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# PHYTO-PHARMACEUTICAL STUDIES AND ANTIOXIDANT ACTIVITY OF SOME MEDICINAL PLANTS USED FOR THE MANAGEMENT OF DISEASE

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**Abstract**

Oxidative stress and free radicals-induced diseases can be managed by introducing clinically proven naturally occurring antioxidants or antioxidant supplements which can prevent the onset of many cardiovascular diseases, neurodegenerative disorders, and cancers. Collection, identification, and preservation of plant material, sample preparation from plant sources using suitable solvents. Rapid screening of plant extracts for antioxidant activity and Electron Transfer methods (ET) assay. In-vitro Antioxidant Methods Hydrogen Atom Transfer methods (HAT). These antioxidants have a strong potential in the conventional treatments of diseases especially in inflammatory disease, neurodegenerative diseases, cancer, and diabetes. Hence, with a balanced diet and best supplementation of fruits, vegetables, grains, oils, and nuts having adequate essential antioxidants such as vitamin A, E, C, lipoic acid, etc., are sufficient to improve our body's immune system.

**Keywords**

ROS (Reactive oxygen species), TPTZ (2, 4, 6-tri (2-pyridyl)-s-triazine), ORAC (Oxygen radical absorbance capacity).

**Introduction**

In the Human Life process, Oxygen plays a vital role in aerobic conditions about 5% or more of the inhaled $O_2$ is converted to reactive oxygen species (ROS). A free oxygen radical (FOR) can be defined as a chemical species possessing an unpaired electron. It FOR shows thebe positively charged, negatively charged, or electrically neutral. The generation of Reactive Oxygen Species takes the antioxidant defense of several physiological disorders of the cells and free radicals start attacking the cell proteins, lipids, and

carbohydrates and this leads to (Eugenio José Garcia *et al.,* 1012). Free radicals have been shown to cause pathological disorders such as diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, and neurological disorders, and the process of aging **15**(Patel Rajesh M *et al.,* 2011; Vana). Antioxidants are chemical substances that transfer an electron to the free radical and convert it to a reactive species molecule. They may reduce the energy of the free radical formation or break chain and repair damaged membranes (Mrinal *et a*l., 2012). Antioxidants have gained a considerable protective role in inhibiting free radical reactions and damage caused by ROS. It was proved that the bioavailability of antioxidants from natural sources is considerably high compared to that of synthetic antioxidants (Deborah EC 2016; Akande *et al*., 2011). The main characteristic of an antioxidant is its ability to trap free radicals. These free radicals may oxidize nucleic acids, proteins, lipids, or DNA and can initiate degenerative disease (Vandecreek L., *et al* 1999; Prasad KN. *et al.*, 1999). Antioxidant compounds like phenolic acids, flavonoids, Polyphenols scavenge free radicals such as peroxide, hydroperoxide, and lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Several clinical studies are suggesting that the antioxidants in fruits, vegetables, tea, and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancersm (Choudhury A *et al*., 2017). The leaf extract shows strong antioxidant activity (Chibueze *et al.,* 2016). The researchers reported free radical scavenging activity of antioxidants in foods was substantially investigated and (UCHEWA *et al*., 2017).

**Methodology**

Various methods have been used to monitor and compare the antioxidant activity of different foods. These methods require special equipment and technical skills for the analysis. These methods can be time-consuming because they depend on the oxidation of a substrate which is influenced by temperature, pressure, matrix, etc., and may not be practical when large numbers of samples are involved (Seifter et *al.,* 1984).

A high antioxidant potential observed in many tropical plants is part of their natural defense mechanism against noxious events causing oxidant damage, e.g. microbial infections (Schmitt CA *et al.,* 1999).

**List of In-vitro Antioxidant Methods**

**Oxygen radical absorbance capacity (ORAC) method**

The oxygen radical absorbance capacity (ORAC) assay is a method that measures the antioxidant capacity of a substance (Alagumanivasagam G., et al., 2012). The ORAC assay is unique in that it's ROS generator, AAPH (2, 2'-azobis (2-methylpropionamidine) dihydrochloride), produces a peroxyl free radical upon thermal decomposition. This free radical is commonly found in the body, making this reaction biologically relevant. Furthermore, AAPH is reactive with water and lipid-soluble substances, so it can measure total antioxidant potential (Badarinath AV et al., 2010).

**Total radical trapping antioxidant parameter (TRAP)**

Another essay that has been applied in human plasma is the total radical trapping antioxidant parameter (TRAP). In this assay, the rate of peroxidation induced by AAPH (2'-azobis (2-amidinopropane) hydrochloride) is monitored through the loss of fluorescence of the protein R-phycoerythrin (R-PE). In the TRAP assay, the lag-phase induced by plasma is compared with that induced by Trolox in the same plasma sample (Zhang J., et al., 2006).

**Scavenging of $H_2O_2$ radicals**

Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food, and beverages. It is widely used as a bleaching agent in the textile, paper, and pulp industries. Human beings exposed to $H_2O_2$ indirectly via the environment are estimated as 0.28 mg/kg/day with intake from leaf crops contributing most to this exposure (Cao G., et al., 1993). Hydrogen peroxide enters the human body through inhalation of vapor or mist and eye or skin contact. In the body, $H_2O_2$ is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\cdot}$) that can initiate lipid peroxidation and cause DNA damage. A solution of hydrogen peroxide (40 mM) is prepared in a phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. Extract (20-60 µg/ml) in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

% scavenged ($H_2O_2$) = (A0 – A1/A0) × 100Where; $A_0$ is the absorbance of the control and $A_1$ is the absorbance of the test. Ascorbic corrosive, rutin BHA, α-tocopherol, or quercetin can be utilized as a positive control.

