

## Significance of Antioxidants and Methods to Evaluate Their Potency

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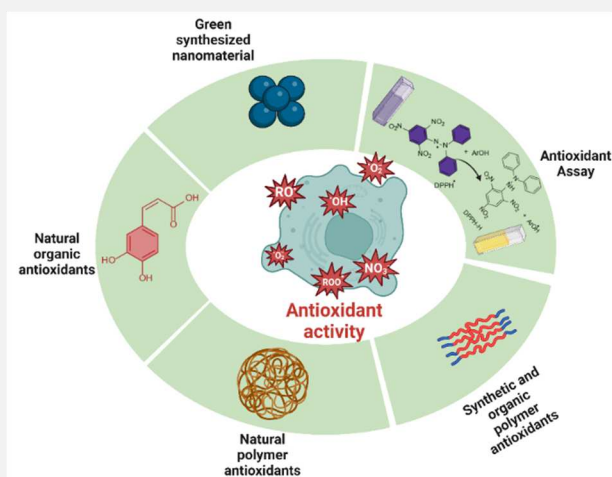


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### ABSTRACT

Antioxidants are vital bioactive components that garnered the attention of various researchers in the area of pharmacy, medicine, and food engineering. Here we have endeavored our effort to highlight the significance of antioxidants and critical assay methods to analyze the inhibitory activity of the antioxidants. Various *in vitro* and *in vivo* assay methods are available to estimate the inhibitory activity of which, the hydroxyl radical antioxidant capacity (HORAC) test, the oxygen radical absorption capacity (ORAC) test, the total oxyradical scavenging capacity (TOSC) test, and the total peroxyl radical trapping antioxidant parameter (TRAP) test are based on the transfer of hydrogen atom. The ferric reducing antioxidant power (FRAP) test, cupric reducing antioxidant power (CUPRAC) test, and the folin–ciocalteu test is based on a transfer of an electron. Whereas, the [2,2-di(4-tert-octylphenyl)-1-picrylhydrazyl] (DPPH) test and, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) tests are based on transfer of both hydrogen and electron. All these assays preliminarily assess the chemical kinetics to reach the chemical equilibrium state and develop chromogenic color or discoloration or develop fluorescence or quenches the fluorescence which can be analyzed in colorimeter or spectrophotometer respectively. In the present review, we have summarized the synthesis of antioxidant materials and their significance and the assay methods which were employed to estimate the inhibitory activity of the antioxidants.



**Keywords:** Green synthesis, natural and polymer-based nanomaterial, antioxidant assay, DPPH

## 1. Introduction

Antioxidants are compounds that can prevent damage to cells caused by molecules called free radicals. Free radicals are produced by normal physiological processes such as metabolism, but they can also be generated by environmental factors such as pollution, radiation, and cigarette smoke. When free radicals build up in the body, they can cause oxidative stress, which can damage cells, proteins, and DNA. This damage has been linked to a variety of health problems, including cancer, heart disease, and Alzheimer's disease. Antioxidants are either natural or synthetic molecules that inhibit the oxidation of other molecules. Oxidation is a chemical reaction in which an electron or a hydrogen atom is transferred from a substance to an oxidant. Oxidative reactions can generate free radicals that can set off chain reactions, and these reactions can lead to cell damage or death when they occur within cells. Antioxidants scavenge free radical intermediates and inhibit other oxidative reactions by self-oxidation [1, 2]. Typically, antioxidants are reducing agents such as thiols, ascorbic acid, or polyphenols [3]. An antioxidant refers to any substance that can prevent or significantly retard the oxidation of a substance that is readily oxidized in small amounts

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or any substance present in lower concentrations than oxidizable materials or substrates that significantly delays or prevents the oxidation of these substances [4].

Antioxidants are bioactive compounds that prevent oxidation of food and pharmaceutical products, thus enhancing their shelf-life and preserving their quality and safety. Antioxidants play a crucial role in the food industry because they prevent spoilage and rancidity of fats and oils. These antioxidants will be used in food industries to retain the color, flavor, and nutritional value of food products, and also due to their significant anti-oxidation property they are used to enhance the shelf-life of processed foods. These antioxidants are also used in pharmaceutical industries or companies to prevent degradation and to preserve the potency of active ingredients and they also play a critical role in prolonging the shelf-life of pharmaceutical products. Antioxidants are also used in various polymer industries where these antioxidants are added to polymers such as plastics, rubber, and coatings to prevent degradation caused by exposure to heat, light, and oxygen. The antioxidants will enhance the overall durability and lifespan of the materials. Common antioxidants used in the polymer industry include hindered phenols, phosphite's, and thioesters. These antioxidants are also used in petroleum and fuel industries where they mix these antioxidants to fuels such as gasoline and diesel to prevent oxidation and improve their stability. This can prevent the formation of harmful by-products and improve the efficiency of the fuel. Common antioxidants used in the fuel industry include phenolic antioxidants and amine-based antioxidants. These antioxidants are also used in personal care products such as lotions, creams, and shampoos to prevent oxidation and extend the shelf life of the products. They can also protect the skin and hair from damage caused by free radicals. Common antioxidants used in personal care products include vitamin E, vitamin C, and green tea extract. They are used to retain the color, flavor, and nutritional value of food products and to enhance the shelf-life of processed foods. Antioxidants are also used in pharmaceutical products to prevent their degradation and preserve the potency of active ingredients, playing a critical role in prolonging the shelf-life of these products.

Antioxidants have been studied extensively for their potential role in preventing or treating various diseases. Extensive research on antioxidants suggests antioxidants may have beneficial effects on certain diseases. Antioxidants play a crucial role in cardiovascular diseases (CVD), antioxidants such as vitamins C and E have the potential to reduce the risk of cardiovascular disease by trapping the organic free radicals or by neutralizing the oxygen free radicals which can induce tissue damage. Though there are no critical studies related to the direct role of vitamin E in preventing cardiovascular disease, few outputs are very promising in evaluating the cardioprotective role of vitamin E in patients with high levels of oxidative stress such as those individuals on hemodialysis (SPACE) or in diabetic individuals with the Hp 2-2 genotype (ICARE) [5]. These antioxidants have a high significance in preventing cancer due to their potential to neutralize the generated free radical which can induce oxidative stress and can damage the DNA by inducing single or double-strand breaks which finally leads to acquired mutations in the genetic material. Extensive studies have revealed that increased levels of exogenous antioxidants have been shown to prevent free radicals-induced cancer [6]. These antioxidants also play a critical role in Alzheimer's disease by reducing the damage caused by free radicals to the DNA, neuronal cell death, and the aggregation of  $\beta$ -amyloid in the brain. Extensive studies on the overexpression of inflammatory cytokines have shown direct evidence in augmenting Alzheimer's disease. High intake of vitamin E and C have shown a lower incidence of Alzheimer's disease [7]. oxidative stress has a direct role in the development of diabetes complications such as diabetic retinopathy and neuropathy these antioxidants play a protective role in reducing the incidence of diabetes by preventing oxidative stress and neutralizing generated free radicals.

In other words, antioxidants are involved in the defense mechanism of organisms against the pathologies associated with free radical attacks. These antioxidants are enzymes, like catalase, glutathione, superoxide dismutase, and peroxidase, or non-enzymes, such as uric acid, bilirubin, albumin, and metallothionein. When these endogenous antioxidants fail to protect organisms, they rely on exogenous antioxidants, which can neutralize the generated free radicals to avoid triggering a catastrophic signal cascade. Nutritional supplements or pharmaceutical products containing active ingredients act as exogenous antioxidants. Vitamin E, vitamin C,  $\beta$ -carotene, vitamin E, flavonoids, and minerals are among the most important exogenous antioxidants. Exogenous antioxidants can be derived from natural sources (vitamins, flavonoids, anthocyanins, and some mineral compounds), but they can also be synthetic compounds, like butylhydroxytoluene, butylhydroxyanisole, and, gallates [8]. These antioxidants have attracted

considerable attention, particularly for preventing the potentially catastrophic effects of free radicals in the human body, as well as the deteriorative effects of fats and other food constituents [9].

Antioxidants protect encapsulated drug molecules from oxidation and degradation, thereby enhancing the shelf-life and stability of drugs. This is particularly crucial for drugs that are prone to oxidation, such as those with unsaturated bonds or those that contain iron or other metals. Antioxidants can also protect nanocarriers from oxidative degradation, thereby preserving their integrity and functionality. Oxidative degradation can alter the physical and chemical properties of nanocarriers, affecting their ability to interact with the target cells and tissues. Antioxidant activity can also enhance the biocompatibility of nanocarriers, thus reducing their toxicity and improving their interactions with biological systems. Moreover, oxidative stress can cause cellular damage and increase inflammation, which can lead to adverse effects and reduced efficacy. Antioxidants can also enhance the pharmacokinetic and pharmacodynamic properties of encapsulated drugs, as they can scavenge free radicals and reduce oxidative stress in the target cells and tissues, leading to improved drug efficacy and reduced toxicity.

Free radicals generated by oxidation can be lethal to cells and cause oxidative DNA damage, resulting in single- or double-strand breakage and ultimately cell death. These radicals can also cause lipid peroxidation and protein carbonylation. Table 1 lists some highly reactive and lethal radical species.

**Table 1.** Free radical and non-free radical species

Free radical species		Non-free radical species	
Hydroxyl radical	HO•	Hydrogen peroxide	H <sub>2</sub> O <sub>2</sub>
Superoxide radical	O <sub>2</sub> •	Singlet oxygen	<sup>1</sup> O <sub>2</sub>
Hydroperoxyl radical	HOO•	Ozone	O <sub>3</sub>
Lipid radical	L•	Lipid hydroperoxide	LOOH
Lipid peroxy radical	LOO•	Hypochlorous acid	HOCl
Peroxy radical	ROO•	Peroxynitrite	ONOO•
Lipid alkoxy radical	LO•	Dinitrogen trioxide	N <sub>2</sub> O <sub>3</sub>
Nitrogen dioxide radical	NO <sub>2</sub> •	Nitrous acid	HNO <sub>2</sub>
Nitric oxide radical	NO•	Nitryl chloride	NO <sub>2</sub> Cl
Thiyl radical	RS•	Nitroxyl anion	NO <sup>-</sup>
Protein radical	P•	Nitrosyl cation	NO <sup>+</sup>

Nanomaterials show immense potential as antioxidants due to their high surface area-to-volume ratio, which enhances their potency to scavenge free radicals and protect against oxidative damage. The most important classes of nanoparticles, including organic (lignin and melanin), metal oxide (cerium oxide), and metal (platinum and gold) nanoparticles exhibit intrinsic redox potential for trapping radical ions and/or superoxide dismutase-like and catalase-like activities. Redox inactive nanomaterials can be transformed into antioxidants by grafting them with low-molecular-weight antioxidants [10]. Designing new antioxidant compounds is crucial because of free radical-associated damage to cellular macromolecules caused by reactive oxygen species (ROS). Therefore, extensive research is being conducted to develop novel potent antioxidants to restrain radical-induced oxidative damage [11]. Furthermore, the orientation of macromolecules in the presence of a plasticizing agent increases the suitability of quercetin for food packaging, whereas the commercial viability of terpenes for replacing existing non-renewable polymers is reinforced by the recyclability of the precursors such as thyme, cannabis, lemon, orange, and mandarin, with their marginal ecological effects and antioxidant properties. Emerging antioxidant polymers have a broad range of applications in tumor-targeted drug delivery, food fortification, biodegradation of synthetic polymers, antimicrobial treatment, and corrosion inhibition [12, 13]. Antioxidant stability refers to the ability of an antioxidant to maintain its potency and activity over time when exposed to different environmental conditions. The stability of an antioxidant can be impacted by temperature, light, humidity, and the presence of other compounds. An antioxidant must be stable to effectively prevent oxidative damage to food or other substances to which it is applied. Antioxidant stability can be assessed by the following methods:

1. High-Performance Liquid Chromatography (HPLC): This method allows the separation and quantification of antioxidants and their degradation products.

