A Comprehensive Review on the Antioxidant Properties of Green Synthesized Nanoparticles: *in vitro* **and** *in vivo* **Insights**

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ABSTRACT

The use of green-synthesized nanoparticles has emerged as a promising avenue for enhancing antioxidant activity in various applications, including medicine, environmental protection and food preservation. This review provides a comprehensive overview of the role of nanotechnology in antioxidant activity, focusing on the green synthesis of nanoparticles using plant-based extracts. The paper begins by discussing the types of antioxidants, categorizing them into enzymatic, non-enzymatic and synthetic compounds and highlighting their mechanisms of action in scavenging free radicals. Various antioxidant assay methods, including DPPH, ABTS and FRAP, are examined for their effectiveness in evaluating antioxidant potential. The review also delves into the role of medicinal plants in the green synthesis of nanoparticles, detailing how bioactive compounds in plant extracts contribute to the reduction and stabilization of metal ions into nanoparticles. The types of green synthesized nanoparticles covered include silver, gold, titanium oxide, starch, iron oxide, zinc oxide, copper, cerium oxide, nickel oxide, selenium, platinum and palladium, each with unique properties that influence their antioxidant activity. The interaction between these nanoparticles and free radicals, as well as their potential synergistic effects with other antioxidants, is discussed. Finally, the review highlights the benefits of using green synthesis methods over conventional chemical synthesis, emphasizing sustainability, cost-effectiveness and the reduced environmental impact. This work underscores the growing potential of green-synthesized nanoparticles as powerful antioxidant agents, offering new insights into their applications and future directions in both scientific research and industrial innovation.

Keywords: Antioxidants, Bioactive compound, Free radical, Green synthesis, Medicinal, Nanoparticle.

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INTRODUCTION

Antioxidants are vital molecules that neutralize Reactive Oxygen Radicals (ROS), which are waste products of metabolism and shield cells from oxidative damage. These ROS have the potential to seriously harm proteins, lipids and DNA, which can then contribute to the onset of a number of illnesses, including cancer, heart problems and neurological conditions.1,2 Exogenous antioxidants from the diet and endogenous enzymes like catalase and superoxide dismutase make up the body's antioxidant defense system.3 When there is an imbalance between the body's antioxidant defenses and the production of Reactive Oxygen Species (ROS), oxidative stress occurs, increasing the risk of disease and leading to cellular dysfunction.⁴ Dietary

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antioxidants potential to foster health and reduce the risk of illness has attracted a lot of attention due to the link between oxidative stress and disease.⁵ Free radicals are extremely unstable molecules with an unpaired electron that play a significant role as intermediates in physiological processes like neurotransmission, cytotoxicity and vascular tone regulation. Numerous illnesses in humans, including cancer, Alzheimer's disease, irregularities in heart perfusion, kidney disease and fibrosis, are brought on by free radicals. When found in diet, antioxidants perform a variety of essential roles in cells and have numerous positive health impacts.^{6,7}

When the body is under stress, it generates more Reactive Oxygen Species (ROS)-such as Hydrogen peroxide, hydroxyl radicals and superoxide anion radicals -than it can neutralize with its natural defense systems. These defenses include enzyme-based antioxidants like catalase, Superoxide Dismutase (SOD) and glutathione peroxidase, along with non-enzymatic antioxidants such as carotenoids, flavonoids, glutathione, vitamin E, vitamin C. This imbalance, where oxidative stress exceeds the body's antioxidant capacity, may result in cell damage and play a role in various health issues.⁸⁻¹²

A wide range of substances, including vitamins E and C, flavonoids, polyphenols and carotenoids, that are present in vegetables, fruits and whole grains are considered natural antioxidants. As an example, vitamin C is a strong water-soluble antioxidant that increases total antioxidant capacity by scavenging free radicals.6 Rich in plant-based foods, polyphenols demonstrate a variety of biological functions, such as scavenging free radicals and modifying redox signalling pathways.⁷ Apart from organic sources, artificial antioxidants such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) are frequently utilized in cosmetics and food preservation. But due to worries about possible health dangers, there's been more investigation and a hunt for natural, safer substitutes.^{13,14}

