SEEDS: ANTI-OXIDANT POWERHOUSE

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ABSTRACT

An antioxidant is a chemical that prevents the oxidation of other chemicals. They protect the key cell components by neutralizing the damaging effects of free radicals, which are natural by- products of cell metabolism. Seeds possess significant antioxidant potential due to the presence of various bioactive compounds. These compounds, such as phenolics and flavonoids, help protect against oxidative damage caused by free radicals. Antioxidant activity in seeds can be measured using assays like DPPH, FRAP, and ABTS. Antioxidants prevent oxidative stress and lower the risk of chronic diseases like cancer, cardiovascular disease, and neurodegenerative conditions by neutralizing free radicals in the body. The presence of an antioxidant defense is universal, and plants produce it to protect themselves from the ultraviolet light of the sun and the reactive oxygen species (ROS) generated during photosynthesis that would cause irreparable damage to the plant tissues. The aim is to raise awareness about the nutritional and therapeutic advantages of consuming these seeds daily, supporting their role in preventive health care and developing functional foods.

Key words: Seeds, anti-oxidant, reactive oxygen species, oxidative stress.

INTRODUCTION

There are various normal reactions within our bodies that produced free radicals as byproducts. Some of these reactions are generation of calories, the degradation of lipids, the catecholamine response under stress, and inflammatory processes. An antioxidant can be defined as any substance which significantly delays or prevents oxidation of oxidizable substrate when present at low concentration compared to that of an oxidizable substrate. There are two groups named as natural enzymatic antioxidants and non-enzymatic ones. Superoxide dismutase, catalases are natural enzymatic antioxidants that are located mostly in peroxisomes. Vitamin E, Vitamin C, carotenoids, glutathione and derivatives, phenolic compounds, flavonoids and alkaloids are natural and synthetic antioxidants [1].

Plant seeds have been consumed since ancient times due to their nutritional value, especially for their macro- and micro-nutrients as well as their polyphenols with antioxidant activity in recent times. Polyphenols are compounds with a di- or trihydroxy phenyl group that are directly involved in the stabilization of reactive species by donating a hydrogen atom or transferring an electron. Moreover, these phenolic compounds can be mainly found in the coats (hull, husk, or skin, for instance) covering the cotyledon(s) of seeds such as reported for lentil, pea, beans, peanuts, and pistachio and can be separated from the seed matrix by extraction with appropriate solvent [1,2].

Antioxidants are molecules that prevent or slow damage to cells caused by free radicals, which are unstable molecules produced by the body in response to environmental and internal stresses. Their primary function is to neutralize free radicals, thereby preventing oxidative stress in the body. Free Radicals are defined as Any atom or molecule with an outer shell containing an unpaired electron is a free radical. Because of this, they are extremely reactive with DNA, proteins, and lipids, among other cellular structures [2-4].

Sources of free radicals:

- **Internal (endogenous):** Mitochondrial respiration, Inflammation, Exercise.
- **External (exogenous):** Pollution, Tobacco smoke, Radiation, UV rays, Chemicals from industry.

When free radicals interact with important cellular components, they can damage cells and contribute to aging and disease. Oxidative stress occurs when there is an imbalance between free radicals and

antioxidants. If free radicals outnumber antioxidants, they cause damage to: DNA, which can cause mutations and even cancer. Proteins - impairing enzyme function, Lipid peroxidation causes damage to cell membranes. Oxidative stress has been implicated in: Aging, Cancer, Diseases of the heart, Neurodegenerative disorders like Parkinson's and Alzheimer's, Diabetes, Inflammation that persists.

- **Endogenous enzymatic antioxidants:** These are made by the body and change harmful molecules into substances that are good for you: Superoxide dismutase (SOD) – converts superoxide to hydrogen peroxide. Hydrogen peroxide is broken down by catalase into water and oxygen. Glutathione peroxidase - breaks down peroxides [2,4,5].