2. Spectrophotometry: This technique measures the light absorption capability of antioxidants and can be used to estimate how much antioxidant is present over time.
3. Thermogravimetric Analysis (TGA): This method analyzes the weight loss of an antioxidant as it is subjected to increasing temperatures, providing information about its stability.
4. Antioxidant Activity Assays: These assays measure the antioxidant capacity of a sample by monitoring its ability to scavenge free radicals. The most commonly used assays are the Trolox Equivalent Antioxidant Capacity (TEAC) and Ferric Reducing Antioxidant Power (FRAP) assay.
5. Electron Paramagnetic Resonance (EPR) Spectroscopy: This method measures the stability of antioxidants by monitoring their ability to trap free radicals.

In this context, the present review article describes the synthesis of important antioxidant materials such as green nanomaterials, synthetic organic molecules, as well as synthetic and natural polymer-based materials. In addition, the review focuses on the methods used to determine the antioxidant potential and their detection mechanisms, as well as the applicability, stability, advantages, and disadvantages of antioxidants. Out of the many tests reported in the literature, the following are described in this review: Oxygen radical absorbance capacity (ORAC), Ferric reducing antioxidant power (FRAP), Trolox equivalent antioxidant capacity (TEAC), Trapping antioxidant parameter (TRAP), Dichloro-dihydro-fluorescein diacetate (DCFH-DA), Total oxidant scavenging capacity (TOSC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH). The most important advantages and disadvantages of each method are summarized in **Table 2**[14].

**Table 2.** Antioxidant activity detection methods

Assay methods	Principle of method	End product determination	Reference
DPPH	Antioxidant reaction with an organic radical	Colorimetry	[15]
H <sub>2</sub> O <sub>2</sub> Scavenging activity	Oxidation by hydroxyl free radicals	Spectrophotometer	[16]
Nitric oxide scavenging activity	Nitric oxide free radical scavenging	Colorimetry	[16]
Peroxynitrite radical scavenging activity	Antioxidant reaction with a peroxynitrite radical	Spectrophotometer	[16]
ABTS radical cation	Antioxidant reaction with an organic cation radical	Colorimetry	[15]
TRAP Assay	Antioxidant capacity to scavenge luminol-derived radicals, generated from AAPH decomposition	Spectrometer	[17]
FRAP	Antioxidant reaction with a Fe (III) complex	spectrophotometer	[18]
SOD radical scavenging activity	Antioxidant reaction with superoxide anion radical	Colorimetry	[16]
OH Radical scavenging activity	Antioxidant reaction with hydroxyl free radical	Colorimetry	[16]
HORAC	Antioxidant capacity to quench OH radicals generated by a Co (II) based Fenton-like system	spectrophotometer	[16]
ORAC	Antioxidant reaction with peroxy radicals, induced by	spectrophotometer	[19]

*In vitro*

	2,2'-azobis-2-amidino-propane (AAPH)		
Reducing power method	Reducing the ability of antioxidants	Colorimetry	[20]
Phosphomolybdenum method	Reducing the ability of antioxidants to reduce Mo (VI) to Mo(V)	Colorimetry	[15]
FTC method	Oxidation of ferric ion	Colorimetry	[21]
TBA Method	Oxidation of TBA to TBARS	Colorimetry	[21]
DMPD method	Antioxidant reaction with DMPD cation radical	Colorimetry	[21]
$\beta$ -carotene linoleic acid method	Oxidation of unsaturated linoleic acid	Colorimetry	[21]
Cupric ion reducing antioxidant capacity method	Cu (II) reduction to Cu (I) by antioxidants	Colorimetry	[21]
Metal chelating activity	Antioxidant capacity to quench OH radicals by Fenton-like system	Colorimetry	[21]
The ferric-reducing ability of plasma	Antioxidant capacity to reduce $Fe^{3+}$	Colorimetry	[21]
Reduced glutathione (GSH) estimation	Antioxidant capacity to reduce chromophore	Colorimetry	[21]
Glutathione peroxidase (GSHPx) estimation	The reaction of hydroperoxides with reduced glutathione	Colorimetry	[21]
Glutathione-S-transferase (GSt)	Conjugation of Glutathione to CDNB	Colorimetry	[21]
<i>In vivo</i> Superoxide dismutase (SOD) method	Redox reaction of transition metals	Colorimetry	[21]
Catalase (CAT)	Decomposition of Hydrogen peroxide	Colorimetry	[21]
$\gamma$ -Glutamyl transpeptidase activity (GGT) assay	Transfer of gamma-glutamyl group	Colorimetry	[21]
Glutathione reductase (GR) assay	Reduction of Glutathione	Colorimetry	[21]
Lipid peroxidation (LPO) assay	Formation of Malondialdehyde	Colorimetry	[21]
LDL assay	Formation of Hydrogen peroxide	Colorimetry	[21]

## 2. Antioxidant activity

Antioxidants molecule inhibits or reduces the overall damage caused by the generated free radicals by trapping or reducing the ROS formation. These antioxidants enable their activity by reducing the rate of self-oxidation in oxidizable molecules, or by restraining the free radical generation during the oxidation process to prevent the self-oxidation of substances [18]. In recent years, antioxidants substances draw the attention of researchers due to their

potential to reduce the risk of oxidative stress which is formed by the oxidation of atmospheric oxygen which results in the formation of reactive oxygen species, including  $O_2^{\bullet-}$ ,  $HO^{\bullet}$ ,  $H_2O_2$ , peroxy radicals ( $ROO^{\bullet}$ ), alkoxy radicals ( $RO^{\bullet}$ ), singlet oxygen ( $^1O_2$ ), and reactive nitrogen species (RNS), including peroxyxynitrite ( $ONOO^-$ ) and peroxy radicals [19]. The generated reactive oxygen species have to be neutralized in the body, otherwise, they augment oxidative stress and enhance the risk of various diseases [20]. The intracellular activity of SOD, CAT, and GSH-PX constitutes the primary defense against oxidative stress [21].

The term "antioxidant" is generally used for two completely different groups of compounds such as natural compounds (natural polymers and compounds) and industrial chemicals (synthetic polymers and nano-metals)[22]. Plant-based antioxidants are a type of natural compound employed in the food industry and green synthesis of metal- and carbon-based nanoarchitectures[22]. The use of natural antioxidants is often restricted, owing to their high sensitivity to pH, light, oxygen, etc. Also, polyphenols in natural antioxidants interact with several proteins, decreasing their antioxidant activity[23]. Based on their mechanism of action these antioxidant materials are classified into three categories:[24]

**Protective agents:** This class of antioxidants consists of sunscreens, metal chelating agents, hydroperoxide-decomposing agents, GSH-PX, and superoxide dismutase enzymes. They exhibit their antioxidant activity by reducing the rate of initiation [24].

**Chain-breaking agents:** These compounds trap the free radicals and restrain the autoxidation reaction by competing with the propagation reactions. For example, the reaction between the antioxidant material (AH) and a  $ROO^{\bullet}$  produces a  $ROOH$  and  $A^{\bullet}$ , which traps a second  $ROO^{\bullet}$  producing neutral end products[25].

**Nanoantioxidants:** These nanomaterials exhibit their antioxidant activity by decelerating the rate of autoxidation or declining the initiation step. Nanoantioxidants exhibit higher stability compared to other counterparts such as vitamin E and  $\beta$ -carotenes, enabling them to avoid quick metabolic activity and to target precisely. Nanomaterials, such as Ag and ZnO nanoparticles, have intrinsic antioxidant properties as cerium oxide nanoparticles are used for various purposes. These nanoparticles could also be employed as nanocarriers to carry antioxidant compounds as shown in Table 3 [26].

**Table 3:** Nanoparticles and nanocarriers antioxidants

Type of nanomaterials	Antioxidant mechanism	Affect	Applications	Ref.
$Co_3O_4$ nanoparticles	Electron transfer	Peroxidase mimics	To screen enzymes inhibitors	[26]
Cs-f- $SiO_2@Fe_3O_4$	Electron transfer	DPPH mimics	Tumor tissues and imaging	[27]
MXene@ $CeO_2$	Electron-transfer	Antioxidant	ROS, Wound healing	[28]
Au/ $CeO_2$ core-shell nanoparticles	Electron transfer	Peroxidase, catalase, and superoxide dismutase mimic	Glucose detection	[29]
PEG-Au nanoparticles	Oxidative stress	DPPH mimics	In human neuroblastoma SHSY5Y cells, ROS generation	[30]
$Fe_3O_4@MoS_2$ -Ag	Electron transfer	Peroxidase mimics	Antibacterial activity	[31]
Ag-PDA-g- $C_3N_4$	Electron transfer	ROS mimics	Antibacterial activity	[32]
$CeO_2/Mn_3O_4$	Electron transfer	ROS mimics	Radioprotectants for preventing ARS induced by TBI	[33]
$MoS_2$ nanosheets	Electron transfer	Superoxide dismutase, catalase, and peroxidase mimic	Hepatic fibrosis therapy	[34]
$MnO_2$ nanosheets	Electron transfer	catalase, and superoxide dismutase mimics	Support for neuroblastoma cells	[35]

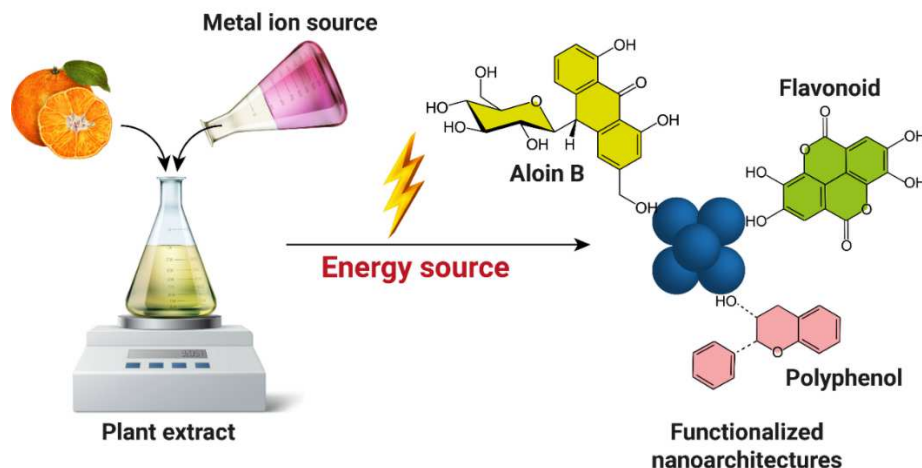
DPPH: 2,2-diphenyl-1-picrylhydrazyl; ROS: Reactive oxygen species; PEG: Polyethylene glycol; GA: Gallic acid; CS: chitosan; TMDs: Transition metal dichalcogenides. ARS Androgen Receptor Aggravates; TBI Traumatic Brain Injury

### 3. Antioxidant materials

#### 3.1. Green synthesized nanomaterials

In addition to the fact that plants, fruits, vegetables, and polysaccharides are rich in natural antioxidants, the synthesis of nanoparticles using green technology also has intrinsic antioxidant properties which make them attractive for medical applications [36]. Biosynthesis methods can positively affect the antioxidant activity of nanomaterials lacking intrinsic antioxidant properties [37]. This positive effect is probably due to the presence of antioxidants in plant-based materials. Natural antioxidants, such as amino acids, carboxylic acids, and phenolic compounds, act as antioxidants and stabilizers for metal structures [38]. During the synthesis process, antioxidants are often present on the surface of nanostructures and synergistically augment antioxidant activity [38]. According to the above, the reduction of free radicals can be found in biosynthesized nanoparticles.

