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Metal chelators, metal chelating, and metal ions assessments serve as methods for assessing antioxidant activity

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ABSTRACT

The creation of biomolecules, immunological function, cell development and reproduction, and other biological activities all depend on heavy metals, yet excessive ingestion of these elements can be harmful. In particular, they produce oxidative stress (OS). The metabolism generates reactive oxygen species (ROS) and free radicals. The respiratory, neurological, reproductive, and digestive systems are among the organs that might be harmed by an accumulation of heavy metals. One popular treatment for metal poisoning is metal chelation therapy. Through interactions with a core metal atom, the ligand creates a complex ring structure. By using the right chemicals to chelate metals, the body can effectively remove them and avoid damage during metabolism. The correct operation of proteins and enzymes during metabolism depends on heavy metals like zinc, iron, and copper. The buildup of metals in excess can have detrimental effects on biomolecules. Peroxidation of biological components, including lipids in the plasma membrane, is brought on by the production of ROS and nitrogen species (RNS). The preventive benefits of antioxidants are making them more and more popular, especially in food and pharmaceutical items. Metal chelating assays and other suitable techniques are needed to screen compounds for antioxidant properties. The properties of metal bonding and chelation are thoroughly reviewed in this study. This article describes the fundamental chemistry ideas of both in vitro and in vivo metal chelation processes. This article discusses metal ions, metal chelating, antioxidants, the biological function of metal chelation, and metal chelating tests, which are frequently employed to evaluate a substance's antioxidant qualities. Additionally, the page offers scientific information, chemical characteristics, and important details regarding the chelation techniques used.

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1. "INTRODUCTION"

1.1. "The heavy metal"

The proper functioning of living creatures depends on metal ions. For many millennia, humans utilized metal-based products with no knowledge of the risks and outcomes associated with their use. Animals, vegetation, and water resources are all harmed by metal ions. Typically, mining, acid rain, industrial effluent, urban waste, fossil fuel residues, fertilizers, and pesticides are the sources of metal pollution [1]. The elements classified as heavy metals (HMs) have densities more than 5 g/mL [2, 3]. They have been used for thousands of years in a variety of ways. Among the most common metals are Cr, Pb, Cd, Hg, Cu, and Zn. Substances that share physical and chemical characteristics with heavy metals are included in this group [4]-[6]. The heavy metals Mn, Co, and Fe are less common. Based on their toxicity, HMs are categorized as either essential or non-essential.