Throughout ancient times, Indian complementary and alternative medicine systems have effectively employed herbal antioxidants as rejuvenators. Numerous studies have shown that the chemicals found in different herbal treatments are diverse, with many of them possessing antibacterial and radical scavenging properties that can shield the human body from infections and cellular oxidation processes.15

Nanotechnology plays a critical role by increasing antioxidant component stability, bioavailability and targeted distribution thus enhancing antioxidant function. Antioxidants can be encapsulated in nanoparticles to prevent deterioration and to boost their potency. With the use of this technology, antioxidant effects can be maximized where they are most required by targeting distribution to particular areas inside the body. Studies have demonstrated, that the antioxidant qualities of natural substances such as flavonoids and vitamins can be improved by the application of silver, gold and other metal oxide nanoparticles. By increasing the solubility and absorption of antioxidants, these nanocarriers can reduce oxidative stress and neutralize free radicals more effectively.16 Figure 1 illustrates different role of Antioxidants.

Types of antioxidants

Antioxidants are mainly of 2 types which are as follows

- Non enzymatic
- Enzymatic

Non enzymatic

Non-enzymatic antioxidants are substances that help shield cells from oxidative damage without necessitating enzymatic activity. They include vitamins (like C and E), carotenoids, flavonoids

Figure 1: Role of Antioxidants.

and glutathione.17 Glutathione (GSH), a tripeptide consisting of cysteine, glutamate and glycine is often referred to as the "master antioxidant." It features a unique gamma peptide bond between carboxyl group of glutamate and the amine group of cysteine, which is then linked to glycine by a standard peptide bond. Found in every cell of the body, GSH plays a crucial role in optimizing the function of other antioxidants, supporting overall cellular health and protection.18 Vitamin C (Ascorbic Acid) is a water-soluble antioxidant that scavenges free radicals, especially in aqueous environments like the cytoplasm, preventing damage to proteins, lipids and DNA. Vitamin E (Tocopherols) is a fat-soluble antioxidant that helps protect cell membranes from oxidative damage by neutralizing lipid peroxyl radicals. Carotenoids (e.g., Beta-carotene, Lutein) are plant-derived pigments that combat Reactive Oxygen Species (ROS), particularly in the eyes and skin, offering protection against UV and light-induced damage. Flavonoids (e.g., Quercetin, Catechins) are polyphenolic compounds that neutralize free radicals and support the body's overall antioxidant defence system.19-22

Enzymatic

Enzymatic antioxidants function by dissolving and eliminating free radicals. In general, antioxidant enzymes neutralize harmful oxidative byproducts by converting them into hydrogen peroxide, which is then further broken down into water. This multi-step process requires the assistance of various trace

metal cofactors, including copper, zinc, manganese and iron, to function effectively. Glutathione peroxidase, catalase and Superoxide Dismutase (SOD) are important enzymes involved in the neutralization of Reactive Oxygen Species (ROS) that protect cells from oxidative damage. Together, these enzymes preserve redox balance and guard against cellular damage linked to a no of diseases, such as neurodegenerative disorders and cancer.²³ The activity of these enzymes is regulated by a person's diet, genetics and environment, underscoring their significance for the longevity and health of cells.²⁴

Various types of Antioxidants have been described in Figure 2.

Antioxidants Assay Methods

Some of the main methods are as Follows Different antioxidant assay methods have been depicted in Figure 3.

In vitro **assay methods Includes** *DPPH free radical scavenging assay*

A common method for assessing the radical scavengers in natural foods is the DPPH, which is 1 of the most stable free radicals.25 The DPPH test method is a rapid and easy way to manually analyse the levels of antioxidants. The DPPH test relies on the stable 2, 2-diphenyl-1 picrylhydrazyl free radical's capacity to react with hydrogen donors.^{26,27} The process includes measuring the absorbance of DPPH at its 516 nm absorption maxima, which

Figure 2: Types of Antioxidants.

is proportional to the amount of free radical scavenger added to the solution containing the DPPH reagent.^{28,29}

CUPRAC Assay

Super oxide free radical scavenging activity

In vitro superoxide radical scavenging activity is assessed by the riboflavin/light/NBT (Nitro Blue Tetrazolium) reduction method**.** This test is commonly based on the reduction of NBT, which is 1 of the most widely employed techniques for assessing superoxide radical activity**.** The process relies on the auto-oxidation of riboflavin in the presence of light to produce super oxide radicals.30,31 Superoxide anion radical overproduction leads to redox imbalance and has detrimental physiological effects.