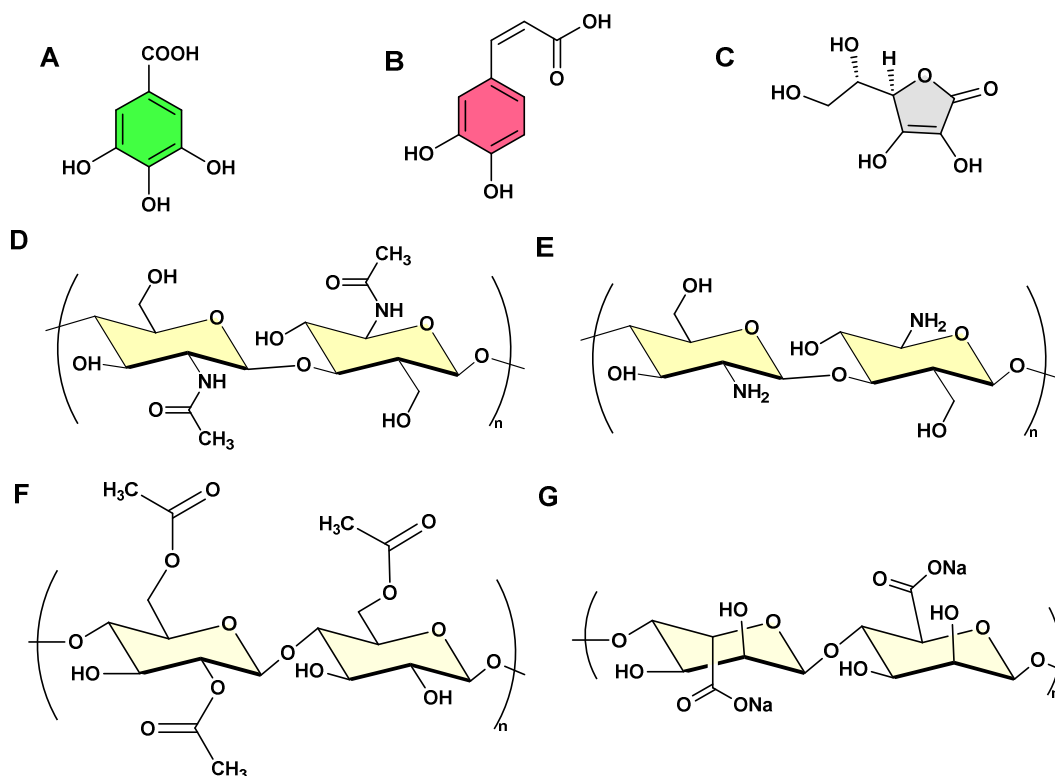
Green synthesis is an appropriate method for the synthesis of metal- and carbon-based nanoarchitectures due to its ease of synthesis, low cost, and environmental friendliness. (**Figure 1**) [39]. The green synthesis can be carried out by plant extract [40], microorganisms (bacteria and fungi) [41], natural polymers[42], and fruit juice [43]. The presence of various compounds such as proteins, polyphenols, and amino acids in natural compounds can influence biological properties (e.g., antimicrobial activity, low toxicity, and antioxidant activity) of metal- and carbon-based nano architectures [44-46]. It was reported that the biosynthesized nanomaterials by plant extract have surprisingly extraordinary biological activities such as antioxidant and antimicrobial properties because of functionalization with organic molecules including polyphenols, flavonoids, etc. [43], compared to non-functionalized nanomaterials.



**Figure 1.** Green synthesis of metal/metal oxide nanoparticles from plant extracts containing natural antioxidants. Heat, light, or microwave have been used as the energy source depending on the type of nanomaterials to be synthesized.

#### 3.2. Natural organic compounds

Plants are immense sources of natural antioxidants, and several medicinal plants have been also found to possess health-boosting antioxidant properties[47]. These natural antioxidants include phenolic compounds, phenolic acids (gallic, caffeic, protocatechuic, and rosmarinic acids), phenolic diterpenes (carnosic acid and carnosol), flavonoids (catechin and quercetin), carotenoids, tocopherols, and volatile oils (carvacrol, eugenol, menthol, and thymol), which can prevent the generation of free radicals and inhibit  $\text{Fe}^{3+}/\text{AA}$ -induced oxidation (**Figure 2A-C**) [48]. Phenolic acids exhibit their antioxidant activity by trapping free radicals; flavonoids can scavenge free radicals and chelate metal ions as well. Multiple hydroxyl groups on flavonoids show better antioxidant properties than those of only having one by suppressing ROS generation either by inhibiting enzymatic activity or by chelating the elements involved in free radical formation [49]. Spices and herbs used in a foodstuff have also been discovered to contain significant concentrations of phenolic compounds that have a strong H-donating capacity. Moreover, many plants derived compounds such as carnosol, rosmanol, rosmariquinone, and rosmaridiphenol illustrate better antioxidant activity than butylated hydroxyanisole (BHA) [50].



**Figure 2.** Chemical structures of natural organic and polymer-based antioxidants (A) Gallic acid; (B) caffeic acid; (C) ascorbic acid; (D) chitin; (E) chitosan; (F) modified starch and (G) sodium alginate.

### 3.3. Natural polymer-based antioxidants

Antioxidants derived from natural polymers and polysaccharides have recently received a lot of attention due to their low cost, biocompatibility, and non-toxic nature, which restricts the use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in food [51]. Natural biopolymers of various sources such as plants (Cellulose, starch, and natural rubber), fungi (chitin), algae (alginate), bacteria (exopolysaccharides and bacterial cellulose), and animals (Chitosan collagen and hyaluronic acid) are among the most studied natural antioxidants [52]. Among the mentioned sources, marine-based polysaccharides and oligosaccharides exhibited extraordinary antioxidant properties that effectively scavenge the superoxide, and hydroxy radicals, reducing oxidative damage to tissue and cells and thus preventing or slowing down the progression of certain chronic diseases. It was reported that the presence of functional groups such as sulfate, phosphate, hydroxyl, and amine group can activate the hydrogen atom of the anomeric carbon thereby enhancing the antioxidant activity of native polysaccharides [53]. Furthermore, low molecular weight (1 < kDa) polysaccharides have greater antioxidant properties than high molecular weight (5- 10 kDa) polysaccharides because of the presence of a higher number of reducing units, thereby scavenging the superoxide, hydroxyl, alkyl, and DPPH radicals *in vitro* [53].

Several polysaccharides such as starch, alginic acid, chitosan, and pectin, are frequently used as stabilizing agents in processing meat products. These polysaccharides have been shown to have excellent antioxidant properties, including the ability to scavenge free radicals and reduce or chelate the ferrous ions thereby preventing lipid hydroperoxides decomposition to form off-flavor compounds [54]. Chitosan is a natural biopolymer made up of polysaccharide units that have been discovered to have exceptional biological properties such as low toxicity, biodegradability, and biocompatibility, as well as the potential to be used as an antioxidant. Chitin can be prepared from crustacean shells composed of  $\beta$ -(1 $\rightarrow$ 4)-linked 2-acetamido-2-deoxy-  $\beta$ -D-glucose units, whereas chitosan can be produced by deacetylation of chitin (**Figure 2 (D-F)**). Chitin and chitosan are both insoluble in water, which limits their potential application in living systems. However, chemical, physical, and enzymatic methods yield water-soluble



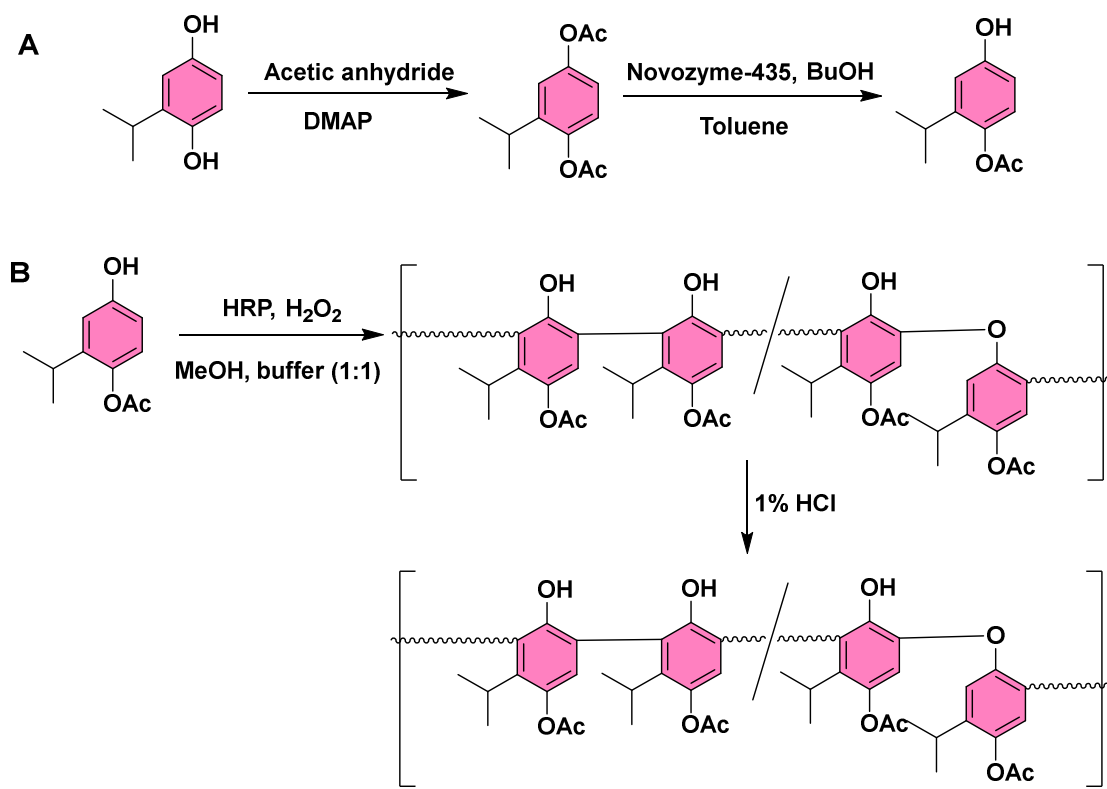
chitin or chitosan which has a potential impact on biomedical applications. Especially, the use of chitin or chitosan can act as antioxidants or free radicals in the human body thereby preventing the generation of ROS. Thus, chemically modified chitosan can be used both *in vitro* and *in vivo* in increasing the antioxidant enzymes such as CAT, SOD, and GSH-PX.