Low concentrations of essential HMs, such as Zn, Cu, Fe, and Co, are either ineffective or innocuous. As, Cr, Hg, Cd, and other non-essential heavy metals (HMs) are extremely dangerous, even in trace concentrations [7, 8]. As cofactors, HMs like Zn, Cu, and Fe are essential for a number of physiological and biological processes. The creation of biomolecules, enzyme activity, oxygen transport, cell formation, and immune system function are all significantly impacted by Cu, Fe, Zn, and Co. Excess HMs in the cytoplasm can alter protein structure, degrade enzyme function, alter pH, and upset redox equilibrium. Necrosis, apoptosis, or cell dysfunction may be the outcome of this illness. Moreover, HMs can bind with carboxyl, imidazole, and thiol groups in proteins [9, 10]. One of the most often eaten and exposed elements in daily life is iron (Fe). The elemental iron content of an average person is 4-5 grams. The oxygen transporter protein hemoglobin stores two-thirds of this amount, The final third of that is stored in iron-retaining proteins called ferritin or hemosiderin [11]. Iron is present within cytochrome, hemoglobin, and myoglobin. It is also required for enzymes like peroxidases, catalase, succinate dehydrogenase, aconitase, aldehyde oxidase, and oxygenase [3,12]. The body can tolerate large amounts of iron, but too much can be dangerous. Numerous cases of metal poisoning in young infants have been caused by excessive iron absorption from food supplements. While chronic Fe overload is frequently experienced by _-thalassemia patients as a result of recurrent whole blood transfusions, acute poisoning is less prevalent in adulthood [11,13]. The effects of elevated iron levels on the liver and heart can be disastrous. A sequestering agent can increase the pace of metal ion evacuation. Currently, one of the recommended drugs for Fe^{3+} removal is desferrioxamine B. Ten times the typical amount of available iron can be achieved using desferrioxamine B [11,13,14]. Drugs that compete with the transferrin protein for binding to and transporting metal ions, can help prevent iron overloads in people. This study discovered that under some conditions, drugs with a greater Fe^{3+} affinity than transferrin can deplete natural iron stores. The natural siderophore enterobactin has a strong affinity for Fe^{3+} ions [15]. Research has shown recent studies on the effects of bacterial 'siderophores', which are small molecules,can make iron soluble and useful for plants. The potent components of microbial siderophores, such as hydroxamate, catecholate, and _-hydroxocarboxylates, enable them to chelate iron efficiently. Mucineic acid and its derivatives are examples of phytosiderophores, which are polydentate ligands that chelate metals by using carboxylate and amine groups. The ability of Gramibactin, a novel class of diazeniumdiolate siderophores, to effectively generate Fe^{3+} indicates that it may be used to separate iron. Across a broad pH range, the study discovered that gramibactin and Fe^{3+} ions form stable complexes (Table 1) [16]. Water pipeline corrosion is one of the main causes of Fe pollution, among other things. Soil and groundwater are contaminated by Fe-containing human waste from industry and agriculture. The steel industry is responsible for both particle iron and iron oxide in today's air pollution. Dehydration, fatigue, nausea, diarrhea, vomiting, and stomach discomfort are common signs of iron poisoning [17,18]. About 8% of all minerals are aluminum (Al), making it the second most common metal in the crust of the earth. It now has a big impact on many different businesses. A human's daily aluminum intake should not exceed 3–10 mg. Living things are at risk from excessive and erratic aluminum consumption [19]. One important aspect of living things is Al3+ toxicity. The chemical characteristics of Al3+ cause an imbalance in the metabolism of free radicals, which leads to oxidative damage to proteins, polysaccharides, nucleic acids, and membrane lipids. This disrupts regular cell processes. Numerous theories have been put out, but the chemical reasons of aluminum toxicity are still unknown. Al has the potential to significantly increase antioxidant activity in biological systems [20]. ROS levels rise noticeably when someone is poisoned by aluminum. It makes people more susceptible to neurological conditions including encephalopathy and dementia. Biomolecules suffer severe harm as a result of this toxicity. There are numerous negative impacts of aluminum's presence in living systems. Changes in the structure and function of proteins/enzymes (glycolysis/TCA pathway) can affect virtually every part of the body: the central nervous system, tissues, cells, etc [21]. Strong Lewis acid Al prefers ligands that donate oxygen, including carboxylates, nucleotides, phosphates, and nucleic acids. It causes normal proteins to become hyperphosphorylated. According to a recently established paradigm, Al may have direct interactions with the backbones of proteins. Al appears to form stable structures with a 5-membered ring that interacts with protein backbones and creates strong covalent bonds with carbonyl oxygen and protonated peptide nitrogen, according to this study [22]. EDTA was used as a chelating agent to treat Al poisoning in patients for a brief length of time (see Table 1) [23]. Certain metabolic enzymes are dependent on copper (Cu) to function such as cytochrome C oxidase, SOD (superoxide dismutase), tyrosinase, ceruloplasmin, and dopamine hydroxylase [19]. The liver has many cells where excessive levels of copper will produce reactive oxygen species (ROS), causing cell death; Wilson's Disease (WD) is an inherited disorder in which the body cannot properly remove excess copper. WD patients accumulate copper in their liver, brain, and other organs, including their eyes. For health, a small amount of dietary copper is adequate, but too much can be detrimental. Excess Cu2+ can be effectively bound by ligands that have both soft and hard donors. In the treatment of WD, penicillamine is the medication most frequently used for this reason.