Food sources rich in antioxidants

Top sources: Grapes, pomegranate, blueberries, strawberries, oranges, and other fruits, sweet potatoes, spinach, kale, broccoli, and carrots are all vegetables. Nuts/Seeds: Walnuts, almonds, sunflower seeds.

Beverages: Green tea, black tea, coffee, red wine.

Spices and herbs: clove, cinnamon, turmeric, and oregano Food typically contains more antioxidants than the more colorful it is [6,7].

Benefits of antioxidants for health:

Reducing the risk of chronic diseases: Antioxidants can neutralize free radicals and minimize oxidative stress, which is linked to various chronic diseases.

Improving heart health: A diet rich in antioxidants may improve blood vessel function, reduce LDL cholesterol oxidation, and lower blood pressure, all of which contribute to a healthier heart.

Boosting cognitive function: Some studies suggest that antioxidants can help protect brain cells from oxidative damage, potentially preventing age-related cognitive decline and reducing the risk of neurodegenerative diseases.

Enhancing immunity: Antioxidants, especially vitamins C and E, support immune cell function, improving the body's ability to fight infections.

Improving skin health: Antioxidants can help reduce signs of aging and protect the skin from damage caused by UV rays and pollution.

Supporting eye health: Many antioxidants are beneficial for eye health, potentially helping to prevent age-related macular degeneration and other vision problems.

Aiding in weight management: Antioxidants can help balance blood sugar levels and reduce hunger, contributing to overall weight management.

Reducing inflammation: Antioxidants can help reduce inflammation in the body, which is linked to various chronic diseases.

Supporting healthy aging: By protecting cells from damage and reducing oxidative stress, antioxidants may help support healthy aging processes [8-10].

Table 1: Herbal plants as anti-oxidants

Name	Image	Synonym	Biological source	Chemical constituents	Use
Pumpkin seed		Squash seed, nicker nut.	*Cucurbita pepo* Cucurbitaceae	Linoleic acid, Oleic acid, Palmitic acid, Stearic acid	Protein and Fiber, Nutrient Rich, Antioxidants, Immunity Booster, Heart, Health, Blood Sugar Control, Digestive Health, Sleep Support, Hair Growth, Prostate Health
Watermelon seed		Magaj Seeds	*Citrullus lanatus* Cucurbitaceae	Linoleic acid, Oleic acid.	Heart health, digestive health, blood sugar control, and anti-inflammatory.
Muskmelon seed		Kharbooja seeds	*Cucumis melo* Cucurbitaceae	Linoleic acid, Oleic acid, Alpha-linolenic acid	Good for the heart. Improves digestion. Boosts immunity. Promotes healthy skin Boosts weight loss Reduces inflammation.
Flax seed		Linseed	*Linum usitatissimum* Linaceae	Linoleic acid, Oleic acid, Alpha-linolenic acid	Heart health, reduces inflammation, and improves overall cardiovascular health. digestive health, weight management, regulate blood sugar levels.
Fenugreek seed		Methi, Greek hay seed	*Trigonella foenum-graecum* Fabaceae	**Alkaloids**: Trigonelline **Steroidal saponins**: Diosgenin, Yamogenin **Flavonoids**: Vitexin, isovitexin **Mucilage**: Galactomannan **Volatile oil**: Gives characteristic odor **Proteins**, amino acids (especially lysine), fixed oils	Used as a galactagogue (increases milk flow in nursing. Helps in diabetes management. Act as digestive aid. inflammation and menstrual discomfort.