Starch is another natural biopolymer that has received a lot of attention for wound healing applications because of its low-cost, availability, biocompatibility, and biodegradable nature. This natural polysaccharide is composed of anhydroglucose units, which can form two different polymers namely amylose and amylopectin. Amylose is a linear polymer chain formed by  $\alpha$ -(1,4)-D-glucose linkage, whereas amylopectin is a branched polymer chain formed linearly by linking with  $\alpha$ -(1,4)-D-glucose as well as with  $\alpha$ -(1,6)-D-glucose linkage with glucose sub units [55]. Because of the presence of hydroxyl groups in the repeating units of the polysaccharide chain, starch could be modified chemically[56]. Carboxymethylated starch, cationic starch, hydroxypropylated starch, and starch acetate are a few examples of modified starch prepared by physical, chemical, or enzymatic modification of native starch in order to improve its physical, chemical, and mechanical properties of the starch. Among these, carboxymethylated starch has received a lot of attention due to its green water-soluble polysaccharide with a high number of carboxymethyl groups which has found widespread use in pharmacy, wound dressing, food processing, textile printing, and in drug delivery [57].

### 3.4.Synthetic polymer and organic-based antioxidants

During the past few decades, a great deal of attention has been given to synthetic antioxidants for their potential use in biomedical fields including the development of artificial muscle, nerve regeneration, and drug release due to their low cytotoxicity and good biocompatibility [58, 59]. The synthetic antioxidants were classified into two subgroups namely synthetic polymers and organic-based antioxidants. The most commonly used synthetic antioxidants include butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, tertiary butylhydroquinone, and several synthetic conductive polymers including polypyrrole, and polyaniline have also been employed [60]. These synthetic antioxidants have various advantages including ease of preparation, low cost, and higher antioxidant efficiency than natural antioxidants. Moreover, the presence of polar functional groups like phenolic and amine functional groups in synthetic antioxidants increases the solubility of lipids and hence gained much importance in food packing industries [61].

2-tert-butylhydroquinone as a substituted polyphenolic compound is the most widely used antioxidant in the food industry. It can be polymerized through horseradish peroxidase (HRP) as a catalyst. The process is as follows: First, the hydroxyl groups of 2-tert-butylhydroquinone were protected by acetylation and one of the acetyl groups is hydrolyzed using lipase enzyme resulting in the formation of 4-acetoxy-3-tert-butylphenol (**Figure 3A**). Next, 4-acetoxy-3-tert-butylphenol (**Figure 3A**) was polymerized using HRP and hydrogen peroxide to form 2-tert-butylhydroquinone polymer which has good antioxidant activity (**Figure 3B**) [62].



**Figure 3.** Synthesis of 4-acetoxy-3-tert-butylphenol as an antioxidant in the food industry (A); synthetic route of polymerization of tert-butylhydroquinone (t-BHQ) polymer using horseradish peroxidase (HRP) as catalyst (B).

Inherently conductive polymers (ICPs) are another class of synthetic antioxidants with distinct electronic and ionic properties. They can be synthesized using various methods including electrochemical polymerization, self-assembly, chemical oxidation, hydrothermal, template-assisted, electrospinning, photochemical, inclusion method, solid-state, and plasma polymerization methods [63-65]. Different types of ICPs are known and their classification is based upon the delocalization of  $\pi$  electrons which are freely movable, and their electrical and optical properties. Polyacetylene (PA), polythiophene (PT), poly[3,4 (ethylenedioxy)thiophene] (PEDOT), polypyrrole (PPy), and polyaniline (PANI) are the most explored ICPs owing to their high thermal conductivity, flexibility, and quick charge-discharge capability (Figure 4A) [66]. CPs are known to interact with biological samples, and thus find extensive applications in biomedicine as antioxidants, free radical scavengers, photosensitizers, and as antimicrobial agents,[67] because of their rapid response to high electrical conductivity from various types of tissues, good biocompatibility caused by its hydrophilic nature, low cytotoxicity and good environmental stability.

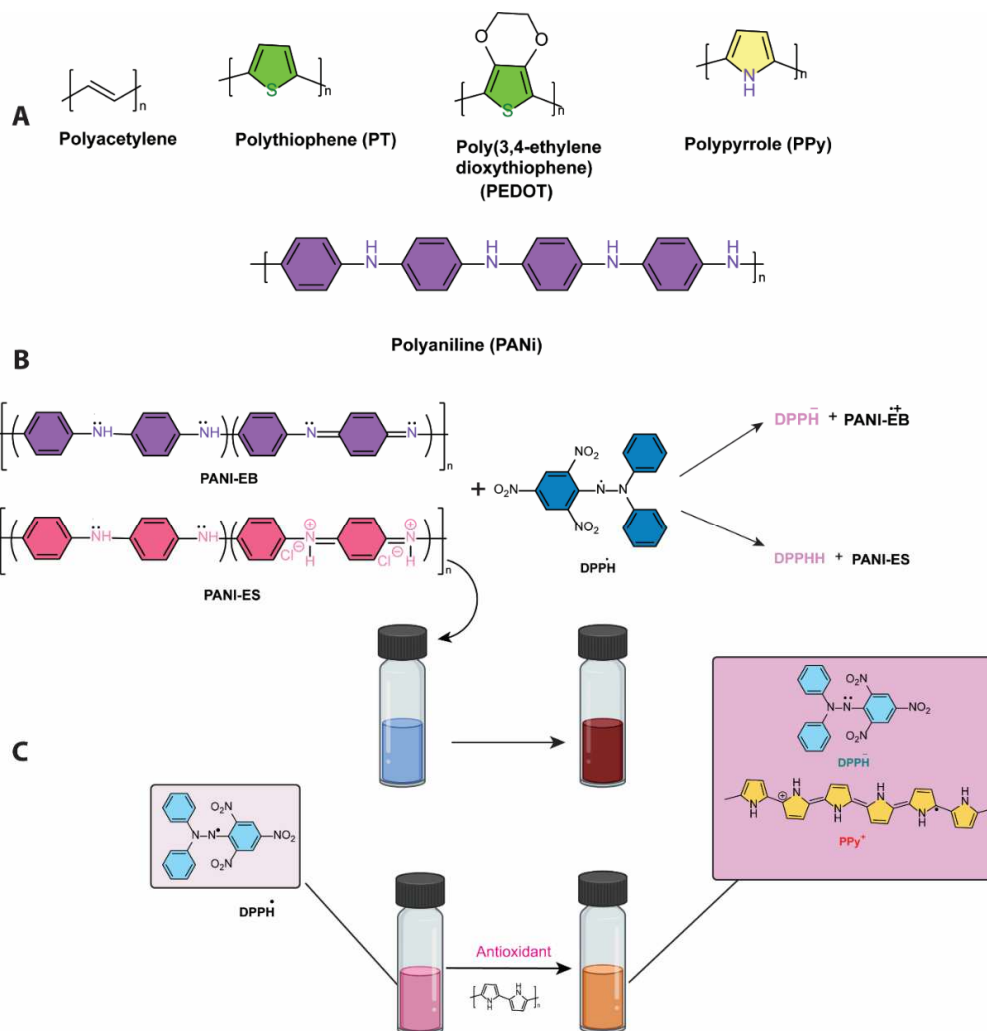
Among ICPs, polyaniline gained a lot of attention in biomedical fields due to its low cost, ease of preparation, low cytotoxicity, biocompatibility, and environmentally friendly nature [68]. Recently, polyaniline nanocomposites with improved antioxidant activity are extensively used in the field of biomedical applications such as tissue reengineering and antimicrobial applications, etc.[69, 70]. They play a crucial role by scavenging free radicals in food and tissues, lowering the risk of chronic diseases. The ICPs such as PANI grafted to lignin, poly(aniline sulphonic acid), and polypyrrole (PPy) demonstrated good antioxidant activity in the presence of 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a free radical scavenger [71]. The antioxidant activity of carbon dots in doped PANI nanofibers showed better antioxidant properties when compared to undoped PANI nanostructures [72]. It has been reported that PANI nanofiber/carbon dot nanohybrid can scavenge approximately 86% of DPPH within 30 min and outperforms HCl-doped PANI [73]. It is noteworthy that the antioxidant potency of any material is determined by its ability to reduce DPPH by donating hydrogen, thus both PANI and carbon dots contained lone pair of electrons that can delocalize over their conjugate structure and exhibits better antioxidant properties. There have been few other studies on the antioxidant activity of PANI nanocomposites in biomedical fields such as wound healing applications [74]. The

antioxidant activity of poly( $\epsilon$ -caprolactone) (PCL) and quaternized chitosan-*graft*-polyaniline (QCSP) polymer biocomposite is enhanced upon increasing the QCSP ratio [75]. This is due to the redox nature of PANI, it can be observed that PCL/QCSP composite can effectively scavenge DPPH free radicals up to 80% upon increasing the concentration of QCSP polymer. Further, these electroactive materials become electric conductors by the reversible exchange of ions between oxidation and reduction states [76]. Besides this PCL/QCSP, biocomposite has profound potential in wound dressing due to its electroactive nature [75]. Based on the literature data the plausible mechanism involved in displaying antioxidant activity of PANI in the presence of DPPH is depicted in **Figure 4B**.

Polypyrrole is another example of ICPs made up of heterocyclic monomers joined together at the 2- and 5-positions [77]. This polymer can be prepared either alone or in combination with other nanomaterials and exhibited remarkable properties such as improved physicochemical, and mechanical properties, superior electrical conductivity as well as good biocompatibility [78]. Thanks to the polypyrrole, the composites consisting of polypyrrole have shown potential applications in bioscience including surgical implants, tissue engineering, antimicrobial, and photothermal agents [79, 80]. Another advantage of polypyrrole composites is their antioxidant activity, they can compensate ROS such as  $H_2O_2$ ,  $O_2^{\bullet-}$ ,  $HO^{\bullet}$ , and RNS generated in food and tissues during metabolism in plants and animals [81]. Polypyrrole is a potent antioxidant that can neutralize free radicals by donating electrons or hydrogen atoms to radicals, thereby preventing oxidative stress. These free radicals can be neutralized or removed *via* an intracellular antioxidant mechanism generated during the oxidation of lipids causing food poison which adversely affects human health causing various diseases including cancer, and cardiovascular diseases [82]. Several dopants such as HCl or  $H_2SO_4$  are used in the synthesis of polypyrrole which enhances the antioxidant activity by creating an active site in the polypyrrole composite [83]. The antioxidant property of polypyrrole mainly depends on various parameters such as the size of the polymer, synthesis method, and chemical structure. Increasing the surface area of the polymer containing polypyrrole enhances the antioxidant activity property of that material [84]. Composite films prepared from micro fibrillated cellulose, chitosan, and polypyrrole have demonstrated better antioxidant activity [85]. Therefore, these materials can be ideal candidates in the biomedical field such as antimicrobial agents as well as in biodegradable food packing industries.