The drug binds to Cu2+ ions preferentially and contains both kinds of donor atoms [15]. Using murexide as a chromogenic reagent, the ternary H-point standard addition method is used to test the presence of Cu^{2+} ions. Use of the reddish-purple compound murexide in spectroscopy and chemical analysis has grown in popularity (Table 1) [24]. Metal Ion Indicators called murexides are used as Chromogenic Reagents in Traditional Spectrophotometry for determining metals (especially Copper) through chelating of metals. Utilizing a spectrophotometer, murexide complexation reactions with Co^{2+}, Cu^{2+}, Ni^{2+}, Cd^{2+}, Zn^{2+}, and Pb2+ ions were shown (refer to Table 1) [25]. The murexide method uses murexide, an ammonium salt of purpuric acid, as a straightforward and sensitive spectrophotometric technique to detect Cu^{2+}. For this method to work, a stable yellow-greenish compound with a maximum absorbance at 476 nm must be produced (at pH 5.0). In analytical chemistry, murexide is frequently used as a complexometric indicator for rare earth metals, Pb^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, Zn^{2+}, Ni^{2+}, and Ca^{2+} [26].

Table 1. The chelating agents of some metals.

Metal	Binding Agent
Zn^{2+}	N,N,N,N-tetrakis (2-pyridylmethyl)-ethylenediamine
Ni^{2+}	Murexide
Cu^{2+}	Penicillamine EDTA
Hg^{2+}	Dimercaprol Murexide
Cd^{2+}	Murexide
Pb^{2+}	Murexide
Ca^{2+}	Calbindins
Mn^{2+}	Desferrioxamine
Co^{2+}	Murexide
Al^{3+}	EDTA
Fe^{3+}	Deferoxamine, Enterobactin, Desferrioxamine

1.2. Biological system of metal chelating

The main treatment for heavy metal poisoning is metal chelation therapy. The Chelation Process is the method by which Ligands; ions, or molecules combine in a circular fashion with a central metal ion/atom via an ion/molecule interaction. The term ligand refers to a single ion/molecule that contains at least two atoms capable of donating two electron(s) to form a covalent bond.Depending on how well they interact with covalent atoms, ligands are divided into three categories. Interactions between ligands and metal ions determine how stable complexes are. Sulfur and nitrogen ligands are preferred above oxygen by Hg and Pb ions, although Ca atoms are attracted to the reverse. When choosing chelating agents, affinity differences are very important [3,51]. Metal toxicity is often treated by chelating agents that include dimercaprol, EDTA, deferoxamine, penicillamine, dimercaptosuccinic acid, and analogues [3].