Black basil seed		Sabja seeds, tukmaria seeds	*Ocimum basilicum* Linn. Lamiaceae	**Fixed Oils**: Rich in **linoleic acid**, **oleic acid**, **palmitic acid** Contains small amounts of **essential oils Limonoids**: Such as **limonin** – bitter compounds with anticancer potential.	Cooling agent in traditional medicine (Ayurveda, Unani) Digestive aid – relieves bloating and acidity. Helps in weight management, Anti-inflammatory and antioxidant action. Mild laxative effect. Nutritional / Culinary: Used in drinks like falooda, sherbet.
Sunflower seeds		Helianthus seeds	*Helianthus annuus* Asteraceae	Linoleic acid, Oleic acid, Palmitic acid, Stearic acid. Flavonoids: Hesperidin, naringin, eriocitri. Coumarins: Found in trace amounts – may support antimicrobial activity. Vitamins & Minerals: Trace amounts of vitamin C, calcium, potassium, magnesium. Proteins and Carbohydrates.	Blood pressure, cholesterol levels, reduce inflammation, protect skin cells, digestion and promote regularity,
Grape seeds		Vitis seed, Grape pip, Seed of grapevine.	*Citrus limon* Rutaceae	Proanthocyanidins (OPCs – Oligomeric Proanthocyanidins): Powerful Antioxidants Flavonoids: Quercetin, catechin, epicatechin Tannins: Astringent properties Phenolic Acids: Gallic acid, caffeic acid. Fatty Acids (from grape seed oil): Linoleic acid (Omega-6) Oleic acid.	Antioxidant – protects against oxidative stress and aging. Cardioprotective – improves circulation and lowers blood pressure. Supports collagen and skin health, Used in varicose veins, edema, and chronic venous insufficiency. Helps in eye health and blood sugar control

Orange seeds		Linum usitatissimum, Linseed	*Citrus sinensis* Rutaceae	**Essential Oils**: **Limonene** (major component) **Flavonoids**: **Hesperidin** – **Rutin** – **Carotenoids**: **Beta-carotene**, **Coumarins**: **Amino Acids & Proteins**: Rich in **amino acids**, including **glutamic acid**, **proline**, and **glycine.**	**Antioxidant** – protects against oxidative stress due to high flavonoid and limonene conten. **Anti-inflammatory**– helps reduce swelling and inflammation in the body. **Antimicrobial** – the essential oils in orange seeds have antimicrobial and antifungal properties. It is used traditionally for digestive health and detoxification**.**
Lemon seeds		Citrus seed, Nimbu beej, Lemon pip.	*Citrus limon* Rutaceae	**Fixed Oils**: Rich in **linoleic acid**, **oleic acid**, **palmitic acid** Contains small amounts of **essential oils** **Limonoids**: Such as **limonin** **Flavonoids**: Hesperidin, naringin, eriocitri. **Coumarins**: **Vitamins & Minerals**: Trace amounts of vitamin C, calcium, potassium, magnesium, Proteins and Carbohydrates**.**	**Antioxidant** and **anti-**inflammatory activity **Antimicrobial** – extracts can inhibit bacterial growth. Traditionally used to support detoxification and digestive health. Lemon seed oil used in **skincare.**

Anti-oxidant activity assay methods

DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl- Hydrate) Assay: A popular technique for evaluating the capacity of antioxidants to scavenge free radicals is the DPPH assay. In this test, a stable free radical (DPPH) is combined with the plant extract, and the amount of color change is measured. A greater amount of discoloration denotes greater antioxidant activity.

Principle: The extracts' ability to scavenge free radicals was assessed using the DPPH radical scavenging assay, as described by Blois and Desmarchelier. The ability of plant extractives to donate hydrogen atoms was tested by decolorizing a methanol solution of 2,2-diphenyl-1- picrylhydrazyl (DPPH). In the presence of antioxidants, DPPH generates a violet/purple color in methanol solution and diminishes to shades of yellow.

FRAP (Ferric Reducing Antioxidant Power) Assay: Antioxidants' capacity to convert ferric ions into ferrous ions is assessed using the FRAP assay. By measuring the reduction potential, this assay can estimate a sample's antioxidant capacity. An increase in reduction potential corresponds to an increase in antioxidant activity.