One of the most widely used assays for the evaluation of the antioxidant activity of polymer composites is by using DPPH as a free radical scavenger. During the antioxidant activity between a composite containing polypyrrole and DPPH, the polymer inactivates the DPPH via electron or hydrogen transfer [61]. The plausible mechanism of polypyrrole composite to scavenge DPPH radical is shown in **Figure 4C**.

Recently, polypyrrole composites such as polypyrrole-*co*-poly indole doped carboxylated CNT was developed as an antibacterial agent, in which polypyrrole works as an antioxidant material. In addition, this polypyrrole composite found application range from wound dressing to antibacterial agents [86]. Furthermore, a recent study shows that polypyrrole composites could be used as a hydrogel for antibacterial applications.



**Figure 4.** Chemical structures of conjugated polymers (CPs) (A); Plausible mechanism of antioxidant activity of polyaniline in the presence of DPPH radical scavenger (B); Proposed mechanism of antioxidant activity of polypyrrole in the presence of DPPH radical scavenger (C).

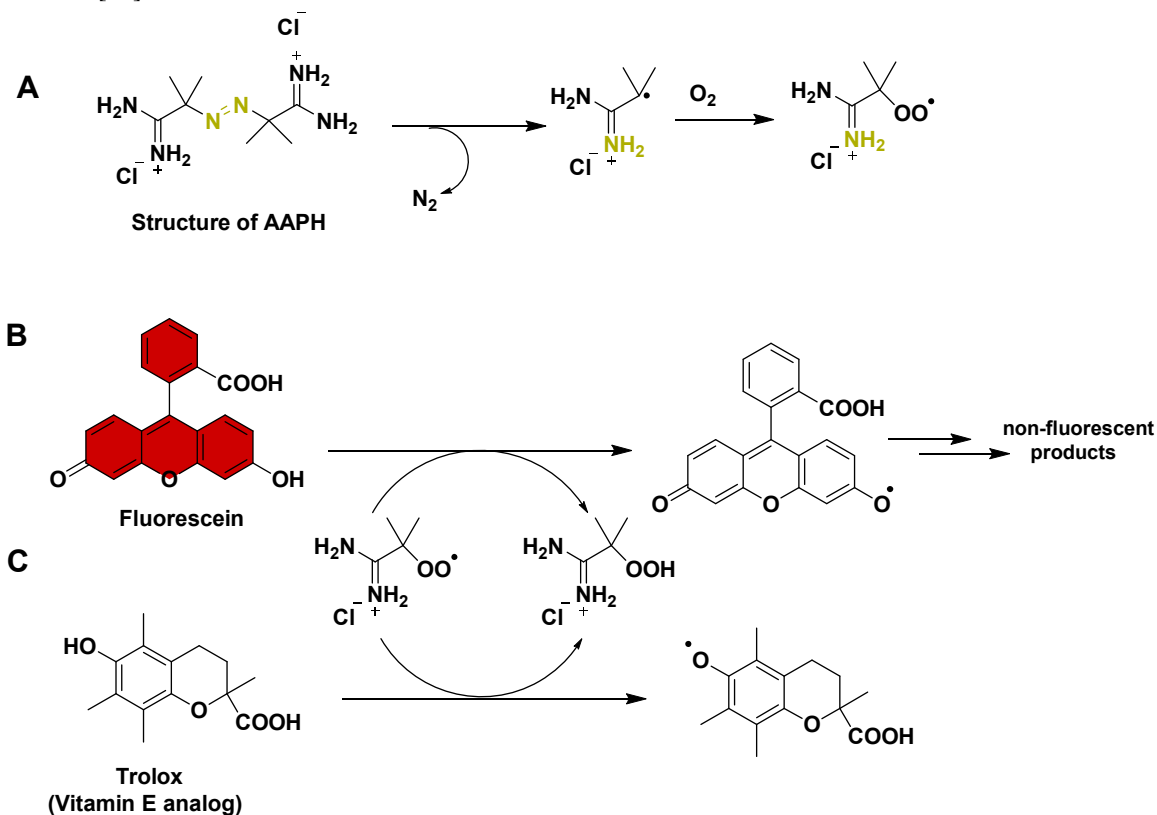
#### 4. Measuring and mechanism of antioxidant activity

There are several methods to evaluate the antioxidant potential of the compounds which are either based on direct interaction with reactive molecules or free radicals reacting with metal ions [87]. Various assay methods can be employed to measure antioxidant activity *in vitro* and *in vivo*. There are several methods used to measure antioxidant potential *in-vitro*, these include Oxygen Radical Absorbance Capacity (ORAC), Ferric Reducing Antioxidant Power (FRAP), Trolox Equivalent Antioxidant Capacity (TEAC), Trapping Antioxidant Parameter (TRAP), Dichlorofluorescein-Diacetate (DCFH-DA), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH•).

The methods used to measure antioxidant potential *in vivo* include Total Antioxidant Capacity (TAC), which measures the capacity of biological fluids such as blood or plasma to neutralize free radicals, Glutathione assay, which measures the levels of oxidized glutathione in blood, urine, and tissue samples, Super Oxide Dismutase Activity assay, which measures enzymatic activity in blood, plasma, or tissues, and electrochemical methods such as Cyclic Voltammetry, Differential Pulse Voltammetry, and Chronoamperometry.

**Oxygen radical absorbance capacity (ORAC):** This method determines the efficiency of different natural antioxidants, existing in plasma/tissue, in inhibiting the ROO• oxidation of the fluorescein. The reaction proceeds with the thermal decomposition of azo compounds, such as 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) (**Figure 5A**), which is the source of free radicals such as ROO• for degradation of fluorescein (**Figure 5B**). This

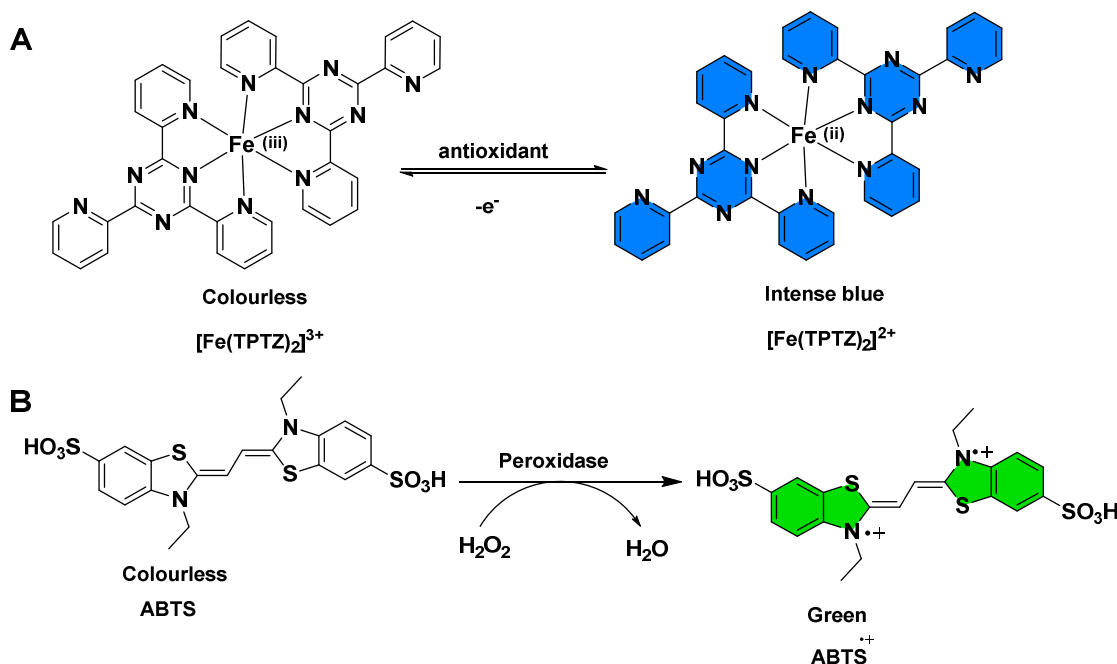
technique relies on the ability of antioxidants to inhibit  $\text{ROO}^\bullet$  oxidation, which can be evaluated by loss of fluorescence intensity, during  $\text{ROO}^\bullet$  damage. This technique is not a proper approach to determining a single antioxidant. Further, this method uses Trolox as an analytical standard (**Figure 5C**), therefore the antioxidant property is expressed in Trolox equivalence. Besides, the ORAC technique was restricted to hydrophilic antioxidants owing to the aqueous environment [88].



**Figure 5.** Radical chemistry of ORAC assay. The water-soluble AAPH forms radicals that react with fluorescein resulting in loss of fluorescence. The presence of antioxidants (Trolox) slows down the loss of fluorescence.

**Ferric reducing antioxidant power (FRAP):** This technique determines the power of antioxidants with the assistance of an oxidant, i.e., ferric ions ( $\text{Fe}^{3+}$ ) (**Figure 6A**). Values of FRAP are achieved by comparing the absorbance variation at 593 nm in examination reaction mixtures with those including  $\text{Fe}^{2+}$  in defined concentration. In this method, antioxidants in the Equivalence range present in the Fe (III)/tripyriddytriazine reduce samples to form a blue ferrous. Indeed, the absorbance change is related to the combined  $\text{Fe}^{3+}$  reducing/antioxidant which determined the power of the antioxidants in the sample [89].

**Trolox equivalent antioxidant capacity (TEAC):** When 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) is incubated in the existence of peroxidase and  $\text{H}_2\text{O}_2$  or the existence of  $^\bullet\text{OH}$ ,  $\text{ROO}^\bullet$ ,  $\text{RO}^\bullet$  and inorganic radicals,  $\text{ABTS}^{+\bullet}$  is produced. Once antioxidants are added before the  $\text{H}_2\text{O}_2$  addition, they hunt the radicals produced by the  $\text{H}_2\text{O}_2$ , delaying the  $\text{ABTS}^{+\bullet}$  formation, thus inducing an increase in the inhibition absorbance percentage (**Figure 6B**). This method is based on the inhibition through antioxidants of the  $\text{ABTS}^{+\bullet}$  absorbance, which has a specific long-wavelength absorption displaying maxima at 660, 734, and 820 nm [90].



