IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
January 2023 Vol.:26, Issue:2

Review of Various Techniques for Estimating Antioxidant Activity

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Anil Kumar[1], P.G. Jadar[*2], Ram V[3]

[1,3] PG Scholar, Department of Rasashastra & Bhaishajya Kalpana, India.

*[*2]Dean and Professor, Department of Rasashastra & Bhaishajya Kalpana, India.*

[3]KAHER's Shri B. M.K Ayurveda Mahavidyalaya, Belagavi, Karnataka, India.

Submitted: 22 December 2022
Accepted: 28 December 2022
Published: 30 January 2023

Keywords: Antioxidants, *In vitro* Antioxidant, Chromatography, Spectrometry, and Electrochemical techniques.

ABSTRACT

The disproportion between the number of antioxidants and free radicals is the main cause of oxidative damage to proteins, lipids, and DNA. One of the important issues in biotechnology, medicine, and food technology is the excess free radical's diminution or reduction to obtain healthy food. Many terms like antiradical activity, antioxidant activity, antioxidant capacity, antioxidant power, and antioxidant ability are widely used. In vitro Antioxidant methods include various techniques such as antioxidative activity assay (ABTS), Cupric Reducing Antioxidant Capacity assay (CUPRAC), Ferric Reducing Antioxidant Power assay (FRAP), the Total Peroxyl Radical Trapping Antioxidant Parameter (TRAP) test, Trolox equivalent capacity assay (TEAC), Total Oxyradical Scavenging Capacity (TOSC) test, Oxygen radical absorbance capacity (ORAC), the Hydroxyl Radical Antioxidant Capacity (HORAC) test, Folin-Ciocalteu test, Mixed tests, including the transfer of both a hydrogen atom and an electron, include the 2,20-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) test, and the [2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl] (DPPH) test, total antioxidant capacity (TAC assay). Even Chromatography, Spectroscopy, and Electrochemical techniques are utilized to determine the antioxidant capacity, and their concepts are explained Due to the variety of antioxidant methods of action, evaluating antioxidant activity could not be performed using a single methodology. This review tries to clarify the knowledge of the various methods used to assess the antioxidant activity of various antioxidants.

INTRODUCTION

The meaning of an Antioxidant is "against oxidation". The occurrence of degenerative processes is linked with molecular biology with the existence of an excess of free radicals, enhancing oxidative processes that are injurious to the body. Antioxidants play a vital role in preserving the quality of food materials and maintaining the health of human beings. Halliwell and Guttridge defined an antioxidant as "Any substance that, when present at low concentration compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate"[1]. Antioxidants are the main reactive oxygen species (ROS) and reactive nitrogen species (RNS) scavengers[2]. ROS initiate and participate in chain reactions and these are chemical oxidizing agents. Both of them participate in oxidative stress appearance and include compounds of radical and non-radical nature[3]. A particular role of an antioxidant in neutralizing the special effects of the oxidative stress associated with the existence of free radicals is played by the enzyme called superoxide dismutase (SOD). SOD is an enzyme that alternately catalyzes the dismutation of the superoxide radical into normal molecular oxygen and hydrogen peroxide. This enzyme catalyzes the recombination reaction of the oxygen radicals, it prevents the formation of hydrogen peroxide and triplet oxygen. High levels of ROS in biological cells have a large impact on their functioning, leading to deficient cell operation, aging, or disease[4].

Terminology:

1. **Antiradical properties:** Characterizing the ability of components to react with free radicals.

2. **Antioxidant properties:** It is defined as "antioxidant capacity"[5] and "antioxidant activity", "antioxidant power, and "antioxidant ability", an antioxidant property/activity represents the capability to inhibit (reduce) all molecules having high red potential, which makes them disparaging for body structures[6]. This makes the term "antioxidant property" more general, that is, more correct. It is easy to see that the term "antioxidant activity" is the most commonly used[7].

Benefits of antioxidants:

- The relationship between antioxidants and cancer prevention, treatment, longevity, and oxidative stress has received a lot of attention recently[8].

- Recent research suggests that eating more vegetables, fruits, and less processed food offers the best protection against oxidative stress-related diseases such as cancer, coronary heart disease, hypertension, obesity, diabetes, and cataract[9].

- Drinks, fruit juices, and hot beverages all include significant levels of antioxidants like polyphenols, carotene, lycopene, vitamin C, and vitamin E[10].

- Total antioxidant potential is a concept that can be used to examine and better understand the relationship between dietary antioxidants and oxidative stress (TAP). Recent studies have demonstrated that the total antioxidant potential of the diet lowers the incidence of both stomach malignancies and heart issues[11].

