International Journal of Engineering Technology and Management Sciences
Website: ijetms.in Issue: 1 Volume No.8 January - February – 2024
DOI:10.46647/ijetms.2024.v08i01.007 ISSN: 2581-4621

a Review Of Certain Medical Plants With Antioxidant Qualities And In Vitro Approaches For Measuring Antioxidant Activity

M. Joshi[1], K.S. Dahiya[2]
[1] Research Scholar-Madhav University, Pindwara (Raj)
[2] Professor -Madhav university, pindwara (Raj)

Reactive oxygen species (ROS) are a type of extremely reactive molecule that results from oxygen metabolism. ROS, which include superoxide radicals, hydroxyl radicals, and hydrogen peroxide molecules, are frequently produced as byproducts of biological reactions or as a result of external causes. There is substantial evidence that ROS play a role in the development of degenerative illnesses. Evidence suggests that chemicals, particularly those derived from natural sources, can provide free radical protection. This has piqued the curiosity of researchers in natural antioxidants. Screening medicinal plants for antioxidant capacity is required. As a result, an attempt was undertaken to analyze various in vitro methods for assessing antioxidant activities of natural compounds derived from medicinal plants. All of the models are detailed, as well as the various estimation criteria. Finally, a great number of plants with antioxidant activity in vitro are described, although in vivo research is sparse.

Key words: Antioxidant assay, *in vitro* models, antioxidant medicinal plants.

Introduction

Oxidative stress is characterized by the presence of products known as free radicals and reactive oxygen species (ROS), which are generated under normal physiological settings but become harmful when not removed by endogenous systems. Indeed, oxidative stress is caused by an imbalance in the production of reactive oxygen species and endogenous antioxidant mechanisms. ROS are important sources of primary catalysts that initiate oxidation in vivo and in vitro, resulting in oxidative stress and a variety of diseases and disorders such as cancer, cardiovascular disease, neural disorders, Alzheimer's disease mild cognitive impairment, Parkinson's disease, alcohol-induced liver disease, ulcerative colitis, aging, and atherosclerosis. Free radicals formed from oxygen, such as superoxide anions, hydroxyl radicals, and hydrogen peroxide, are cytotoxic and cause tissue damage. Excess ROS is hazardous because it initiates bimolecular oxidation, which causes cell death and oxidative stress. Furthermore, oxidative stress induces unintentional enzyme activation and oxidative damage to the biological system.[1]

When oxygen catches a single electron, it becomes unstable and consequently extremely reactive, causing damaging chain reactions against several biological components. The tremendous toxicity of oxygen is due to its propensity to generate free radicals, which destroys many major biological components. They are capable of attacking lipids and proteins and destroying membranes. ROS can cause DNA damage, resulting in mutation and chromosomal damage.

To induce membrane lipid peroxidation, oxidized cellular thiols extract hydrogen atoms from unsaturated fatty acids. ROS can target a variety of substrates in the body, contributing to the development of chronic illnesses. For example, it has been proposed that oxidatively changed LDL is a causal agent in the development of cardiovascular illnesses.[2]

Exogenous substances and endogenous metabolic processes in the human body generate free radicals, particularly oxygen-derived radicals, capable of damaging biomolecules and causing cell death.

DOI:10.46647/ijetms.2024.v08i01.007 ISSN: 2581-4621

Under stress conditions like as hard exercise, some medicines, illness, and other disease states, superoxide anion radicals rise. The human body produces more than 2 Kg of.O - every year via typical metabolic activities.
Cells are equipped with many methods to combat ROS and maintain the redox homeostasis of the cell. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), for example, play critical roles in scavenging free radicals and avoiding cell harm. Vitamins C and E, for example, reduce lipid peroxidation in cells. When the antioxidant defense mechanism in the human body becomes imbalanced, an antioxidant supplement may be administered to assist prevent oxidative damage.[3]