1.3. Oxidative Stress (OS) and Reactive Oxygen Species (ROS)

A powerful and extremely reactive oxidizing agent, oxygen may swiftly form oxides with a variety of elements and compounds. It is in a ground state and progressively diminishes in the atmosphere [52]–[55]. Oxygen (molecular) contains 2 electrons, each having a similar spin to one another in 2 separate antibonding molecular orbital; therefore, it will accept 2 electrons from any common electron donor [56]-[58]. Redox reactions are major metabolic pathways in living organisms through which electrons can pass from one species to another. Most biological systems have this as their main reaction. Living organisms convert molecular oxygen in the atmosphere into the form of usable energy (ATP). [59]-[61]. Oxygen is commonly used in both biological enzymatic biocatalysis and in reduction-oxidation (redox) reactions occurring within cells and tissues. Also, oxygen can transfer electrons from one atom to another. Oxygen plays an important role in the structure of aerobic organisms and aerobic metabolic pathways and is the final electron acceptor in the electron transport system [62]-[65]. The biggest issue occurs when the electron flow becomes interrupted. Otherwise, everything appears normal. This scenario leads to the creation of free radicals with an odd electron. ROS are produced through the degradation of free radicals; these are highly reactive and unstable compounds that react with a wide variety of molecules and intermediates [66]-[68] including those that have been identified as being made up of oxygen, sulfur, and nitrogen. The main types of ROS include lipid hydroperoxides (LOO), peroxyl (ROO), nitric oxide (NO), alkoxyl (RO), superoxide anion (O2), and hydroxyl (HO) radicals O2, NO, and LOO showed lower reactivity [69]-[71]. Nonradical ROS forms found in biological systems include H_2O_2, $1O_2$, and HOCl [70,71]. ROS can be generated in biological systems by elemental ions such as Fe^{2+} [72,73]. An imbalance between the generation of ROS and the antioxidant system leads to OS. OS disrupts a variety of cellular processes and results in pathogenic events in organisms [74–76]. Lipids, proteins, DNA, and RNA are all oxidatively altered by this situation [77,78]. A higher risk of a number of conditions, such as cancer, diabetes, arthrosclerosis, aging, arthritis, and neurological problems, has been associated with OS [79, 80]. Because they reduce the harmful effects of ROS and inhibit oxidative processes, antioxidants are essential for good health [81]. In the body, toxic levels of HMs can damage the reproductive, neurological, respiratory, and digestive systems [38]. Reactive oxygen species (ROS) are created when lipids peroxide in the plasma membrane. ROS like OH_ can be produced by Fenton and Haber-Weiss reactions when transition metals like Fe and Cu are present [82]–[84]. H2O2 can be changed into OH_ by the Fenton reaction [85] in the presence of oxygen and metal ions. Ferrous ions catalyze the Haber-Weiss process, which converts O2 and H2O2 into OH_. First, Fritz Haber and his student hypothesized the reaction [86]. These mechanisms are the main generator of radicals and damage cells, according to later research.

$$Fe^{2+} + H_2O_2 \quad \rightarrow \quad Fe^{3+} + OH^- + OH \left(Fenton\ reaction\right)$$

$$O_2 + H_2O_2 \quad \rightarrow \quad O_2 + H_2O + OH \left(Haber - Weiss\ reaction\right)$$

The production of ROS and radicals, which can damage biomolecules, is inhibited by metal ion chelation. It is preferable to use natural metal chelating compounds, including flavonoids and phenolics, rather than artificial ones. These have to do with the problem of toxicity [87].

1.4. The antioxidant

Mitigate the negative effects of both synthetic and natural substances [88]–[90]. Chemicals known as antioxidants can scavenge reactive oxygen species (ROS), alter antioxidant defenses, or stop ROS from forming [91,92]. Antioxidants prolong the shelf life of products by delaying lipid peroxidation. Food and pharmaceutical products can be kept fresh while being prepared and stored [93]–[95]. Because they are more affordable, more readily available, more stable, and more efficient than natural antioxidants, synthetic antioxidants are frequently preferred [96]. Synthetic antioxidants including propyl gallate (PG), tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA) and hydroxytoluene (BHT) are frequently used, as (*Fig. 1*) illustrates [97]-[99].