Classification of Antioxidants:

The natural antioxidant generally of two types are

(A) Enzymatic antioxidant (in vivo methods) (B) Non-Enzymatic antioxidant

(A) Enzymatic antioxidant: Those enzymes which function either by removing dissolved or headspace oxygen, e.g., glucose oxidase, or by removing highly oxidative species, e.g., superoxide dismutase.

Enzymatic antioxidants are further divided into two types i.e., Primary and secondary antioxidant.

Table No:1 Difference between Primary antioxidant and Secondary antioxidant[12].

Content	**Primary antioxidant**	**Secondary antioxidant**
Description	It is also called chain-breaking antioxidants, which are those compounds, mainly phenolic substances that terminate the free radical chains in lipid oxidation and function as hydrogen and electron donors. It chelates transition metals acting as a catalyst in lipid oxidation.	The compounds function by decomposing the lipid hydro-peroxide into nonradical nonreactive and thermally stable end products called Secondary antioxidants.
Example	• Super oxide dismutase • Catalase • Glutathione peroxidase	• Glutathione reductase • Glucose6-phosphate dehydrogenase
Mechanism of action	Work in three main ways to control the oxidation reaction. **1. Chain Breakers or Free Radical Interceptors:** They work on a hydrogen atom transfer mechanism. **2. Single Electron Transfer Mechanism:** An electron is donated to the free radical to form an energetically stable anion, while the antioxidant forms a cation radical which is also a less reactive species. **3. Metal Chelating:** As transition metals act as a catalyst and also prooxidants in the oxidation reaction.  **(M-Metallic ion), (CH-Chelating agent), (MCH-Metallic chelate-stable complex).**	They are often used in combination with primary antioxidants to yield synergistic effects. Typical commercial secondary antioxidants are trivalent phosphorus compounds. **1. Phosphate-based secondary antioxidants: They** support melt processing stability by accepting oxygen atoms from hydroperoxides, becoming themselves phosphates and leaving behind stable alcohol species. **2. Thioester-based secondary antioxidants: These** are sulfur-based secondary AO'S that are often called **thio-synergists** when combined with primary AO'S.

(B) Non- Enzymatic antioxidant:

Non-enzymatic antioxidants work by interrupting free radical chain reactions.

Table No: 2 List of antioxidants with their examples.

S.no	Antioxidants	Example
1.	Phenolic acids (Polyphenols)	Hydroxycinnamic acid: Ferulic acid, p-coumaric acid Hydroxybenzoic acid: Gallic acid, Ellagic acid
2.	Flavonoids (Polyphenols)	Flavonoles: Quercetin, Kaempferol Isoflavonoids: Genistein, Flavanois: Catechine, pelagonidin, EGCG. Flavanones: Hesperidin Flavones: Chrysin Anthocyanidins: Cyanidin, Pelargoridin
3.	Antioxidant enzyme Cofactors	Coenzyme Q10
4.	Oxidative enzyme inhibitors	Aspirin, ibuprofen
5.	Transition metal chelators	EDTA (Ethylenediamine tetra-acetic acid)
6.	Radical scavengers	Ascorbic acid(C), Tocopherols(E)

MATERIALS AND METHOD

A literature view was carried out through various modern texts, journals, and internet sources about the different antioxidant activities.

Antioxidant activity measurement techniques

There is a different types of methods for assessment of the antioxidant capacity categorise into three main distinct categories namely, spectrometry, electrochemical assays, and chromatography.

Table no: 3 List of different antioxidant techniques.

Spectrometry	Electrochemical Techniques	Chromatography
1. ORAC 2. HORAC 3. TRAP 4. CUPRAC 5. FRAP 6. ABTS 7. DPPH 8. Fluorometric Analysis	1. Voltammetry 2. Amperometry 3. Bi-amperometry 4. Pulse polarography 5. Volt-amperometry	1. Gas chromatography 2. High-performance liquid chromatography (HPLC)

1. Spectrometry

A measurement method for the interactions between light and matter is spectrometry. In the field of medicine, spectroscopic techniques such as magnetic resonance imaging and Fourier transform infrared imaging are employed for the diagnosis of various disorders[13].

The majority of analyses of antioxidant properties use spectrometric techniques. However, these techniques depend on a variety of factors, including temperature, the length of the study, the nature of a molecule or mixture of compounds (extracts), the number of antioxidants and prooxidants present, and a variety of other substances.