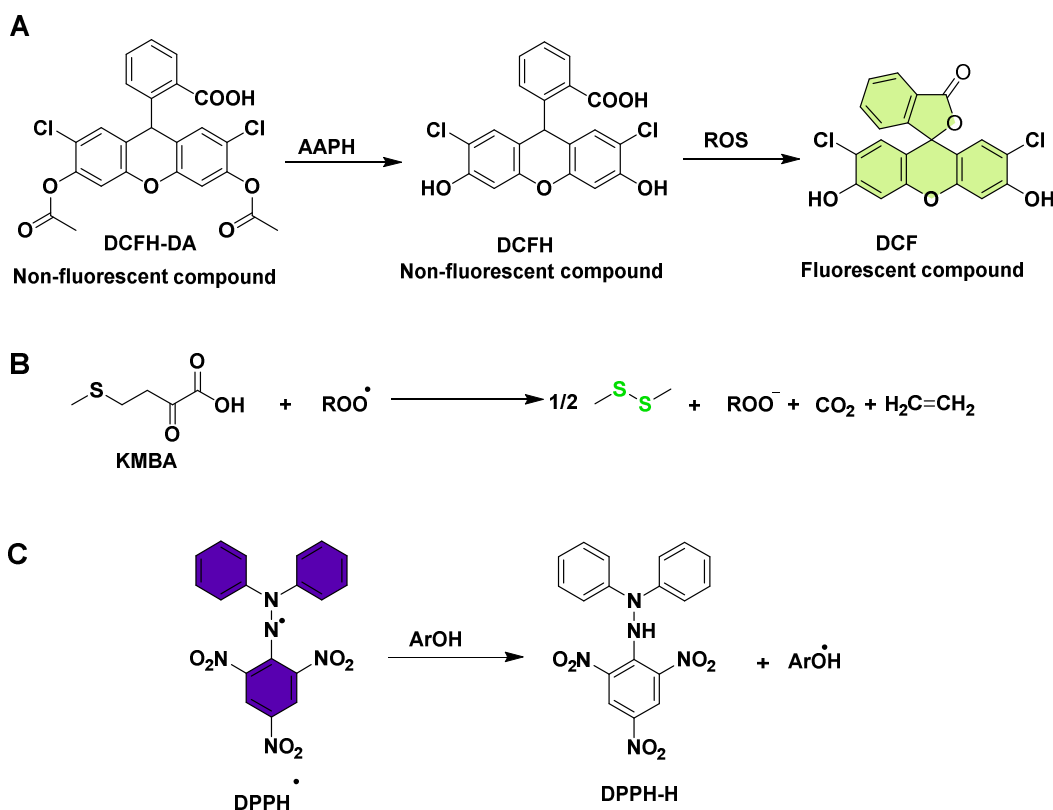
**Figure 6.** Antioxidant assay by using Fe (III)/tripyridyltriazine as ferric ions ( $\text{Fe}^{3+}$ ) source (A); TEAC assay using ABTS as a radical initiator in the presence of peroxidase as an enzyme (B).

**Trapping antioxidant parameter (TRAP):** This method is based on the determination of  $\text{O}_2$  consumption during a lipid peroxidation reaction produced through azo-compound decomposition such as AAPH. TRAP is the most extensively employed technique for determining plasma/serum antioxidant capacity. Once AAPH is added to the plasma, the oxidation of the oxidizable materials is observed by determining the  $\text{O}_2$  consumed during the reaction.

**Dichlorofluorescein-diacetate (DCFH-DA):** The AAPH used in this method is to peroxy radicals and DCFH-DA as the oxidizable compound for the peroxy radicals. The oxidation of DCFH-DA resulted in dichlorofluorescein (DCF) (Figure 7A). Moreover, the formed DCF can be observed either by spectrophotometric or fluorometric techniques [91].

**Total oxidant scavenging capacity (TOSC):** With the assistance of this method, the absorbance capacity values of antioxidants towards three strong oxidants (e.g.,  $\cdot\text{OH}$ ,  $\text{ROO}\cdot$  and  $\text{ONOO}^-$ ) can be measured (Figure 7B). These oxidants were produced through the reaction of iron plus ascorbate-driven Fenton, AAPH thermal homolysis, and 3-morpholinopyridone *N*-ethylcarbamide, respectively. They react with alpha-keto-gamma-methylbutyric acid (KMBA), which results in the production of ethylene via the oxidation process. The antioxidant potency of the materials is evaluated based on their ability to inhibit ethylene formation relative to a control reaction [92].

**2,2-diphenyl-1-picrylhydrazyl (DPPH $\cdot$ ):** DPPH $\cdot$  is a stable free radical, because of the electron delocalization on the whole structure. Consequently, it does not dimerize, as occurs with most free radicals. The delocalization on the DPPH $\cdot$  molecule defines the happening of a purple color, with a maximum absorption peak around 517 nm. Once DPPH $\cdot$  reacts with a hydrogen donor, the DPPH-H or DPPH-R is produced, attended by the violet color disappearance (Figure 7C). Consequently, the absorbance decrease depends directly on the antioxidant concentration. The spectrophotometric technique was employed to determine the antioxidant capacity of materials in the DPPH $\cdot$  solution. The dependence on tested antioxidant materials can be utilized by either of the above methods [93-95].



**Figure 7.** The Non-fluorescent dichlorofluorescein-diacetate (DCFH-DA) reacts with water-soluble AAPH to form a nonfluorescent compound (DCFH). Later reactive oxygen species (ROS) induced oxidation of DCFH to form highly fluorescent dichlorofluorescein (DCF) molecule (A); Reaction of alpha-keto-gamma-methylbutyric acid (KMBA) with oxidant  $\text{ROO}^\bullet$  (B); The Reduction of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH $^\bullet$ ) with hydrogen donor (antioxidant phenol ArOH) DPPH-H is produced (C).

## 5. Conclusions and perspective

Free radicals are highly active and lethal radicals that can deteriorate cells. Antioxidants are gaining a lot of attention as a countermeasure to free radicals, protecting cells from oxidative damage, and preventing oxidative stress-mediated pathological processes. The identification of efficient antioxidants and the design of new ones are crucial to counteract free radical mutilations. Enumeration of antioxidant capability and stability is essential to evaluate the radical scavenging activity of designed antioxidants. Reliable methods to enumerate this activity are critical to assess the potential of antioxidants in *in-vitro* and *in-vivo* analyses.

In this review, we discuss the significance of antioxidants in neutralizing free radicals and protecting biological macromolecules. We also cover the latest developments in designing new synthetic and natural antioxidants. Additionally, we explore the applications of synthetic, natural, and green-synthesized nanoparticles and nanocarriers in delivering antioxidant molecules.

We made sincere efforts to discuss both conventional and advanced methods employed to estimate the antioxidant capacity of any antioxidants. Assay methods to measure both hydrophilic and lipophilic antioxidants are crucial. Methods such as ABTS and CUPRAC can measure both types of antioxidants and evaluate their inhibition activity. Furthermore, there is always a demand to develop novel assay methods to evaluate antioxidant capacity in foods, fruits, vegetables, and other antioxidant molecules. Electrochemical biosensors are vital in determining antioxidant capacity and studying process kinetics. The development of novel electrochemical biosensors to measure accurate inhibition activity will provide an efficient method to evaluate the antioxidant potential of antioxidants.

## Conflict of interest

The authors declare no conflict of interest.

## References

- [1] B. Hazra, S. Biswas, N. Mandal, Antioxidant and free radical scavenging activity of *Spondias pinnata*, *BMC Complement Altern Med.* 8(1) (2008) 63.
- [2] R. Sarwar, U. Farooq, A. Khan, S. Naz, S. Khan, A. Khan, A. Rauf, H. Bahadar, R. Uddin, Evaluation of Antioxidant, Free Radical Scavenging, and Antimicrobial Activity of *Quercus incana* Roxb, *Front.Pharmacol.* 6 (2015) 277.
- [3] R. Tsao, Chemistry and biochemistry of dietary polyphenols, *Nutrients.* 2(12) (2010) 1231-46.
- [4] B. Halliwell, How to characterize a biological antioxidant, *Free Radic.Res.Commun.* 9(1) (1990) 1-32.
- [5] M. Vardi, N.S. Levy, A.P. Levy, Vitamin E in the prevention of cardiovascular disease: the importance of proper patient selection, *J Lipid Res.* 54(9) (2013) 2307-14.
- [6] V. Fuchs-Tarlovsky, Role of antioxidants in cancer therapy, *Nutrition.* 29(1) (2013) 15-21.
- [7] M.C. Morris, D.A. Evans, J.L. Bienias, C.C. Tangney, D.A. Bennett, N. Aggarwal, R.S. Wilson, P.A. Scherr, Dietary Intake of Antioxidant Nutrients and the Risk of Incident Alzheimer Disease in a Biracial Community Study, *JAMA.* 287(24) (2002) 3230-3237.
- [8] M.S. Brewer, Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications, *Compr. Rev. Food Sci. Food Saf.* 10(4) (2011) 221-247.
- [9] M. Pateiro, F.J. Barba, R. Domínguez, A.S. Sant'Ana, A.M. Khaneghah, M. Gavahian, B. Gómez, J.M.J.F.R.I. Lorenzo, Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review, *Food Res. Int.* 113 (2018) 156-166.
- [10] H. Kumar, K. Bhardwaj, E. Nepovimova, K. Kuča, D.S. Dhanjal, S. Bhardwaj, S.K. Bhatia, R. Verma, D. Kumar, Antioxidant Functionalized Nanoparticles: A Combat against Oxidative Stress, *Nanomaterials.* (Basel) 10(7) (2020).
- [11] M. Dizdaroglu, P. Jaruga, M. Birincioglu, H. Rodriguez, Free radical-induced damage to DNA: mechanisms and measurement, *Free Radic. Biol. Med.* 32(11) (2002) 1102-15.
- [12] S.C. Lourenço, M. Moldão-Martins, V.D. Alves, Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications, *Molecules.* 24(22) (2019).
- [13] R. Jamaledin, C.K.Y. Yiu, E.N. Zare, L.-N. Niu, R. Vecchione, G. Chen, Z. Gu, F.R. Tay, P. Makvandi, Advances in Antimicrobial Microneedle Patches for Combating Infections, *Adv Mater.* 32(33) (2020) 2002129.
- [14] M.C. Christodoulou, J.C. Orellana Palacios, G. Hesami, S. Jafarzadeh, J.M. Lorenzo, R. Domínguez, A. Moreno, M. Hadidi, Spectrophotometric Methods for Measurement of Antioxidant Activity in Food and Pharmaceuticals, *Antioxidants.* 11(11) (2022) 2213.
- [15] C. Wan, Y. Yu, S. Zhou, W. Liu, S. Tian, S. Cao, Antioxidant activity and free radical-scavenging capacity of *Gynura divaricata* leaf extracts at different temperatures, *Pharmacogn Mag.* 7(25) (2011) 40-5.
- [16] A. Phaniendra, D.B. Jestadi, L. Periyasamy, Free radicals: properties, sources, targets, and their implication in various diseases, *Indian J. Clin. Biochem.* 30(1) (2015) 11-26.
- [17] K. Schlesier, M. Harwat, V. Böhm, R. Bitsch, Assessment of antioxidant activity by using different in vitro methods, *Free Radic. Res.* 36(2) (2002) 177-87.
- [18] R.P. Fernandes, M.A. Trindade, F.G. Tonin, C.G. Lima, S.M. Pugine, P.E. Munkata, J.M. Lorenzo, M.P. de Melo, Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers, *J Food Sci. Technol.* 53(1) (2016) 451-60.
- [19] B.J.R. Gregório, I.I. Ramos, L.M. Magalhães, E.M.P. Silva, S. Reis, M.A. Segundo, Microplate ORAC-pyranine spectrophotometric assay for high-throughput assessment of antioxidant capacity, *Microchem. J.* 158 (2020) 105156.
- [20] N.R. Bhalodia, P.B. Nariya, R.N. Acharya, V.J. Shukla, In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of *Cassia fistula* Linn, *Ayu.* 34(2) (2013) 209-14.
- [21] R.R. Kotha, F.S. Tareq, E. Yıldız, D.L. Luthria, Oxidative Stress and Antioxidants; A Critical Review on In Vitro Antioxidant Assays, *Antioxidants.* 11(12) (2022) 2388.
- [22] A. Augustyniak, G. Bartosz, A. Čipak, G. Duburs, L.U. Horáková, W. Łuczaj, M. Majekova, A.D. Odysseos, L. Rackova, E. Skrzydlewska, M. Stefek, M. Štrosová, G. Tiritis, P.R. Venskutonis, J. Viskupicova, P.S. Vranka, N. Žarković, Natural and synthetic antioxidants: An updated overview, *Free Radic. Res.* 44(10) (2010) 1216-1262.
- [23] F. Arriagada, G. Günther, J. Nos, S. Nonell, C. Olea-Azar, J. Morales, Antioxidant Nanomaterial Based on Core-Shell Silica Nanospheres with Surface-Bound Caffeic Acid: A Promising Vehicle for Oxidation-Sensitive Drugs, *Nanomaterials.* 9(2) (2019) 214.
- [24] L. Valgimigli, A. Baschieri, R. Amorati, Antioxidant activity of nanomaterials, *J. Mater. Chem. B* 6(14) (2018) 2036-2051.
- [25] K.U. Ingold, D.A. Pratt, Advances in Radical-Trapping Antioxidant Chemistry in the 21st Century: A Kinetics and Mechanisms Perspective, *Chem. Rev.* 114(18) (2014) 9022-9046.
- [26] J. Dong, L. Song, J.-J. Yin, W. He, Y. Wu, N. Gu, Y. Zhang, Co<sub>3</sub>O<sub>4</sub> Nanoparticles with Multi-Enzyme Activities and Their Application in Immunohistochemical Assay, *ACS Appl Mater Interfaces.* 6(3) (2014) 1959-1970.