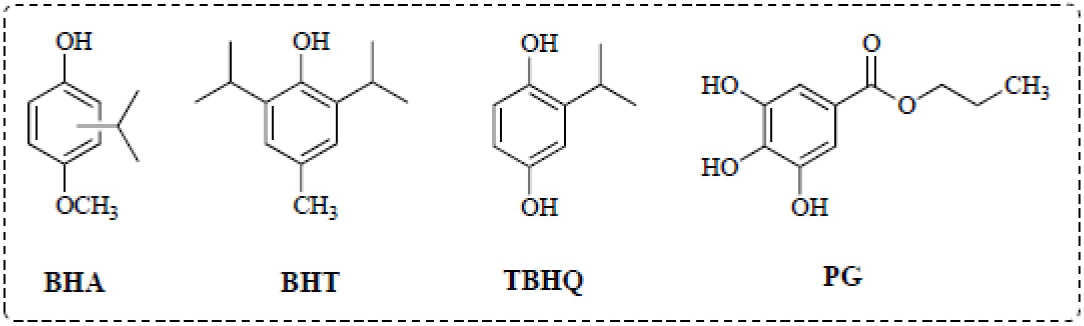


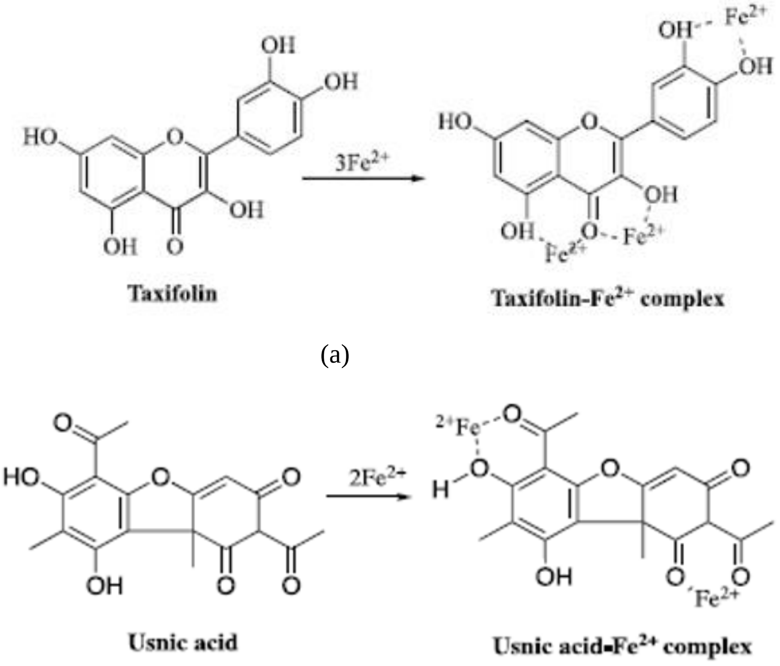
Figure 1. The molecular configurations of the predominantly utilized synthetic antioxidants.

Despite the prevalent usage of these synthetic antioxidants, reports indicate that prolonged consumption may lead to health issues, including fatty liver, carcinogenesis, skin allergies, and gastrointestinal disorders [79,100,101]. Consequently, people favor natural antioxidants in their regular diets and express concern about exposure. On the negative consequences of artificial antioxidants. Unlike synthetic antioxidants, natural antioxidants are safe and come from a variety of plants, such as fruits, vegetables, herbs, and spices [102]–[104]. As a result, more food makers and consumers may choose to use natural antioxidants instead of synthetic ones as their popularity grows. people [105,106]. Due to their high content in various compounds (tannins, catechins, theines, flavonoids), aqueous extracts of tea, anise and fennel are among the most used natural sources of antioxidants [107]–[109]. The quality of the natural source as well as the extraction and technological processes used for the production of the extract determine both the quality and antioxidant potential of natural antioxidants and extracts. According to the findings of toxicity studies, the safety of well-known natural antioxidants has also been assessed using information on chemicals and their cumulative effects [79,110,111].