1.1 The oxygen radical absorbance capacity (ORAC)

The oxygen radical absorbance capacity assay (ORAC), which was first created by Cao, Sofi, and Prior, uses a measurement of fluorescence quenching to gauge the impact of alleged antioxidants. The oxygen radical absorbance capacity (ORAC) measures the scavenging capacity against peroxyl radicals induced by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH)[14] at 37°C induced the antioxidant scavenging activity against peroxyl radical and this activity is measured by this method. Commonly used peroxyl radical generators in ORAC assays include azo compounds such as the lipophilic AIBN (α, α-azobisisobutyronitrile), ABAP (2,2-azobis(2-amidinopropane) dihydrochloride), and AMVN (2,2′-azobis(2,4-dimethylvaleronitrile)) and the hydrophilic AAPH (2,2′-azobis(2-amidinopropane) dihydrochloride)[15]. The ORAC method is frequently used to assess the antioxidant capacity of water-soluble antioxidants, including those found in juices, wines, teas, and other beverages. It is also used to examine the antioxidant capacity of different types of antioxidants, including those found in flavonoids, catechins, and vitamin E.

1.2 HORAC (Hydroxyl Radical Antioxidant Capacity)

HORAC is hydroxyl radical averting capacity. This is the method that measures the metal chelating activity of the antioxidant and the conditions that provide Fenton reaction. Cobalt (II) complex is used in this method, which prevents the formation of hydroxyl radicals.

HORAC assay is based on the oxidation of fluorescein by hydroxyl radicals via a classic hydrogen atom transfer (HAT) mechanism. Free radicals are generated by hydrogen peroxide(H_2O_2). The hydroxyl radicals thus generated quench the fluorescence of fluorescein over time.

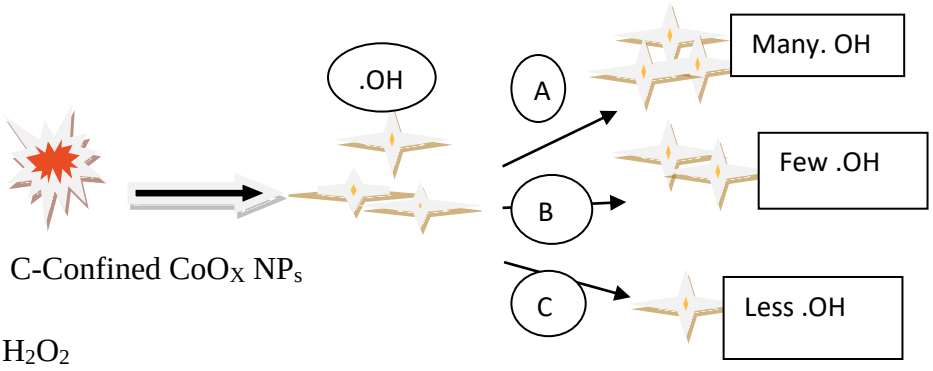

Fig: No 1 Mechanism of HORAC (Hydroxyl Radical Antioxidant Capacity)

HORAC test provides a direct measurement of antioxidant capacity against hydroxyl radicals by interrupting the radical reaction[16].

1.3 TRAP (Total Peroxyl Radical Trapping Antioxidant Parameter)

The TRAP test is based on the antioxidant's capacity to inhibit the reaction between peroxyl radicals and a target molecule, which initially represented the O_2 consumption (as a sample) in the peroxidation process triggered by the thermal decomposition of 2,20 azobis (2 amidino propane) dihydrochloride (ABAP). The retardation time of the O_2 absorption, i.e., the induction period may be quantitatively measured and used to express the total antioxidant capacity of the samples as the TRAP value. One of the methods uses luminol (o-aminofthalhydrazide) and pyranine (8-hydroxy-1,3,6–pyrene trisulfonicacid) as target molecules. Luminol reacts with ROO, emitting photons that can be measured by a luminometer, while pyranine oxidation, triggered by ROO, may be tracked by fluorescence measurements[17]. It can be seen that the intensity of the light emission during the incubation of luminol with AAPH is directly linked to the stable state concentration of the ROO generated in the AAPH thermolysis.

1.4 CUPRAC (Cupric Reducing Antioxidant Capacity assay)

The CUPRAC assay for determining the total antioxidant capacity was devised in the early 2000s[18]but it has already been modified for various methods of measuring the antioxidant activity based on the reduction of cupric (Cu^{2+}) to cuprous (Cu). It was also applicable to measure the hydrophilic and lipophilic antioxidants, due to the high solubility of the reagent in a polar and non-polar solvent[19].