Natural sources of antioxidants

Antioxidants are abundant in medicinal plants. Natural antioxidants boost plasma antioxidant capacity and lower the risk of certain diseases such as cancer, heart disease, and stroke. Plant secondary metabolites such as phenolics and flavonoids have been shown to be effective free radical scavengers. They can be found in all parts of the plant, including the leaves, fruits, seeds, roots, and bark. Many synthetic antioxidants are in use. However, it has been observed that they have various negative effects, including the danger of liver damage and carcinogenesis in laboratory animals. As a result, more effective, less harmful, and cost-efficient anti-oxidants are required. Medicinal plants appear to provide these desired comparative benefits, which is why there is a growing interest in natural antioxidants derived from plants.[4]
Evaluation of antioxidant activity
A great number of *in vitro* methods have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. *In vitro* methods can be divided into two major groups: 1) Hydrogen atom transfer reactions like Oxygen Radical Absorbance Capa- city (ORAC), Total radical trapping antioxidant potential (TRAP) and β carotene bleaching; 2) Electron transfer reactions like trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant power (FRAP), α, α- diphenyl-β-picryl-hydrazyl radical scavenging assay (DPPH), Superoxide anion radical scavenging assay, Hydroxyl radical scavenging assay, Nitric oxide radical scavenging assay and Total phenol assay. Because of their speed and sensitivity, these approaches are widely used. However, due to the complexity of phytochemicals, it is necessary to apply more than one approach to evaluate antioxidant capacity of plant materials. The following sections detail the most regularly and infrequently used antioxidant assays, as well as several standards that can be employed as positive controls.[5]

SCREENING METHODS OF ANTIOXIDANT ACTIVITY:AN OVERVIEW

Total phenolic content (TPC)

Plant polyphenols, a wide set of phenolic chemicals (flavanols, flavonols, anthocyanins, phenolic acids, and so on), have a perfect structural chemistry for free radical scavenging. Polyphenols' antioxidative properties stem from their high reactivity as hydrogen or electron donors, the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron (chain-breaking function), and their ability to chelate metal ions (Fenton reaction termination).
The Folin-Ciocateu reagent method can be used to calculate the total phenol content.0.5 ml of extract is combined with 0.1 ml of Folin-Ciocalteu reagent (0.5 N) and incubated at room temperature for 15 minutes. Then 2.5 mL of saturated sodium carbonate is added, and the absorbance at 760 nm is measured after 30 minutes at room temperature. Positive controls include gallic acid, tannic acid, quercetin, chlorogenic acid, pyrocatechol, and guaiacol. The total phenolic content is given in standard equivalents (mgg-1 of extracted compound).[6]

DOI:10.46647/ijetms.2024.v08i01.007 ISSN: 2581-4621

Total antioxidant activity

Because hydrogen atoms are abstracted from the diallylic methylene groups of linoleic acid during oxidation, peroxyl free radicals are produced. The highly unsaturated beta carotene will then be oxidized by the free radicals. As a result, the orange chromophore of beta carotene is destroyed, and the findings can be measured spectrophotometrically.[7]

The conjugated diene technique is used to assess antioxidant activity. To accelerate oxidation, each extract (0.1 - 20 mg/ml) in water or ethanol (100 l) is mixed with 2.0 ml of 10 mM linoleic acid emulsion in 0.2 M sodium phosphate buffer (pH 6.6) in a test tube and stored in the dark at 37°C. After 15 hours, 0.1 ml from each tube is combined with 7.0 ml of 80% methanol in deionized water, and the absorbance of the combination is measured in a spectrophotometer at 234 nm against a blank. The antioxidant activity is calculated in the following way:

$$\text{Antioxidant activity (\%)} = (A_0 – A_1 / A_0) \times 100$$

Where; A_0 is the absorbance of control and A_1 is the absorbance of test. Ascorbic acid, BHA, α-tocopherol or trolox can be used as a positive control.

Oxygen radical absorbance capacity (ORAC) assay

Using the ORAC assay, the capacity of a chemical to scavenge peroxyl radicals generated by spontaneous decomposition of 2, 2'- azo-bis, 2- amidinopropane dihydrochloride (AAPH) was measured in terms of standard equivalents.

For estimation, the approach is utilized. The reaction mixture (4.0 ml) is made up of 0.5 ml extract in phosphate buffer (75 mM, pH 7.2) and 3.0 ml fluorescein solution, which are both combined and pre-incubated for 10 minutes at 37°C. Then, for 35 minutes, 0.5 ml of 2, 2'-azo-bis, 2-amidinopropane (AAPH) dihydrochloride solution is added and the loss of fluorescence (FL) is measured at 1 min intervals. The final results are presented as micromole trolox equivalents (TE) per gram (mol TE g-1) and are derived using the differences in areas under the FL decay curves between the blank and a sample[8-10].