1.5. Ability of metal for chelating

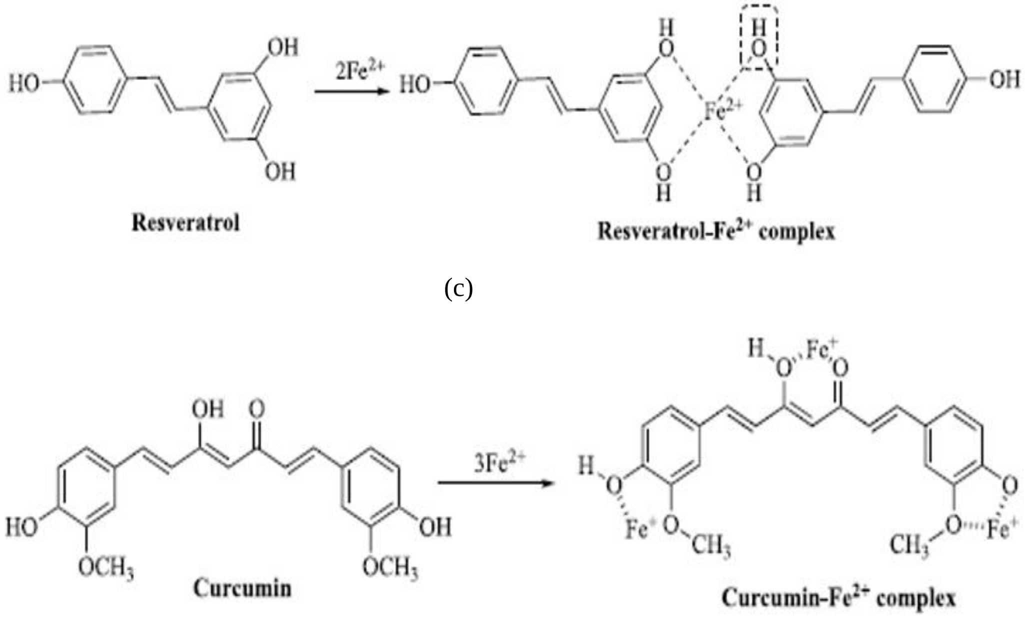
Transition metals are necessary for all living things to maintain their vital processes. In biological systems, metal ions have a variety of functional roles. Most of the time, metalloproteins and metals are firmly joined by coordination bonds, which causes the proteins to take on a three-dimensional shape. This is how you should organize [112,113]. The high chemical affinity that proteins have for metal ions is an important factor in how many enzymes are able to display such powerful catalytic properties Generally speaking, metal chelation is seen to be the most practical and widely used antioxidant method. It is said that antioxidants' functional groups, which aid in metal binding, provide them potent iron-binding properties. Fe ions' biological effects, including their antioxidant capacities, may also be altered by their interactions with antioxidant molecules [114,115].

(*Fig. 2A*) illustrates possible chelation sites for Fe to the known antioxidant, taxifolin (which includes 5-OH and 4-oxo groups as the binding sites located between the heterocyclic and A rings; the catechol moiety of the B ring; and the 3-OH and 4-oxo groups within the heterocyclic ring). The taxifolin molecule's groups prevent the iron ions-ferrozine complex from forming. Before ferrozine or 2,2'-bipyridine reagents, which have a high affinity for Fe^{2+}, are added, taxifolin can chelate Fe^{2+}. Taxifolin either sterically blocks interactions between lipid intermediates and metals or aids in the transformation of ferrous ions into metal complexes. Taxifolin's functional carbonyl (-C=O) and hydroxyl (-OH) groups have the ability to bind many Fe^{2+} ions [116]. The semiquinone-metal combination is stabilized by metallic centers. numerous sites on a single substrate or a reagent operating on numerous substrates can both provide binding selectivity. Both steric and electronic interactions between substrates and receptors have an impact on this. The binding of chemical species is contingent upon distinct affinities. Functional biomolecules typically function as systems with several linker sites. [15]. Moreover, specific binding is crucial in chelation therapy. Various methodologies exist about metal binding affinities. A different method was employed by Harris and colleagues [13]. This method used the concentrations of metal ions in solution to assess how efficiently different tris catecholates bind to Fe3+ ions [11]. A separate investigation indicated that incorporating a diamino unit into the pyrolic tripodal structure markedly enhanced its binding capacity relative to the parent aminopyrolic receptors [118]. The primary stage in examining interactions among types of binding by assessment of affinities of binding. A binding constant is a quantitative measure of the binding affinity of two compounds that form a stable complex. However, a binding constant will only adequately characterize the affinity of one compound for another if the compound forms a complex with that other compound. Cooperative effects can sometimes occur during the binding of an atom or group of atoms to a ligand (or chelate) as well. The binding of oxygen to hemoglobin is an excellent example of a cooperative effect. Hemoglobin binds oxygen in the lungs and releases it at the peripheral tissues by forming a reversible complex. To achieve this objective, four distinct complexation processes involving the absorption of four O_2 molecules have been found [46]. The initial O_2 molecule can attach to the Fe^{2+} ions present in the prosthetic group of heme of monomers within tetrameric hemoglobin. The stability constant for the coordination of the first O2 to deoxyhemoglobin is relatively small; however, when the second O2 coordinates to the heme of hemoglobin, it facilitates an increase in the oxygen affinity of the hemoglobin thereby making easier the subsequent coordination of the next two oxygen molecules to the binding site. Oxygen coordination causes alterations in the spatial configuration of the oxygen binding sites on hemoglobin [15].