The CUPRAC method is based on the utilization of the pigment-oxidizing agent (chromogenic), namely bis-copper (II) neocuproine. When the antioxidants mix with the

reagent, the reduction of the chromogenic probe occurs and changes the color of the solution. The degree of color change is correlated with the concentration of antioxidants in the sample. The change is measured at a wavelength of 450 nm[20].

1.5 FRAP (Ferric Reducing Antioxidant Power assay)

The FRAP test is a typical SET-based method measuring the reduction of the complex of ferric ions (Fe^{3+}) ligand to the intensely blue ferrous complex (Fe^{2+}) using antioxidants in acid environments. Antioxidant activity is determined as an increase in absorbance at 593 nm, and the results are expressed as micromolar equivalents of Fe^{2+} or about a standard antioxidant[21]. The original FRAP test uses tripyridyl triazine (TPTZ) as the linking ligand to the iron ion, while alternative ligands were also used to bind the iron ion, such as ferrozine to assess the reducing power of ascorbic acid[22].

$$\text{Antioxidants} + Fe^{3+} \rightleftharpoons Fe^{2+} + \text{Oxidized Antioxidants}$$

$$Fe^{2+} + Fe(CN)_6^{3-} \rightleftharpoons Fe[Fe(CN)_6]^{-} \text{ OR}$$

$$\text{Antioxidants} + Fe(CN)_6^{3-} \rightleftharpoons Fe(CN)_6^{4-} + \text{Oxidized Antioxidants}$$

$$Fe(CN)_6^{4-}\ Fe^{3+} \rightleftharpoons Fe[Fe(CN)_6]^{-}$$

The FRAP test is used globally on a large scale, providing results for a variety of purposes, including the estimation of the antioxidant content in foods and their contribution to the supply of antioxidants, to investigate the effect of storage, growth, drought, solar radiation, processing, genetic modification of dietary agents and pet foods.

1.6 The ABTS (TEAC) Test

The TEAC test was first developed by Miller and his team (1993) as a simple and convenient method used to measure the total antioxidant capacity (TAC)[23]. $ABTS^{+}$ may be generated from ABTS in the presence of powerful antioxidant agents. The degree of discoloration of theblue–green color, quantified as a sudden drop in absorbance to 734 nm, depends on the reaction duration, intrinsic antioxidant activity, and sample concentration. Furthermore, the ABTS radical is soluble in water and organic solvents, enabling the determination of the antioxidant capacity of both hydrophilic and lipophilic compounds. The TEAC antioxidant test may be used over a wide pH range, although, in many cases, the sample for which

antioxidant activity is measured may influence the pH value [24]. This is because the reaction mechanism may vary with pH, e.g., the electron transfer is facilitated by acid conditions[25].

1.7 DPPH([2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl]) test)

The DPPH test is a simple technique and requires only a Vis spectrophotometer or an electronic paramagnetic resonance (EPR) spectrometer. However, DPPH is not a natural radical but the mechanism of reaction with antioxidants is similar to that with peroxyl radicals ROO• [26].

λ = 517 nm

Deep purple Pale yellow

The purple color appeared due to the delocalization of the DPPH molecule with an absorption band around 520 nm. The DPPH method was used in the determination of antioxidant capacity in many fruit extracts and many fruit juices. The result is expressed in uM Trolox Equivalents/g fresh mass and the standard curve is linear between 25 and 800 mM Trolox. Guava fruit methanol extract has antioxidant activity between 16.2 ± 1.0 and 32.0 ± 5.1 µM TE/ fresh mass[27,28.]

1.8 Fluorometric Analysis

This method is also being used for the determination of antioxidant content. Fluorescence occurs when an electron moves from a lower energy state to a higher energy state and then comes back to its lower energy state by emitting radiation. By this method, we can determine the phenolic compound in oils. The very strict control of pH is required if a fluorometric method is used for the determination of ascorbic acid. With the help of fluorescence assay, it can be determined how sterol lateral organization affects antioxidant potency, and also the extent of sterol oxidation in lipid bilayers can also be determined by fluorescence assay[29,30,31.]

2. Electrochemical Techniques

Electrochemical techniques are used for the determination of the characteristics of the antioxidants in plant samples and in clinical diagnostics, for which electrochemical methods

have been proposed and developed. These provide a rapid, simple, and sensitive alternative method in the analysis of bioactive compounds associated with the scavenging of the radicals as well as the antioxidant capacity itself.

It is of two types i.e., (i) Stationary systems and (ii) Dynamic systems.