Principle: The FRAP assay is based on the rapid reduction of ferric-tripyridyl triazine (Fe^3-TPTZ) in samples by antioxidants, resulting in ferrous-tripyridyl triazine (Fe^2- TPTZ), a blue-colored product. By adding the FRAP reagent to a range of known concentrations of Fe^{2+} solutions, a standard curve was produced, allowing the Fe^{2+} concentration of the samples to be determined and thereby indicating "antioxidant capacity." The FRAP approach was inspired by Benzie and Strain's work. The assay is used for the measurement of the antioxidant potential of various samples. FRAP assay does this by reducing the ferric ions to ferrous ions through antioxidants present in the samples. Once the ferric iron is reduced, a blue color is developed which is read calorimetrically. Determination of the antioxidant

potential of samples is done using a ferrous iron standard curve. Results are expressed as Fe^{2+} equivalents which are in μm or FRAP value.

Hydrogen Peroxide Free Radical Scavenging Activity Assay: The H_2O_2 assay evaluates the ability of the plant extract to scavenge hydrogen peroxide, which is a harmful reactive oxygen species. Hydrogen peroxide can cause oxidative damage to cells and tissues. The assay measures the reduction in hydrogen peroxide concentration after exposure to the plant extract, indicating its antioxidant potential.

Principle: The scavenging activity of natural antioxidants found in plant extracts against hydrogen peroxide (H_2O_2) has been widely tested by detecting the decrement of H_2O_2 in an incubation system containing H_2O_2 and the scavenger using the classical UV method at 230 nm.

Oxygen radical absorbing capacity (ORAC) assay: Basically, the same principle is applied as in the TRAP assay. The ORAC assay is another commonly applied antioxidant assay based on the ability of a test substance to inhibit the oxidation of B-phycoerythrin by reactive oxygen species, relative to Trolox. Proteins interfere with the analysis, partially protecting R-PE when all plasma antioxidants are exhausted. Determination of the lag-phase TRAP and ORAC assays can be performed with different radicals and thus different results will be obtained depending on the radical. For these reasons, results obtained with the TRAP or the ORAC assay in plasma have to be interpreted with care.

Trolox equivalent antioxidant capacity (TEAC): This assay is based on the ability of molecules to scavenge the stable free radical of 2,2'-azinobis (3- ethylbenzothiozoline-6-sulfonic acid) in comparison with Trolox, a water-soluble analogue of vitamin E. The activity of a compound is therefore expressed as TEAC. Of these assays, the Enhanced chemiluminescence (ECL) seems the least suitable to determine plasma antioxidant capacity because it relies on enzymatic activity. This technique has not been widely applied, which limits the possibility to compare results from different studies. All the other assays have been applied in plasma reproducibility.

Total radical trapping antioxidant parameter (TRAP): Another assay which 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-amidino propane)-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 [10-20].

CONCLUSION

Oxidants and free radicals are harmful for the body health when their overload cannot steadily be destroyed and consequently generate an occurrence called oxidative stress. This course of action happening due to disproportionate production of free radicals and antioxidants plays a key role in the formation and development of chronic diseases such as cancer, rheumatoid arthritis, cardiovascular and autoimmune disorders or even aging. The field of free radical chemistry has gained a great deal of attention in recent years. Free radicals' reactive oxygen species generated by our body by various endogenous systems leads to various pathological conditions. A balance between free radicals and antioxidants is prerequisite for proper physiological function. Oxidative stress caused by generation of free radicals adversely alters lipids, proteins, and DNA and provokes a number of human ailments. The antioxidant properties are contributed by the presence of polyphenolics, flavonoids, vitamin C, and monophenolics making the identification and quantification of these compounds important. As a conclusion, the use of DPPH assay coupled with various other useful methods such as FRAP and ORAC, preferred because they are able to reflect the antioxidant properties more accurately. Antioxidants prevent free radical induced tissue damage by inhibiting the formation of radicals, scavenging them, or by enhancing their decomposition. Synthetic antioxidants are reported to be harmful to human health. Thus, the search for effective, nontoxic natural compounds with antioxidant activity has been escalated in recent years.

Conflict of interest

The authors declare no conflict of interest.

Sponsorship

Nil.