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is based on antioxidants' ability to decrease Fe3+ to Fe2+ in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), resulting in a bright blue Fe2+-TPTZ complex with a maximum absorption at 593 nm.[11] This reaction is pH-dependent (pH 3.6 is optimal). The decrease in absorbance is related to the antioxidant content. 0.2 ml of the extract is mixed with 3.8 ml of FRAP reagent (10 parts 300 mM sodium acetate buffer at pH 3.6, 1 part 10.0 mM TPTZ solution, and 1 part 20.0 mM FeCl3. 6H2O solution), and the absorbance at 593 nm is determined after 30 minutes at 37°C. Calibration is done with FeSO4. The antioxidant capacity is computed from the linear calibration curve and expressed as mmol FeSO4 equivalents per gram of sample based on the ability to reduce ferric ions. As a positive control, BHT, BHA, ascorbic acid, quercetin, catechin, or trolox might be employed.[12]

Trolox equivalent antioxidant capacity (TEAC) assay

The ABTS + produced by the reaction ABTS-e- ABTS + quickly combines with ethanol/hydrogen donors to create the colorless 2, 2'-azinobis (3-ethyl-benzothiazoline-6-sulfonate (ABTS). The reaction is pH insensitive. The concentration of ABTS+ decreases linearly with the concentration of antioxidant. The radical cation ABTS. $^+$ is generated by persulfate oxidantion of ABTS. A mixture (1:1, v/v) ofABTS (7.0 mM) and potassium persulfate (4.95 mM) is allowed to stand overnight at room temperature in dark toform radical cation ABTS $^+$. At 734 nm, a working solution is diluted with phosphate buffer solution to achieve absorbance values between 1.0 and 1.5. After 10 minutes at 37°C in the dark, an aliquot (0.1 ml) of each sample is mixed with the working solution (3.9 ml), and the decrease in absorbance is recorded at 734 nm. As a control, 3.9ml of aqueous phosphate

International Journal of Engineering Technology and Management Sciences
Website: ijetms.in Issue: 1 Volume No.8 January - February – 2024
DOI:10.46647/ijetms.2024.v08i01.007 ISSN: 2581-4621

aerial parts	bark	essential oil	flower	fruit
galls	wood	leaves	peel	rhizomes
root	seed	stem	whole plant	other

buffer solution (without ABTS. + solution) is employed. It is determined the ABTS + scavenging rate. As a positive control, trolox, BHT, rutin, ascorbic acid, or gallic acid might be employed.[13,14]