(i) Stationary systems

e.g. Cyclic voltammetry, Pulse-polarography, and Volt-amperometry.

A stationary system is suitable for the quantification of the limited number of analytes and for studying the formation of complexes of simple as well as more complicated biomolecules with the target molecule(s). A carbon working electrode is the most used sensor in electrochemical detection. This electrode can be based on the fluid (carbon paste electrodes)[32] or solid (printed carbon electrode)[33] matrix (binder). Besides common graphite powders, both paraffin and silicone oils can be also mixed with other carbonaceous materials, forming more or less special carbon pastes[34].

(2) Dynamic systems

Dynamic systems are used to cover rapid and sensitive quantification of simple analytes.

2.1 Voltammetric techniques

The term voltammetry is derived from volt-amperometry, and it expresses that the current is measured as a function of voltage, i.e., electrode potential. Since any electrochemical cell needs two electrodes, it would be impossible to extract unambiguous analytical information, if both electrodes would determine the magnitude of the flowing current. Therefore, one electrode is made much smaller than the other, so the flowing current is limited by this electrode only. This electrode is called the working electrode, and the other (larger) electrode is called the auxiliary electrode.

2.2 Amperometric measurement

The Amperometric method is based on the measurement of an electric current resulting from the oxidation of the substance (or the mixture) being studied on the surface of a working electrode at a certain voltage potential. The nature of the working electrode as well as the voltage potential applied determines the sensitivity of the amperometric method. The antioxidant activity may be measured by using the value of oxidation of such compounds on

the working electrode of the amperometric detector. The signal is registered as differential dynamic curves[35]. Therefore it is concluded that the amperometric method used for an evaluation of antioxidant capacity had the same advantages and disadvantages as the compared spectrophotometric methods.

3. Chromatography

Chromatographic techniques are also employed for the assessment of the antioxidant capacity of the plant extracts. Of the chromatographic techniques, gas chromatography is used for these purposes.

3.1 Gas chromatography

Gas chromatography is one of the primary types of chromatography that is extensively applied for the separation and analysis of stable but vaporizable compounds. The process is carried out by passing the mixture through the liquid stationary phase and the mobile phase in this type of chromatography is gas. The retention time comparison gives its value. Usually, thermal conductivity and flame ionization detectors are used in gas chromatography. The sample was then evaluated for anti-oxidant capacity by employing other two systems the carotene linoleate system and the phosphor molybdenum method[36].

4. *In vivo* methods

The samples that are to be evaluated for all types of in-vivo procedures are typically given to testing animals like mice, rats, etc. at a certain dose regimen as defined by the relevant method. After a specified period, the animals are usually sacrificed and blood or tissues are used for the assay**.**

Table no: 4 Explanation of different In vivo antioxidant methods.

Sr.No.	Antioxidants	Description
1.	The ferric-reducing ability of plasma	It is one of the quickest tests and very valuable for routine analysis[37]. The antioxidative activity is assessed by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent containing TPTZ (2,4,6-tripyridyl-s-triazine) and $FeCl_2$ $6H_2O$.
2.	Reduced glutathione (GSH) estimation	GSH is an intra-cellular reductant and plays a major role in catalysis, metabolism, and transport. It protects cells against free radicals, peroxides, and other toxic compounds. A deficiency of GSH in the lens leads to cataract formation [38]. Glutathione also plays an important role in the kidney and takes part in a transport system involved in the reabsorption of amino acids. The method illustrated by Ellman (1959) can be used for the determination of antioxidant activity [39].
3.	Glutathione peroxidase (GSHPx) estimation	GSHPX is a seleno-enzyme two third of which (in the liver) is present in the cytosol and one-third in the mitochondria. It catalyzes the reaction of hydroperoxides with reduced glutathione to form glutathione disulfide (GSSG) and the reduction product of hydroperoxide. GPS measurement is considered in particular with patients who are under oxidative stress for any reason; low activity of this enzyme is one of the early consequences of a disturbance of the pro-oxidant / antioxidant balance[40].
4.	Glutathione-S-transferase (GST)	Glutathione-S-transferase is thought to play a physiological role in initiating the detoxication of potential alkylating agents, including pharmacologically active compounds. These enzymes catalyze the reaction of such compounds with a the-SH group of glutathione, thereby neutralizing their electrophilic sites and rendering the products more water-soluble. The method can be used as described by Jocelyn (1972)[41].
5.	Superoxide dismutase (SOD) method	This method is well described by Mccord and Fridovich (1969)[42] and can be applied for the determination of the antioxidant activity of a sample. One unit of enzyme activity is 50% inhibition of the rate of autooxidation of pyrogallol as determined by a change in absorbance/min at 420 nm. The activity of SOD is expressed as units/mg protein.
6.	Catalase (CAT)	Catalase activity can be determined in erythrocyte lysate using Aebi's method (Aebi, 1984)[43]. **Method:** Fifty microliters of the lysate is added to a cuvette containing 2 mL of phosphate buffer (pH 7.0) and 1 mL of 30 mM H_2O_2. Catalase activity is measured at 240 nm for 1 min using a