- [27] M. Zare, M.N. Sarkati, Chitosan-functionalized Fe<sub>3</sub>O<sub>4</sub> nanoparticles as an excellent biocompatible nanocarrier for silymarin delivery, *Polym Adv Technol.* 32(10) (2021) 4094-4100.
- [28] H. Zheng, S. Wang, F. Cheng, X. He, Z. Liu, W. Wang, L. Zhou, Q. Zhang, Bioactive anti-inflammatory, antibacterial, conductive multifunctional scaffold based on MXene@CeO<sub>2</sub> nanocomposites for infection-impaired skin multimodal therapy, *Chem. Eng. J.* 424 (2021) 130148.
- [29] S. Bhagat, N.V. Srikanth Vallabani, V. Shutthanandan, M. Bowden, A.S. Karakoti, S. Singh, Gold core/ceria shell-based redox active nanozyme mimicking the biological multienzyme complex phenomenon, *J. Colloid Interface Sci.* 513 (2018) 831-842.
- [30] X. Zhang, X. Guo, X. Kang, H. Yang, W. Guo, L. Guan, H. Wu, L. Du, Surface Functionalization of Pegylated Gold Nanoparticles with Antioxidants Suppresses Nanoparticle-Induced Oxidative Stress and Neurotoxicity, *Chem. Res. Toxicol.* 33(5) (2020) 1195-1205.
- [31] F. Wei, X. Cui, Z. Wang, C. Dong, J. Li, X. Han, Recoverable peroxidase-like Fe<sub>3</sub>O<sub>4</sub>@MoS<sub>2</sub>-Ag nanozyme with enhanced antibacterial ability, *Chem. Eng. J.* 408 (2021) 127240.
- [32] Y. Wu, Y. Zhou, H. Xu, Q. Liu, Y. Li, L. Zhang, H. Liu, Z. Tu, X. Cheng, J. Yang, Highly Active, Superstable, and Biocompatible Ag/Polydopamine/g-C<sub>3</sub>N<sub>4</sub> Bactericidal Photocatalyst: Synthesis, Characterization, and Mechanism, *ACS Sustain.Chem.Eng.* 6(11) (2018) 14082-14094.
- [33] S.I. Han, S.-w. Lee, M.G. Cho, J.M. Yoo, M.H. Oh, B. Jeong, D. Kim, O.K. Park, J. Kim, E. Namkoong, J. Jo, N. Lee, C. Lim, M. Soh, Y.-E. Sung, J. Yoo, K. Park, T. Hyeon, Epitaxially Strained CeO<sub>2</sub>/Mn<sub>3</sub>O<sub>4</sub> Nanocrystals as an Enhanced Antioxidant for Radioprotection, *Adv Mater.* 32(31) (2020) 2001566.
- [34] X. Zhang, S. Zhang, Z. Yang, Z. Wang, X. Tian, R. Zhou, Self-cascade MoS<sub>2</sub> nanozymes for efficient intracellular antioxidant and hepatic fibrosis therapy, *Nanoscale.* 13(29) (2021) 12613-12622.
- [35] O.K. Savchak, N. Wang, M.A. Ramos-Docampo, P. de Dios Andres, A.M. Sebastião, F.F. Ribeiro, A. Armada-Moreira, B. Städler, S.H. Vaz, Manganese dioxide nanosheet-containing reactors as antioxidant support for neuroblastoma cells, *J. Mater. Chem. B.* 10(24) (2022) 4672-4683.
- [36] R. Sriranjani, B. Srinithya, V. Vellingiri, P. Brindha, S.P. Anthony, A. Sivasubramanian, M.S. Muthuraman, Silver nanoparticle synthesis using Clerodendrum phlomidis leaf extract and preliminary investigation of its antioxidant and anticancer activities, *J. Mol. Liq.* 220 (2016) 926-930.
- [37] B.K. Thakur, A. Kumar, D. Kumar, Green synthesis of titanium dioxide nanoparticles using Azadirachta indica leaf extract and evaluation of their antibacterial activity, *S. Afr. J. Bot.* 124 (2019) 223-227.
- [38] N.Y. Stozhko, M.A. Bukharinova, E.I. Khamzina, A.V. Tarasov, M.B. Vidrevich, K.Z. Brainina, The Effect of the Antioxidant Activity of Plant Extracts on the Properties of Gold Nanoparticles, *Nanomaterials.* 9(12) (2019) 1655.
- [39] S. Ahmed, Annu, S.A. Chaudhry, S. Ikram, A review on biogenic synthesis of ZnO nanoparticles using plant extracts and microbes: A prospect towards green chemistry, *J. Photochem. Photobiol. B, Biol.* 166 (2017) 272-284.
- [40] P. Makvandi, Z. Baghbantarghdari, W. Zhou, Y. Zhang, R. Manchanda, T. Agarwal, A. Wu, T.K. Maiti, R.S. Varma, B.R. Smith, Gum polysaccharide/nanometal hybrid biocomposites in cancer diagnosis and therapy, *Biotechnol. Adv.* 48 (2021) 107711.
- [41] N.I. Hulkoti, T.C. Taranath, Biosynthesis of nanoparticles using microbes—A review, *Colloids Surf. B.* 121 (2014) 474-483.
- [42] X. Chen, Z. Xue, J. Ji, D. Wang, G. Shi, L. Zhao, S. Feng, Hedysarum polysaccharides mediated green synthesis of gold nanoparticles and study of its characteristic, analytical merit, catalytic activity, *Mater. Res. Bull.* 133 (2021) 111070.
- [43] E.N. Zare, V.V.T. Padil, B. Mokhtari, A. Venkateshaiah, S. Wacławek, M. Černík, F.R. Tay, R.S. Varma, P. Makvandi, Advances in biogenically synthesized shaped metal- and carbon-based nanoarchitectures and their medicinal applications, *Adv. Colloid Interface Sci.* 283 (2020) 102236.
- [44] J. Virkutyte, R.S. Varma, Green Synthesis of Nanomaterials: Environmental Aspects, Sustainable Nanotechnology and the Environment: Advances and Achievements, *ACS Symp. Ser. Am. Chem. Soc.* (2013)11-39.
- [45] P. Makvandi, G.W. Ali, F. Della Sala, W.I. Abdel-Fattah, A. Borzacchiello, Biosynthesis and characterization of antibacterial thermosensitive hydrogels based on corn silk extract, hyaluronic acid and nanosilver for potential wound healing, *Carbohydr. Polym.* 223 (2019) 115023.
- [46] P. Makvandi, G.W. Ali, F. Della Sala, W.I. Abdel-Fattah, A. Borzacchiello, Hyaluronic acid/corn silk extract based injectable nanocomposite: A biomimetic antibacterial scaffold for bone tissue regeneration, *Mater.Sci.Eng.C.* 107 (2020) 110195.
- [47] N. Nakatani, Phenolic antioxidants from herbs and spices, *BioFactors.* 13(1-4) (2000) 141-146.
- [48] S.-S. Norma Francenia, S.-C. Raúl, V.-C. Claudia, H.-C. Beatriz, Antioxidant Compounds and Their Antioxidant Mechanism, in: S. Emad (Ed.), *Antioxidants*, IntechOpen. (2019) 1-28.
- [49] P.-G. Pietta, Flavonoids as Antioxidants, *J.Nat.Prod.* 63(7) (2000) 1035-1042.
- [50] M.J. del Baño, J. Lorente, J. Castillo, O. Benavente-García, J.A. del Río, A. Ortuño, K.-W. Quirin, D. Gerard, Phenolic Diterpenes, Flavones, and Rosmarinic Acid Distribution during the Development of Leaves, Flowers, Stems, and Roots of *Rosmarinus officinalis*. Antioxidant Activity, *J. Agric. Food Chem.* 51(15) (2003) 4247-4253.
- [51] Y. Zhu, Z. Ma, L. Kong, Y. He, H.F. Chan, H. Li, Modulation of macrophages by bioactive glass/sodium alginate hydrogel is crucial in skin regeneration enhancement, *Biomaterials.* 256 (2020) 120216.
- [52] T.G. Sahana, P.D. Rekha, Biopolymers: Applications in wound healing and skin tissue engineering, *Mol. Biol. Rep.* 45(6) (2018) 2857-2867.
- [53] Q. Zhong, B. Wei, S. Wang, S. Ke, J. Chen, H. Zhang, H. Wang, The Antioxidant Activity of Polysaccharides Derived from Marine Organisms: An Overview, *Mar.Drugs.* 17(12) (2019) 674.