FRAP assay

1 of the simplest tests, FRAP (Ferric Reducing Ability of Plasma), is excellent for routine analysis. Originally employed to ascertain the antioxidant activity of plasma, it was subsequently effectively used to gauge the antioxidant activity of several biological samples and pure compounds.32,33 The rise in absorbance brought on by the production of ferrous ions from the FRAP reagent containing TPTZ (2,4,6-tri (2-pyridyl)-s-triazine) and FeCl36H2O is used to calculate the antioxidative activity.^{34,35}

CUPRAC is basically an ET-based assay that is frequently used to assess a compound's total antioxidant capacity, or its ability to completely scavenge free radicals. The basic redox reaction between antioxidants and free radicals serves as the basis for this technique, which measures antioxidant activity by converting cupric ions to cuprous ions.36,37 It is referred to as Cupric ion reducing antioxidant capacity. It is frequently used to assess the antioxidant potential of biological samples, food, plants, human blood, dietary polyphenols, vitamins C and E and other substances.

FCR, the total phenols assay

Tungsten and molybdenum oxides combine to form FCR. This technique was first applied to the examination of proteins with phenolic groups, such as tyrosine,³⁸ but it was later utilized to determine the overall phenolic content of wine. This approach offers a sensitive and quantitative measurement that is largely unaffected by the presence of proteins, nucleic acids, or ascorbic acid.

Figure 3: Various Antioxidant Assay Methods.

NO free radical scavenging activity

Numerous biological processes, such as neurotransmission, vascular homeostasis, antibacterial and anticancer activity, are influenced by NO. Additionally, it causes oxidative damage. This technique relies on the Griess reagent's measurement of the suppression of nitric oxide radicals produced by sodium nitroprusside in buffer saline.³⁹

ORAC assay

The ORAC assay is a technique for measuring a material's antioxidant power that combines the substance to be evaluated (the antioxidant) with a fluorescent component and a compound that produces free radicals at a defined rate. It determines how well a substance or product guards against potentially harmful free radicals. This analytical process assesses a food's, vitamins, nutritional supplements, or other chemical's capacity to function as an antioxidant or defend against free radical damage. Trolox, a water-soluble vitamin E analogue, is used as a standard in the test to calculate the Trolox Equivalent (TE). After that, the Trolox Equivalent is used to evaluate the ORAC value, which is then converted to ORAC units or value.^{40,41}

ABTS assay

It is known as "2,2-azinobis (3-ethyl benzothiazoline, 6-sulfonic acid)". Unlike antioxidant concentration, which may include a portion of physiologically inactive antioxidants, this measure reflects the actual antioxidant activity. Additionally, it makes it possible to quantify the antioxidant activity of drug mixtures, which aids in differentiating between additive and synergistic effects.^{42,43}

Hydroxyl radical scavenging activity

The hydroxyl radical is a powerful ROS in the biological system that damages cells by reacting with the polyunsaturated fatty acid moiety of phospholipids in cell membranes. The antioxidant activity of an extract is directly correlated with its ability to scavenge hydroxyl radicals. This process uses the Fenton reaction to create hydroxyl radicals *in vitro* utilizing the Fe³⁺/ascorbate/ $EDTA/H_2O_2$ system.⁴⁴

Ascorbic acid content assay

The techniques form the foundation for the High-Performance Liquid Chromatography (HPLC) method of ascorbate determination developed by Lee and Coates.⁴⁵

Phosphomolybdenum assay

The assay is based on the ability of the sample analyte to reduce Mo (VI) to Mo (V), which then forms a green phosphate-Mo (V) complex under acidic conditions. This reduction of molybdenum by antioxidant compounds in plant extracts results in the formation of a green molybdenum complex, allowing for the measurement of antioxidant capacity using the phosphomolybdenum assay.46 The phosphomolybdenum complex is formed in the total antioxidant capacity assay, a spectroscopic technique for quantifying antioxidant capacity.