		spectrophotometer.
7.	c-Glutamyl transpeptidase activity (GGT) assay	According to Singhal et al. (1982)[44], **Method:** The serum sample is added to a substrate solution containing glycylglycine, $MgCl_2$, and g Glutamyl-p-nitroanilide in 0.05 M tris (free base), pH 8.2. The mixture is incubated at 37 ^{0}C for 1 min and the absorbance is read at 405 nm at 1 m intervals for 5 m. The activity of GGT is calculated from the absorbance values.
8.	Glutathione reductase (GR) assay	The ubiquitous tripeptide glutathione (GSH), which is the most abundant low molecular weight thiol in almost all cells, is involved in a wide range of enzymatic reactions. A major function of GSH is to serve as a reductant in oxidation-reduction processes; a function resulting in the formation of glutathione disulfide (GSSG). A heat-labile system capable of reducing GSSG was discovered in the liver. The enzyme is directly involved in the reduction of GSSG.
9.	Lipid peroxidation (LPO) assay	LPO is an autocatalytic process, which is a common consequence of cell death. This process may cause peroxidative tissue damage in inflammation, cancer, and toxicity of xenobiotics and aging. Malondialdehyde (MDA) is one of the end products in the lipid peroxidation process. Malondialdehyde (MDA) is formed during oxidative degeneration as a product of free oxygen radicals, which is accepted as an indicator of lipid peroxidation[45].
10.	LDL assay	The isolated LDL is washed and dialyzed against 150 mmol/L NaCl and 1 mmol/L Na2EDTA (pH 7.4) at 4 ^{0}C. The LDL is then sterilized by filtration (0.45 µM), and kept under nitrogen in the dark at 4 ^{0}C. LDL (100 µg of protein/mL) is incubated for 10 min at room temperature with samples. Then, 5 µ mol/ L of CuSO4 is added, and the tubes are incubated for 2 h at 37 C. Cu2+-induced oxidation is terminated by the addition of butylated hydroxytoluene (BHT, 10 µ M). At the end of the incubation, the extent of LDL oxidation is determined by measuring the generated amount of lipid peroxides and also by the thiobarbituric acid reactive substances (TBARS) assay at 532 nm, using malondialdehyde (MDA) for the standard curve as described by Buege and Aust, 1978; El-Saadani et al., 1989[46].

RESULTS AND DISCUSSION

In addition to the compilation of various methods related to the evaluation of antioxidant activity, it was our interest to see the frequency of each method of a given number of citations being used. For the assay of serum or plasma's total antioxidant activity, a wide range of techniques have been suggested. These reviews make a distinction about the antioxidant

capacity measures the amount of a specific free radical that a test solution can scavenge, regardless of the amount of each antioxidant that is present in the mixture. Depending on the characteristics of the sample materials and the mechanism of action of the antioxidants, each approach has pros and cons. No one method can adequately characterize all antioxidant activity. To measure the various free radical inhibition mechanisms, many methodologies should be used in the evaluation of the antioxidant activity.

CONCLUSION

The evaluation of several antioxidants is the main topic of this review paper. It was created using extensive literature research. The health advantages provided by antioxidants with low molecular weight are primarily responsible for the antioxidants' increased importance. Antioxidants protect the body's essential elements, such as lipids and nucleic acids, against damage by free radicals. Chromatography, spectroscopy, and electro-analytical techniques can all be utilized for antioxidant determination. These methods allow for the determination of the total antioxidant content of the food product.

ACKNOWLEDGEMENT

Nil.