**ABTS {2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)} radical scavenging method**

This assay is based on the principle that when 2, 2'-azinobis-(3- ethyl-benzothiazoline-6-sulphonic acid) {ABTS} is incubated with a peroxidase (such as metmyoglobin and $H_2O_2$, a relatively stable radical cation (ABTS+). The formation of ABTS+ on interaction with ferryl myoglobin produces a relatively stable blue-green color, measured at 600 nm. Antioxidants in the fluid sample suppress this color production to a degree that is proportional to their concentrations (Emad AS *et al*.., 2013).

In this equation,

HX-FeIII=metmyoglobin,

X-[FeIV=0]=ferryl myoglobin,

ABTS=2, 2'-azino-di-[3-ethylbenzthiazoline sulfonate]

**Trolox equivalent antioxidant capacity (TEAC)**

The ABTS˙+ formed from the reaction ABTS-e¯ → ABTS˙+ reacts quickly with ethanol/hydrogen donors to form colorless 2, 2'-azinobis (3-ethylbenzothiazoline 6- sulfonate (ABTS). The reaction is pH-independent. A decrease of the ABTS˙+ concentration is linearly dependent on the antioxidant concentration (Nilima SR et al., 2011). The radical cation ABTS. + is generated by persulfate oxidation of ABTS. A mixture (1:1, v/v) of ABTS (7.0 mM) and potassium persulfate (4.95 mM) is allowed to stand overnight at room temperature in the dark to form radical cation ABTS +. A working solution is diluted with phosphate buffer solution to absorbance values between 1.0 and 1.5 at 734 nm. An aliquot (0.1 ml) of each sample is mixed with the working solution (3.9 ml) and the decrease of absorbance is measured at 734 nm after 10 min at 37°C in the dark. Aqueous phosphate buffer solution (3.9 ml, without ABTS. + Solution) is used as a control. The ABTS+ scavenging rate is calculated. Trolox, BHT, rutin, ascorbic acid, or gallic acid can be used as a positive control (Maan H., et al., 2016).

**Ferric reducing antioxidant power (FRAP) assay**

FRAP assay is based on the ability of antioxidants to reduce Fe3+ to Fe2+ in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe2+- TPTZ complex with an absorption maximum at 593 nm (Chi CW *et al.,* 2004). This reaction is pH-dependent (optimum pH 3.6). The absorbance decrease is proportional to the antioxidant content. 0.2 ml of the extract is added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution, and 1 part of 20.0 mM $FeCl_3$. 6H2O solution) and the reaction mixture is incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. $FeSO_4$ is used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of the sample is calculated from the linear calibration curve and expressed as mmol $FeSO_4$ equivalents per gram of sample. BHT, BHA, ascorbic acid, quercetin, catechin or trolox can be used as a positive control (Adrienne CP., et al., 2013).

**DPPH free radical scavenging assay**

The delocalization gives rise to deep violet color, which is characterized by an absorption band at about 517 nm (in methanol solution). When a solution containing a substance that can donate a hydrogen atom is mixed with DPPH, it gives rise to the reduced form by losing violet color (if picryl group is still present in the mixture then there will be a chance of residual pale-yellow color to be obtained) (Naganuma A., et al., 1984). The primary reaction representing the DPPH radical by Z• and the donor molecule by AH is Z• $^+$ AH = ZH $^+$ A•

## Result and Discussion

### Role of Antioxidants in Human Health

According to the medical plant antioxidants agents have an impact on health. Many antioxidants that penetrate the body through ingestion, inhalation, or skin can be harmful. These substances can generate free radicals that are accumulated. This accumulation can cause damage and even death due to biological consequences (Naganuma A. et al., 1984). Currently, the main causes for reducing the plasma level are due to antioxidants that are produced during smoking and chronic alcoholism (Berry JP *et al.,* 1984). For example, in the skin, there is a defense antioxidant against UV radiation which is formed by melanin and antioxidant enzymes. This defense prevents swelling, wrinkling, and skin cancer. The benefit of antioxidant uptake has been demonstrated in the course of some diseases and certain conditions such as diabetes, asthma, hemodialysis, thalassemia, rheumatoid arthritis, systemic attack, post-menopause, schizophrenia, depression, and leukemia (Prasad KN., et al., 1999).

## Conclusions

Oxidative stress and free radicals-induced diseases can be managed by introducing clinically proven naturally occurring antioxidants or antioxidant supplements which can prevent the onset of many cardiovascular diseases, neurodegenerative disorders, and cancers. These antioxidants have a strong potential in the conventional treatments of diseases especially in inflammatory disease, neurodegenerative diseases, cancer, and diabetes. Hence, with a balanced diet and good supplementation of fruits, vegetables, grains, oils, and nuts having adequate essential antioxidants such as vitamin A, E, C, lipoic acid, etc., are sufficient to improve our body's immune system and to prevent many diseases, and premature aging.

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