REFERENCES

1. Jenifer da Silva Mendonça, Rita de Cássia Avellaneda Guimarães, Verônica Assalin Zorgetto-Pinheiro. Natural Antioxidant Evaluation: A Review of Detection Methods. Molecules. 2022; 27:3563.
2. Hanaa MH, HA Ismail, ME Mahmoud, HM Ibrahim. Antioxidant Activity and Phytochemical Analysis of Flaxseeds *(Linum Usitatisimum L)*. J. of Agric. Res. & Develop. 2017; 37(1):129-140.
3. M Salihović, M Pazalja, A Ajanovic. Antioxidant Activity of Watermelon Seeds Determined by DPPH Assay. Kem. Ind. 2022; 71(5-6):295−300.
4. Lobo V, Patil A, Phatak A, Chandra N. Free Radicals, Antioxidants and Functional Foods: Impact on Human Health. Pharmacognosy Reviews. 2010; 4(8):118-126.
5. Gilgun-Sherki Y, Rosenbaum Z, Melamed E, Offen D. Antioxidant Therapy in Acute Central Nervous System Injury: Current State. Pharmacological Reviews. 2002; 54(2):271-284.
6. Islam S, Nasrin S, Khan MA, Hossain ASMS, Islam F, et al. Evaluation of antioxidant and anticancer *properties of the seed extracts of Syzygium fruticosum* Roxb. growing in Rajshahi, Bangladesh. BMC Complementary and Alternative Medicine. 2013; 13:1-10.
7. Pedgaonkar A, Manish Adithya G, Sandhya Reddy P, Thanuja K, Divyashree L, Vasudha B, Narender B. Assessment of antioxidant activity of *Ficus benghalensis* leaves: An *in-vitro* design. Journal of Drug Delivery and Therapeutics. 2019; 9(2):20-24.
8. Abeyrathne EDNS, Nam K, Ahn DU. Analytical methods for lipid oxidation and antioxidant capacity in food systems. Antioxidants. 2021; 10(10):1587.
9. Munteanu IG, Apetrei C. Analytical methods used in determining antioxidant activity: A review. International Journal of Molecular Sciences. 2021; 22(7):3380.
10. Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences. 2020; 12(1):1-10.
11. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, et al. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. Molecules. 2022; 27(4):1326.
12. Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181(4617):1199-1200.
13. Desmarchelier C, Bermudez MJN, Coussio J, Ciccia G, Boveris A. Antioxidant and prooxidant activities in aqueous extracts of Argentine plants. International Journal of Pharmacognosy. 1997; 35(2):116-120.
14. Benzie IFF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. Analytical Biochemistry. 1996; 239(1):70-76.
15. Madhuri K, Sai Priyanka Kadali, Bindu Priya Kalukuri, Amulya Manthuri, Narender Boggula, Vasudha Bakshi, Ram Mohan Manda. Assessment of Antioxidant Activity of Leaf Extract of *Mussaenda erythrophylla*. Chemistry Research Journal. 2022; 7(5):1-7.
16. Yanala Suresh Reddy, Krishna Mohan Chinnala, K. Vamshi Sharath Nath, E. Madhan Mohan, B. Preethi and B. Narender. *In vitro* evaluation of anti-oxidant activity of different extracts of *Justicia gendarussa* leaf. Scholars Research Library, Der Pharmacia Lettre. 2015; 7(7):295-298.
17. Narender Boggula, Himabindu Peddapalli. Phytochemical analysis and evaluation of *in vitro* anti oxidant activity of *Punica granatum* leaves. International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(8):1110-1118.
18. Munteanu IG, Apetrei C. Analytical Methods Used in Determining Antioxidant Activity: A Review. Int J Mol Sci. 2021; 22(7):3380.
19. Rodrigo R. Oxidative Stress and Antioxidants: Their Role in Human Diseases. Nova; New York, NY, USA: 2009. pp. 9–10.
20. Shahidi F, Zhong Y. Measurement of antioxidant activity. J. Funct. Foods. 2015; 18:757–781.