REFERENCES

1. Halliwell B, Gutteridge J.M.C. Free Radicals in Biology and Medicine, 5th ed.; Oxford University Press: Oxford, UK, 2015.
2. Allamaneni SS, Agarwal A, Nallella KP, Sharma RK, Thomas Jr AJ, Sikka SC. Characterization of oxidative stress status by evaluation of reactive oxygen species levels in whole semen and isolated spermatozoa. Fertility and sterility. 2005 Mar 1;83(3):800-3.
3. Morin D. New Research on Antioxidants; Marín, D., García, P., Eds.; Nova Science Publishers Inc.: New York,NY, USA, 2008.
4. Rodrigo, R. Oxidative Stress and Antioxidants: Their Role in Human Diseases; Nova: New York, NY, USA, 2009; pp. 9–10.
5. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radical Biology and Medicine. 2000 Dec 1;29(11):1106-14.
6. Tirzitis G, Bartosz G. Determination of antiradical and antioxidant activity: Basic principles and new insights. Acta biochimica polonica. 2010 May 11;57(2).
7. Apak R, Gorinstein S, Bohm V, Schaich KM, Özyürek M, Güçlü K. Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). Pure and Applied Chemistry. 2013 Feb 26;85(5):957-98.
8. Kalcher K, Svancara I, Buzuk M, Vytras K, Walcarius A. Electrochemical sensors and biosensors based on heterogeneous carbon materials. Monatshefte für Chemie-Chemical Monthly. 2009 Aug;140(8):861-89.
9. Halvorsen BL, Holte K, Myhrstad MC, Barikmo I, Hvattum E, Remberg SF, Wold AB, Haffner K, Baugerod H, Andersen LF, Moskaug O. A systematic screening of total antioxidants in dietary plants. The Journal of nutrition. 2002 Mar 1;132(3):461-71.

10. Ramadan-Hassanien MF. Total antioxidant potential of juices, beverages and hot drinks consumed in Egypt screened by DPPH in vitro assay. Grasas y aceites. 2008 Sep 30;59(3):254-9.
11. Serafini M, Bellocco R, Wolk A, Ekstrom AM. Total antioxidant potential of fruit and vegetables and risk of gastric cancer. Gastroenterology. 2002 Oct 1;123(4):985-91.
12. Sehwag S, Das M. Antioxidant activity: an overview. J. Food Sci. Technol. 2013;2(3):1-1.
13. Zhu X, Gao T. Spectrometry. In Nano-inspired biosensors for protein assay with clinical applications 2019 Jan 1 (pp. 237-264). Elsevier.
14. Mine K, Nakayama T, Milanese S, Grimmer K. Effects of microwave diathermy on signs and symptoms of delayed onset muscle soreness: a systematic review of Japanese primary studies. Physical Therapy Reviews. 2015 Nov 2;20(5-6):290-8.
15. Becker EM, Nissen LR, Skibsted LH. Antioxidant evaluation protocols: Food quality or health effects. European Food Research and Technology. 2004 Nov;219(6):561-71.
16. Ciz M, Cizova H, Denev P, Kratchanova M, Slavov A, Lojek A. Different methods for control and comparison of the antioxidant properties of vegetables. Food Control. 2010 Apr 1;21(4):518-23.
17. Apak R, Capanoglu E, Shahidi F. Measurement of Antioxidant Activity and Capacity—Recent Trends and Applications; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2018; pp. 1–283.
18. Apak R, Guclu K, Ozyurek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. Journal of agricultural and food chemistry. 2004 Dec 29;52(26):7970-81.
19. Çekic SD, Başkan KS, Tutem E, Apak R. Modified cupric reducing antioxidant capacity (CUPRAC) assay for measuring the antioxidant capacities of thiol-containing proteins in admixture with polyphenols. Talanta. 2009 Jul 15;79(2):344-51.
20. Ozyurek M, Guçlu K, Tutem E, Başkan KS, Erçag E, Çelik SE, Baki S, Yıldız L, Karaman S, Apak R. A comprehensive review of CUPRAC methodology. Analytical methods. 2011;3(11):2439-53.
21. Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods for testing antioxidant activity. Analyst. 2002;127(1):183-98.
22. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL. High molecular weight plant polyphenolics (tannins) as biological antioxidants. Journal of agricultural and food chemistry. 1998 May 18;46(5):1887-92.
23. Miller NJ, Diplock AT, Rice-Evans CA. Evaluation of the total antioxidant activity as a marker of the deterioration of apple juice on storage. Journal of Agricultural and Food Chemistry. 1995 Jul;43(7):1794-801.
24. Zheng L, Zhao M, Xiao C, Zhao Q, Su G. Practical problems when using ABTS assay to assess the radical-scavenging activity of peptides: Importance of controlling reaction pH and time. Food chemistry. 2016 Feb 1; 192: 288-94.
25. Lemanska K, Szymusiak H, Tyrakowska B, Zielinski R, Soffers AE, Rietjens IM. The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxyflavones. Free Radical Biology and Medicine. 2001 Oct 1;31(7):869-81.
26. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. InMethods in enzymology 1999 Jan 1 (Vol. 299, pp. 15-27). Academic press.
27. Su L, Yin JJ, Charles D, Zhou K, Moore J, Yu LL. Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. Food chemistry. 2007 Jan 1;100(3):990-7.
28. Thaipong K, Boonprakob U, Crosby Aebi, H., 1984. Catalase. Methods Enzymol. 105, 121–126.
29. K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of food composition and analysis. 2006 Sep 1;19(6-7):669-75.
30. Borowski J, Szajdek A, Borowska EJ, Ciska E, Zieliński H. Content of selected bioactive components and antioxidant properties of broccoli (Brassica oleracea L.). European Food Research and Technology. 2008 Jan;226(3):459-65.
31. Chong PL, Olsher M. Fluorometric assay for detection of sterol oxidation in liposomal membranes. Methods in Membrane Lipids. 2007:145-58.