- [54] Y. Endo, T. Aota, T. Tsukui, Antioxidant Activity of Alginic Acid in Minced Pork Meat, *Food Sci. Technol. Res.* 21(6) (2015) 875-878.
- [55] F.G. Torres, S. Commeaux, O.P. Troncoso, Starch-based biomaterials for wound-dressing applications, *Starch - Stärke.* 65(7-8) (2013) 543-551.
- [56] T. Szychaj, K. Wilpiszewska, M. Zdanowicz, Medium and high substituted carboxymethyl starch: Synthesis, characterization and application, *Starch - Stärke.* 65(1-2) (2013) 22-33.
- [57] Z. Abdollahi, E.N. Zare, F. Salimi, I. Goudarzi, F.R. Tay, P. Makvandi, Bioactive Carboxymethyl Starch-Based Hydrogels Decorated with CuO Nanoparticles: Antioxidant and Antimicrobial Properties and Accelerated Wound Healing In Vivo, *Int. J. Mol. Sci.* 22(5) (2021) 2531.
- [58] V. Sindhi, V. Gupta, K. Sharma, S. Bhatnagar, R. Kumari, N. Dhaka, Potential applications of antioxidants—A review, *J. Pharm. Res.* 7(9) (2013) 828-835.
- [59] M. Wojcik, I. Burzynska-Pedziwiatr, A.L. Wozniak, A Review of Natural and Synthetic Antioxidants Important for Health and Longevity, *Curr. Med. Chem.* 17(28) (2010) 3262-3288.
- [60] A. Muñoz-Acevedo, L.Y. Vargas Méndez, E.E. Stashenko, V.V. Kouznetsov, Improved Trolox® Equivalent Antioxidant Capacity Assay for Efficient and Fast Search of New Antioxidant Agents, *Anal. Chem. Lett.* 1(1) (2011) 86-102.
- [61] B. Bideau, J. Bras, N. Adoui, E. Loranger, C. Daneault, Polypyrrole/nanocellulose composite for food preservation: Barrier and antioxidant characterization, *Food Packag. Shelf Life.* 12 (2017) 1-8.
- [62] A. Dhawan, V. Kumar, V.S. Parmar, A.L. Cholli, Properties, Applications, Novel polymeric antioxidants for materials. *Antioxidant Polymers* (2012) 385-425.
- [63] A. Hodgson, K. Gilmore, C. Small, G.G. Wallace, I.L. Mackenzie, T. Aoki, N. Ogata, Reactive supramolecular assemblies of mucopolysaccharide, polypyrrole and protein as controllable biocomposites for a new generation of 'intelligent biomaterials'. *Supramol. Sci.* 1(2) (1994) 77-83.
- [64] C.R.J.A.o.c.r. Martin, Template synthesis of electronically conductive polymer nanostructures *Acc. Chem. Res.* 28(2) (1995) 61-68.
- [65] A. Deronzier, J.C. Moutet, Functionalized polypyrroles. New molecular materials for electrocatalysis and related applications, *Acc. Chem. Res.* 22(7) (1989) 249-255.
- [66] G. Jin, K. Li, The electrically conductive scaffold as the skeleton of stem cell niche in regenerative medicine, *Mater. Sci. Eng. C.* 45 (2014) 671-681.
- [67] T. Nezakati, A. Seifalian, A. Tan, A.M. Seifalian, Conductive Polymers: Opportunities and Challenges in Biomedical Applications, *Chem. Rev.* 118(14) (2018) 6766-6843.
- [68] M. Shahadat, S.Z. Ahammad, S.A. Wazed, S. Ismail, Synthesis of Polyaniline-Based Nanocomposite Materials and Their Biomedical Applications, *Electrically Conductive Polymer and Polymer Composites.* (2018), 199-218.
- [69] V. Hasantabar, M.M. Lakouraj, E.N. Zare, M. Mohseni, Synthesis, Characterization, and Biological Properties of Novel Bioactive Poly(xanthoneamide-triazole-ethersulfone) and Its Multifunctional Nanocomposite with Polyaniline, *Adv. Polym. Technol.* 36(3) (2017) 309-319.
- [70] E.N. Zare, P. Makvandi, Antimicrobial metal-based nanomaterials and their industrial and biomedical applications, *Engineered Antimicrobial Surfaces.* (2020) 123-134.
- [71] M. Gizdavic-Nikolaidis, J. Travas-Sejdic, G.A. Bowmaker, R.P. Cooney, C. Thompson, P.A. Kilmartin, The antioxidant activity of conducting polymers in biomedical applications, *Curr. Appl. Phys.* 4(2) (2004) 347-350.
- [72] A. Saikia, N. Karak, Polyaniline nanofiber/carbon dot nanohybrid as an efficient fluorimetric sensor for As (III) in water and effective antioxidant, *Mater. Today Commun.* 14 (2018) 82-89.
- [73] M. Gizdavic-Nikolaidis, J. Travas-Sejdic, G.A. Bowmaker, R.P. Cooney, P.A. Kilmartin, Conducting polymers as free radical scavengers, *Synth. Met.* 140(2) (2004) 225-232.
- [74] P. Moutsatsou, K. Coopman, S. Georgiadou, Biocompatibility Assessment of Conducting PANI/Chitosan Nanofibers for Wound Healing Applications, *Polymers*, 9(12) (2017) 687.
- [75] J. He, Y. Liang, M. Shi, B. Guo, Anti-oxidant electroactive and antibacterial nanofibrous wound dressings based on poly( $\epsilon$ -caprolactone)/quaternized chitosan-graft-polyaniline for full-thickness skin wound healing, *Chem. Eng. J.* 385 (2020) 123464.
- [76] M. Zhang, B. Guo, Electroactive 3D Scaffolds Based on Silk Fibroin and Water-Borne Polyaniline for Skeletal Muscle Tissue Engineering, *Macromol. Biosci.* 17(9) (2017) 1700147.
- [77] B.M. Hryniewicz, R.V. Lima, F. Wolfart, M. Vidotti, Influence of the pH on the electrochemical synthesis of polypyrrole nanotubes and the supercapacitive performance evaluation, *Electrochim. Acta.* 293 (2019) 447-457.
- [78] E.N. Zare, T. Agarwal, A. Zarepour, F. Pinelli, A. Zarrabi, F. Rossi, M. Ashrafizadeh, A. Maleki, M.-A. Shahbazi, T.K. Maiti, R.S. Varma, F.R. Tay, M.R. Hamblin, V. Mattoli, P. Makvandi, Electroconductive multi-functional polypyrrole composites for biomedical applications, *Appl. Mater. Today* 24 (2021) 101117.
- [79] Y. Liang, J.C.-H. Goh, Polypyrrole-Incorporated Conducting Constructs for Tissue Engineering Applications: A Review, *Bioelectricity.* 2(2) (2020) 101-119.
- [80] Y. Park, J. Jung, M. Chang, Research Progress on Conducting Polymer-Based Biomedical Applications, *Appl. Sci.* 9(6) (2019) 1070.
- [81] J. Upadhyay, A. Kumar, B. Gogoi, A.K. Buragohain, Biocompatibility and antioxidant activity of polypyrrole nanotubes, *Synth. Met.* 189 (2014) 119-125.
- [82] P. Poprac, K. Jomova, M. Simunkova, V. Kollar, C.J. Rhodes, M. Valko, Targeting free radicals in oxidative stress-related human diseases *Trends Pharmacol. Sci.* 38(7) (2017) 592-607.

- [83] E.N. Zare, P. Makvandi, B. Ashtari, F. Rossi, A. Motahari, G. Perale, Progress in Conductive Polyaniline-Based Nanocomposites for Biomedical Applications: A Review, *J. Med. Chem.* 63(1) (2020) 1-22.
- [84] E.N. Zare, M.M. Lakouraj, M. Mohseni, A. Motahari, Multilayered electromagnetic bionanocomposite based on alginic acid: Characterization and biological activities, *Carbohydr. Polym.* 130 (2015) 372-380.
- [85] Q. Gao, M. Lei, K. Zhou, X. Liu, S. Wang, H. Li, Preparation of a microfibrillated cellulose/chitosan/polypyrrole film for Active Food Packaging, *Prog. Org. Coat.* 149 (2020) 105907.
- [86] M. Mansour Lakourj, R.-S. Norouzian, M. Esfandyar, S. Ghasemi mir, Conducting nanocomposites of polypyrrole-copolyindole doped with carboxylated CNT: Synthesis approach and anticorrosion/antibacterial/antioxidation property, *Mater. Sci. Eng. B.* 261 (2020) 114673.
- [87] A. Somogyi, K. Rosta, P. Pusztai, Z. Tulassay, G. Nagy, Antioxidant measurements, *Physiol. Meas.* 28(4) (2007) R41.
- [88] G. Cao, R.L. Prior, Comparison of different analytical methods for assessing total antioxidant capacity of human serum, *Clin. Chem.* 44(6 Pt 1) (1998) 1309-15.
- [89] M. Ozgen, R.N. Reese, A.Z. Tulio, J.C. Scheerens, A.R. Miller, Modified 2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) Method to Measure Antioxidant Capacity of Selected Small Fruits and Comparison to Ferric Reducing Antioxidant Power (FRAP) and 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) Methods, *J. Agric. Food Chem.* 54(4) (2006) 1151-1157.
- [90] N.J. Miller, C. Rice-Evans, M.J. Davies, V. Gopinathan, A. Milner, A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates, *Clin. Sci. (London, England: 1979)* 84(4) (1993) 407-12.
- [91] D.D. Wayner, G.W. Burton, K.U. Ingold, S. Locke, Quantitative measurement of the total, peroxy radical-trapping antioxidant capability of human blood plasma by controlled peroxidation. The important contribution made by plasma proteins, *FEBS Lett.* 187(1) (1985) 33-7.
- [92] G.W. Winston, F. Regoli, A.J. Dugas, J.H. Fong, K.A. Blanchard, A Rapid Gas Chromatographic Assay for Determining Oxyradical Scavenging Capacity of Antioxidants and Biological Fluids, *Free Radic. Biol. Med.* 24(3) (1998) 480-493.
- [93] V. Hasantabar, M.M. Lakouraj, E.N. Zare, M. Mohseni, Innovative magnetic tri-layered nanocomposites based on polyxanthone triazole, polypyrrole and iron oxide: synthesis, characterization and investigation of the biological activities, *RSC Adv.* 5(86) (2015) 70186-70196.
- [94] E.N. Zare, M.M. Lakouraj, P.N. Moghadam, R. Azimi, Novel polyfuran/functionalized multiwalled carbon nanotubes composites with improved conductivity: Chemical synthesis, characterization, and antioxidant activity, *Polym. Compos.* 34(5) (2013) 732-739.
- [95] I. Khalil, W.A. Yehye, A.E. Etxeberria, A.A. Alhadi, S.M. Dezfooli, N.B. Julkapli, W.J. Basirun, A. Seyfoddin, Nanoantioxidants: Recent Trends in Antioxidant Delivery Applications, *Antioxidants.* 9(1) (2019) 24.

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