Xanthine Oxidase method

Using xanthine as a sub-substrate, the xanthine oxidase activity can be spectrophotometrically evaluated using the Noro *et al*. method.47

Metal chelating activity

Ferrozine and Fe²⁺ combine to produce chelates, which results in a red complex. The red of the ferrozine-Fe²⁺ complexes decrease as a result of this reaction, which is limited when additional chelating agents are present. The chelating activity to compete with ferrozine for the ferrous ions is determined by measuring the colour reduction.⁴⁸

Some more assays are there which include

TEAC (Trolox Equivalent Antioxidant Capacity), DMPD (N, N-Dimethyl-p-phenylenediamine), TBA (Thiobarbituric Acid) Reactive Substances (TBARS), TRAP (Total Radical-Trapping Antioxidant Parameter), HORAC (Hydroxyl Radical Antioxidant Capacity), LPIC (Lipid Peroxidation Inhibition Capacity), CAA (Cellular Antioxidant Activity).

In vivo **assay methods Includes**

Ferric reducing ability of plasma

Antioxidant potential is evaluated by observing the rise in absorbance resulting from the generation of ferrous ions through a reaction with the FRAP solution, which includes "TPTZ (2,4,6-tripyridyl-s-triazine)" and "FeCl₂·6H₂O. The absorbance is then determined using spectrophotometry at 593 nm. This method is 1 of the fastest assays available and is particularly useful for routine analysis.49

Reduced GSH estimation

GSH is an intracellular reductant that is essential for transport, metabolism and catalysis. It shields cells from harmful substances like peroxides and free radicals.⁵⁰ GSH participates in a transport system that aids in in the process of amino acid reuptake and has a significant function in the kidney as well**.** A cataract develops when the lens's GSH levels are low.

GSHPx estimation

GSHPx is a selenium-containing enzyme, with two thirds located in the cytosol, with 1-3rd present in the mitochondria (liver). In order to create GSH disulfide (GSSG) and the hydroperoxide reduction product, it facilitates the reaction between hydroperoxides and reduced GSH. Four distinct isoenzymes of GSHPx are present in all tissues: Gastrointestinal GSH peroxidase, Cellular GSH peroxidase, extracellular GSH

peroxidase and phospholipid hydroperoxide GSH peroxidase. Patients experiencing oxidative stress for whatever reason should pay particular attention to GSHPx measurement; reduced enzyme activity is among the most $1st$ signs of a disruption in the prooxidant/antioxidant balance.^{51,52}

GSt

Gst, or glutathione S-transferase, is an important enzyme involved in detoxification processes in the body. It is believed that GST has a physiological function in starting the detoxication of chemicals that are pharmacologically active as well as possible alkylating agents. By catalyzing the interaction between these chemicals and GSH's-SH group, these enzymes neutralize the compounds' electrophilic sites and increase the water solubility of the resulting products.53

SOD method

It is a crucial antioxidant enzyme that aids in shielding cells from oxidative damage and is commonly referred to as the SOD method. Superoxide radicals are dismutated into oxygen and hydrogen peroxide by SOD. Mccord and Fridovich, 196954 provide a thorough description of this technique, which can be used to assess a sample's antioxidant activity.

CAT

The enzyme Catalase (CAT) is responsible for breaking down Hydrogen Peroxide $(\mathrm{H}_2\mathrm{O}_2)$ into oxygen and water. Its ability to degrade potentially hazardous hydrogen peroxide, a byproduct of numerous metabolic activities, is essential for shielding cells from oxidative damage.⁵⁵

GSR assay

The Glutathione Reductase (GSR) assay measures the activity of the enzyme glutathione reductase, which plays a crucial role in preserving cellular redox equilibrium involves the conversion of oxidized Glutathione (GSSG) to its reduced form (GSH).⁵⁶ Several enzymatic reactions rely on the widespread tripeptide GSH, which is the most abundant low-molecular-weight thiol found in nearly all living organisms. 1 of GSH's primary roles is to act as a reductant during oxidation-reduction reactions, which produces GSH disulfide (GSSG). It was found that the liver contains a heat-labile mechanism that can reduce GSSG. The enzyme that is directly responsible for GSSG reduction.