32. Arya S, Mahajan M, Jain P. Non-spectrophotometric methods for the determination of Vitamin C. Analytica Chimica Acta 2000; 417(1):1-14.
33. Dospivova D, Smerkova K, Ryvolova M, Hynek D, Adam V, Kopel P, Stiborova M, Eckschlager T, Hubalek J, Kizek R. Catalytic electrochemical analysis of platinum in Pt-DNA adducts. Int. J. Electrochem. Sci. 2012 Apr 1;7(4):3072-88.
34. Brett C, Oliveira-Brett AM. Electrochemical sensing in solution—origins, applications and future perspectives. Journal of Solid State Electrochemistry. 2011 Jul;15(7):1487-94.
35. Gligor D, Muresan LM, Dumitru A, Popescu IC. Electrochemical behavior of carbon paste electrodes modified with methylene green immobilized on two different X type zeolites. Journal of applied electrochemistry. 2007 Feb;37(2):261-7.
36. Yashin YI, Nemzer BV, Ryzhnev VY, Yashin AY, Chernousova NI, Fedina PA. Creation of a databank for content of antioxidants in food products by an amperometric method. Molecules. 2010 Oct 22;15(10):7450-66.
37. Jayaprakasha GK, Jena BS, Negi PS, Sakariah KK. Evaluation of antioxidant activities and antimutagenicity of turmeric oil: a byproduct from curcumin production. Zeitschrift für Naturforschung C. 2002 Oct 1;57(9-10):828-35.
38. Jagtap UB, Panaskar SN, Bapat VA. Evaluation of antioxidant capacity and phenol content in jackfruit (Artocarpus heterophyllus Lam.) fruit pulp. Plant foods for human nutrition. 2010 Jun;65(2):99-104.
39. Sapakal VD, Shikalgar TS, Ghadge RV, Adnaik RS, Naikwade NS, Magdum CS. In vivo screening of antioxidant profile: a review. J. Herbal Med. Toxicol. 2008;2(2):1-8.
40. Ellman GL. Tissue sulfhydryl groups. Archives of biochemistry and biophysics. 1959 May 1;82(1):70-7.
41. Pleban PA, Munyani A, Beachum J. Determination of selenium concentration and glutathione peroxidase activity in plasma and erythrocytes. Clinical Chemistry. 1982 Feb 1;28(2):311-6.
42. Jocelyn PC. Biochemistry of the SH Group. Academic Press, London; 1972. Pp 10.
43. McCord, J., Fridovich, I., 1969. Superoxide dismutase, an enzymic function for erythrocuprin. J. Biol. Chem. 244, 6049–6055.
44. Singhal, P.K., Kapur, N., Dhillon, K.S., Beamish, R.E., Dhalla, N.S., 1982. Role of free radicals in catecholamine cardiomyopathy. Canadian J. Physiol. Pharmacol. 60, 1390.
45. Kakkar, P., Das, B., Viswanathan, P.N., 1984. A modified spectrophotometric assay of superoxide dismutase. Ind. J. Biochem. Biophys. 21, 131–132.
46. Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 52, 302–310.

Image *Author -1*	**Author Name – Dr.Anil kumar** PG Scholar of Department of RS & BK KAHER's Shri B.M.K Ayurveda Mahavidyalaya.
Image *Author -2*	**Author Name- Dr.PG.Jadar** Dean and Professor, Department of RS & BK KAHER's Shri B.M.K Ayurveda Mahavidyalaya.
Image *Author -3*	**Author Name- Dr.Ram V** PG Scholar, Department of RS & BK KAHER's Shri B.M.K Ayurveda Mahavidyalaya.