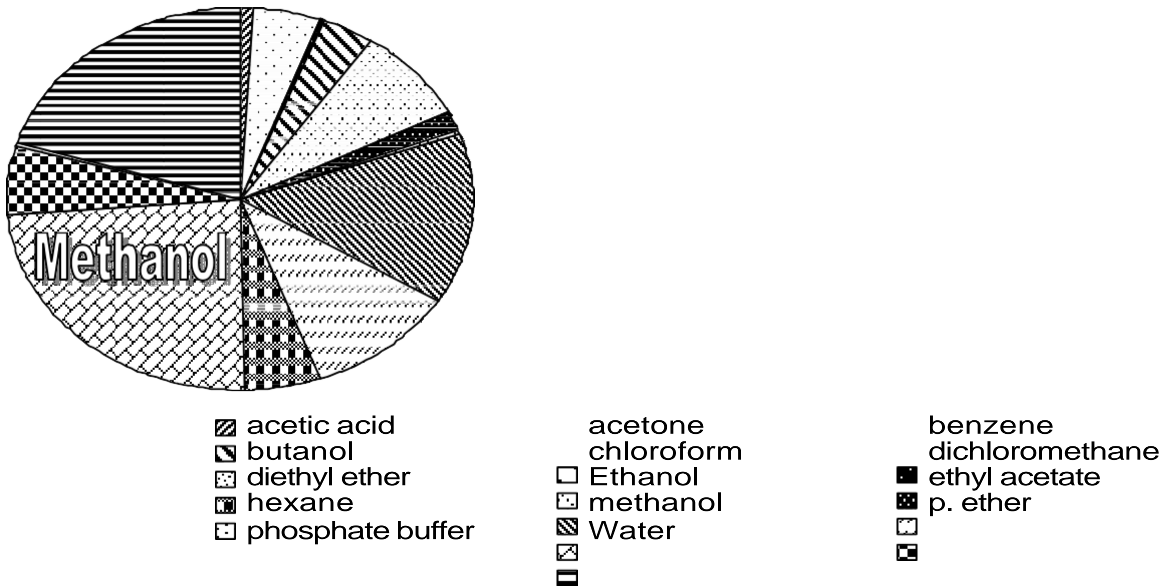

Figure 1. Various solvents used for plants extraction.

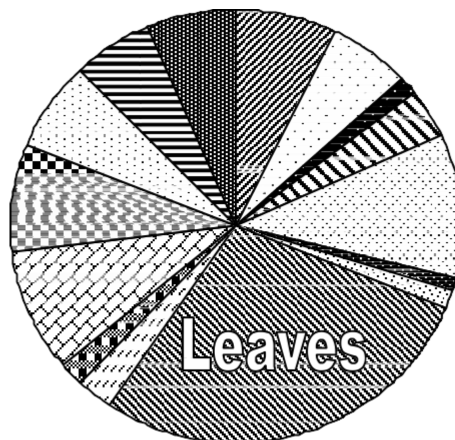

Figure 2. Different plant parts used for the study of antioxidant activity.

Conclusion

DOI:10.46647/ijetms.2024.v08i01.007 ISSN: 2581-4621

Many different solvents are utilized to extract bioactive chemicals from plants. The polarity of the solvents utilized varies. Methanol is the most often used solvent, as illustrated in Figure 1. Water has traditionally been employed for extraction, however it is now second only to methanol. Non-polar solvents are used less frequently, indicating that the active components are only soluble in polar solvents. In general, any part of the plant can be employed for antioxidant research, however the leaf is the most usually used, followed by the fruit (Figure 2).

References:

1. Grosso, G. (2018). Dietary antioxidants and prevention of non-communicable diseases. *Antioxidants*, *7*(7), 94.
2. Engelgau, M. M., Rosenthal, J. P., Newsome, B. J., Price, L., Belis, D., & Mensah, G. A. (2018). Noncommunicable diseases in low-and middle-income countries: a strategic approach to develop a global implementation research workforce. *Global heart*, *13*(2), 131-137.
3. Sies, H., Stahl, W., & Sevanian, A. (2005). Nutritional, dietary and postprandial oxidative stress. *The Journal of nutrition*, *135*(5), 969-972.
4. Ahsan, H., Ali, A., & Ali, R. (2003). Oxygen free radicals and systemic autoimmunity. *Clinical & Experimental Immunology*, *131*(3), 398-404.
5. Saddhe, A. A., Malvankar, M. R., Karle, S. B., & Kumar, K. (2019). Reactive nitrogen species: paradigms of cellular signaling and regulation of salt stress in plants. *Environmental and Experimental Botany*, *161*, 86-97.
6. Huang, Y. J., & Nan, G. X. (2019). Oxidative stress-induced angiogenesis. *Journal of Clinical Neuroscience*, *63*, 13-16.
7. Litescu, S. C., Eremia, S., & Radu, G. L. (2010). Methods for the determination of antioxidant capacity in food and raw materials. In *Bio-Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors* (pp. 241-249). Boston, MA: Springer US.
8. Oroian, M., & Escriche, I. (2015). Antioxidants: Characterization, natural sources, extraction and analysis. *Food Research International*, *74*, 10-36.
9. Ali, S. S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahu, A., & Bora, U. (2008). Indian medicinal herbs as sources of antioxidants. *Food research international*, *41*(1), 1-15.
10. Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi pharmaceutical journal*, *21*(2), 143-152.
11. Nicklisch, S. C., & Waite, J. H. (2014). Optimized DPPH assay in a detergent-based buffer system for measuring antioxidant activity of proteins. *MethodsX*, *1*, 233-238.
12. Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of agricultural and food chemistry*, *53*(10), 4290-4302.
13. Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of food science and technology*, *48*, 412-422.
14. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical biochemistry*, *239*(1), 70-76.