LPO assay

Lipid peroxidation, a sign of oxidative stress, is measured in biological samples using the Lipid Peroxidation (LPO) assay. The term "lipid peroxidation" describes the oxidative breakdown of lipids that produces quantifiable reactive aldehydes like Malondialdehyde (MDA).⁵⁷ 1 frequent result of cell death is LPO, an autocatalytic process. This mechanism results in aging, xenobiotic toxicity, cancer and peroxidative tissue damage in

inflammation. Malondialdehyde (MDA) is 1 of the end products of the Lipid Peroxidation (LPO) process. It is a well-established marker of LPO and is formed as a byproduct of free oxygen radicals during oxidative damage.

Some more assays are there which include

GGT (Gamma-Glutamyl Transferase), LDL (Low-Density Lipoprotein).

GREEN SYNTHESIZED NANOPARTICLES

This approach focuses on avoiding the use of hazardous or polluting materials in manufacturing, minimizing the consumption and waste of non-renewable resources, reducing or, when possible, eliminating pollution during synthesis and shortening the overall synthesis time. As the father of green chemistry, Paul J. Anastas described it as "a work philosophy that involves the use of alternative tools and pathways to prevent pollution," which encompasses both the design of the synthetic approach and the management of possible by-products that may result from that process.58,59

A widely used approach in this process involves utilizing algae, plants or microorganisms such as fungi or bacteria. These organisms work synergistically to produce a range of compounds, including polyphenols, terpenes, alkaloids, proteins, carbohydrates and genetic materials, all of which are crucial to the nanoparticle synthesis process.^{60,61} Factors such as the metal ion concentration, reaction time, pH and temperature influence the size and shape of nanoparticles., in addition to the biological resources (plants, algae, or microbes) that are utilized to carry out the synthesis.⁶²

Figure 4: Various metallic nanoparticles.

Biosynthesized nanoparticles have gained substantial interest because of their potential uses in a variety of industries and their ecologically friendly production processes. Here are some key points regarding their importance

Eco-Friendly Synthesis

Plant extracts are commonly used in green synthesis techniques, which minimize the need for harsh conditions and hazardous chemicals. This strategy improves sustainability while reducing its negative effects on the environment.⁶³

Biomedical Applications

Green manufactured nanoparticles have potential applications as antibacterial agents, medication delivery and imaging. They are appropriate for medical applications due to their biocompatibility.⁶⁴

Catalytic Properties

By acting as efficient catalysts in a range of chemical reactions, these nanoparticles can support more environmentally friendly industrial operations.⁶⁵

Environmental Remediation

Green manufactured nanoparticles can be used to remove pollutants from soil and water, proving their usefulness in cleaning up the environment.⁶⁶

Agricultural Applications

By acting as environmentally friendly herbicides and promoting plant development, these nanoparticles can help with food security concerns.⁶⁷

Various Green synthesized metallic nanoparticles and their antioxidant potential

Different types of metallic nanoparticles are shown in Figure 4.

