity, hydrogen peroxide (H2O2) scavenging power and reducing potential. It was shown from the results that a dose-dependent free radical scavenging action against DPPH and H2O2, concentration dependent reducing potential were demonstrated by the *Porphyra* spp.

**Pandima Devi K *et. al., (2008)***, carried out the evaluation of methanolic extracts of seaweeds for antimicrobial and antioxidant activity. Ten edible seaweeds with wide pharmaceutical applications were evaluated for the antioxidant and antimicrobial activity against food borne pathogens. The results indicated that *Gelidiella acerosa* has the highest antioxidant activity. High phenolic contents were assessed in both *Gelidiella acerosa* and *Haligra species* which associated to their respective antioxidant and antimicrobial activities[17].

**Zahra *et al.* (2007)** investigated the water and ethanol extracts (WE and EE) from the dried sample of brown alga (*Sargassum boveanum*) for its phenolic compounds. The total phenolic compounds estimated in WE was about 17 ± 0.492 mg catechin equivalent (CE)/g of dry sample, using Folin-Ciocalteu method. The antioxidant activity (AOA) of WE was high at about 90% inhibition of peroxidation of linoleic acid with 7 mg dry sample/ml solvent. The IC50 of the WE sample and catechin which was used as the positive control with the hemoglobin catalyzed linoleic acid peroxidation method, were (mg/ml): 3.82 and 0.0713, respectively. The IC50 of the WE sample in terms of CE was 18.76 mg CE/g dry sample. The results concluded that WE sample displayed evident scavenging effects in DPPH free radical scavenging assay. The radical scavenging activity (RSA) was about 94% at 3 mg dry sample/ml solvent. The phenolic constituent appears to be responsible at least in part, for the observed AOA of the algal extract[18].

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