Silver nanoparticles (AgNPs) synthesized from *Mussaenda frondose, Tinospora cordifolia, Curcuma longa, Trigonella foenum-graecum, Emblica officinalis, Salacia oblonga, Brassica oleracea, Psidium guajava, Digitaria radicosa, Salvia officinalis, Prunus japonica, Elephantopus scaber, Bergenia ciliata, Salvia aethiopis, Lenzites betulina, Ficus carica, Lippia nodiflora, Vetiveria zizanioides, Alpinia katsumadai, Phyllanthus amarus, Blighia sapida, Morinda lucida, Eucalyptus leucoxylon, Piper longum, Hyacinthus orientalis, Dianthus caryophyllus, Nepeta leucophylla, Physalis angulata, Thymus kotschyanus, Achillea millefolium, Trichoderma harzianum, Sambucus nigra, Cosmos* *sulphureus*, black currant, *Allium ampeloprasum, Iresine herbstii, Nervalia zeylanica, Passiflora edulis* f. *flavicarpa, Pueraria tubesinens, Echinacea purpurea, Linum usitatissimum* and *Catharanthus roseus* exhibit potent antioxidant properties, enhancing their biomedical applications. **Gold nanoparticles** (AuNPs) derived from *Sumac, Lavandula angustifolia, Vitex negundo, Citrus limetta, Curcumae kwangsiensis, Cannabis sativa, Glaucium flavum, Nerium oleander, Mangifera indica, Pistacia atlantica, Thyme, Acer pentapomicum, Terminalia bellirica* and *Ziziphus nummularia* also show strong antioxidant capabilities, making them beneficial for health applications. **Zinc oxide nanoparticles** (ZnONPs) synthesized from *Capparis zeylanica, Beta vulgaris, Scoparia dulcis*, Mulberry, *Garcinia xanthochymus, Berberis aristata, Pelargonium odoratissimum, Cassia fistula, Polygala tenuifolia, Ceropegia candelabrum, Fumaria officinalis, Peganum harmala* and *Achillea nobilis* demonstrate effective antioxidant activity, contributing to their use in pharmaceuticals and cosmetics. **Iron oxide nanoparticles** (FeONPs) from *Ficus carica* and *Phoenix dactylifera* are recognized for their antioxidant properties, enhancing applications in environmental remediation. **Copper and copper oxide nanoparticles** (CuNPs and CuO NPs) derived from *Abutilon indicum, Eclipta prostrata, Cocculus hirsutus, Cissus vitiginea, Pongamia pinnata, Withania somnifera, Galeopsidis herba, Tinospora cordifolia, Magnolia champaca* and *Achillea nobilis* highlight their significant antioxidant activity, beneficial for agriculture and medicine. **Cerium oxide nanoparticles** (CeO₂ NPs) synthesized from *Euphorbia amygdaloides* and nickel oxide nanoparticles (NiO NPs) from *Calendula officinalis* are noted for their antioxidant properties, enhancing various applications. **Selenium nanoparticles** (SeNPs) from *Crataegus monogyna* and **platinum nanoparticles** (PtNPs) from *Atriplex halimus* and *Tornabea scutellifera* also exhibit considerable antioxidant activity, contributing to health and catalysis. Lastly, **palladium nanoparticles** (PdNPs) synthesized from *Anogeissus latifolia* further emphasizes the effectiveness of plant-derived nanoparticles with antioxidant capabilities. This overview underscores the potential of these nanoparticles in promoting health and combating oxidative stress, driven by the natural antioxidant properties of their plant sources.

The antioxidant activity of green synthesized nanoparticles illustrated in table, the table shows selected research articles of antioxidant activity of nanoparticles synthesized using plant/ plant parts organized according to year of publication, the plant used (around 100 plants), type of extract, Nanoparticle types, used doses, mode of administration, duration of experiment, model of study and observed effects (Table 1).

Table 1: Antioxidant activity of green synthesized nanoparticles.

CONCLUSION

Green-synthesized nanoparticles are emerging as a promising eco-friendly alternative for combating oxidative stress, offering significant antioxidant potential. These nanoparticles, synthesized using plant extracts and microorganisms, provide a sustainable approach to nanotechnology. However, several challenges still need to be addressed. Variability in the composition of natural sources can affect the consistency of nanoparticle size, shape and stability, leading to discrepancies in their antioxidant activity. Additionally, the mechanisms by which these nanoparticles exert their antioxidant effects remain poorly understood, requiring further exploration.

This review highlights the importance of green-synthesized nanoparticles in various applications, from medicine to environmental protection. By leveraging bioactive compounds from plants and microorganisms, these nanoparticles offer a sustainable solution for mitigating oxidative stress-related diseases, aging and environmental pollution. Their eco-friendly nature and versatility make them attractive candidates for future therapeutic and industrial use.

Future research should focus on optimizing synthesis processes to enhance reproducibility and control over nanoparticle characteristics. Greater understanding of their antioxidant mechanisms is needed to advance their biomedical applications. Additionally, *in vivo* studies are crucial to validate their safety and efficacy. Exploring new natural sources could further improve the effectiveness of these nanoparticles, paving the way for their widespread use in pharmaceuticals, food preservation and environmental remediation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BHT: Butylated Hydroxy Toluene, **DPPH Assay:** 2,2-Diphenyl-1-Picrylhydrazyl Assay, **NO radical assay:** Nitric Oxide Radical Assay, **SO scavenging activity:** Superoxide Anion Scavenging Activity, H_2O_2 **scavenging:** Hydrogen Peroxide Scavenging Activity, **MDA** - Malondialdehyde, **TEAC:** Trolox Equivalent Antioxidant Capacity, **BHA:** Butylated Hydroxy Anisole, **ABTS:** 2,2'-Azino-bis (3-ethylbenzothiazoline-6 -sulfonic acid), **TPC:** Total Phenolic Content.

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