(a)

(b)



(c)

(d)

Figure 2. The proposed binding mechanism of ferrous ions (Fe^{2+}) for taxifolin (A), usnic acid (B), resveratrol (C), and curcumin (D).

Moreover, curcumin, a food additive prevalent in turmeric and ginger, chelate Fe2+ ion and inhibit Fe^{2+}-ferrozine complex formation.Thus, curcumin can effectively attach to iron ion with high affinity, similar to ferrozine. A curcumin molecule is proposed to bind three Fe^{2+} ions, as illustrated in (*Fig. 2D*). Curcumin has been reported to chelate iron ions through its biologically active -OH and $-OCH_3$ groups [119]. Moreover, molecules with functional groups like C–OH and C=O readily chelate metal ions. Kazazica et al. demonstrated in another investigation that kaempferol binds to Fe^{2+} and Cu^{2+} ions. They further indicated that this binding was facilitated by functional-OCH_3 and-OH group [120]. Compounds with two or more of the following functional groups: -OH, -COOH, -SH, $-OCH_3$, -C=O, $-PO_3H_2$, $-NR_2$, -O-, and -S-, arranged in an appropriate functional structure, can effectively chelate Fe^{2+} ions [121]–[124]. In a separate investigation, Fiorucci and colleagues demonstrated that quercetin, a prevalent phenolic molecule in plants, exhibited comparable metal chelating capacity [125]. Our research group has demonstrated the potential binding mechanism of Fe^{2+} by usnic acid [126]. Usnic acid was found to inhibit the formation of the Fe^{2+}-ferrozine complex (*Fig. 2B*). Usnic acid can chelate Fe^{2+} ions through the -OH and -COOH groups associated with the phenolic ring. A further investigation demonstrated that resveratrol binds to Fe^{2+} ions at the meta locations of its -OH groups [127]. It has been suggested that the potential for the natural, powerful antioxidant, resveratrol, to serve as an important primary antioxidant is related to its ability to bind iron. This work convincingly revealed that resveratrol binds to Fe^{2+} and disrupts the formation of the Fe^{2+}-ferrozine complex. One strategy for assessing chelation capacity involves measuring free iron ions (Fe^{2+}) with a chelating agent such ferrozine or 2,2'-bipyridine, which forms a readily visible complex through spectroscopic examination. Metal chelators create complexes that diminish the reactivity of metals like iron, rendering them inert [128]. The majority of the metal-binding activity of this compound arises from the presence of the catechol moiety as demonstrated by the larger bathochromic shift exhibited upon copper binding to quercetin than the chelation activity of kaempferol [129]. Flavonoids are bioavailable due to their ability to chelate excessive levels of metal ions that exist within a human body. Chelation is the process through which certain flavonoid compounds bind with the hazardous metal ions (chromium, tin, cadmium, lead) to provide a means of detoxifying these harmful substances. Additionally, flavonoids can bind to an excess of aluminum to form complexes [114,130].

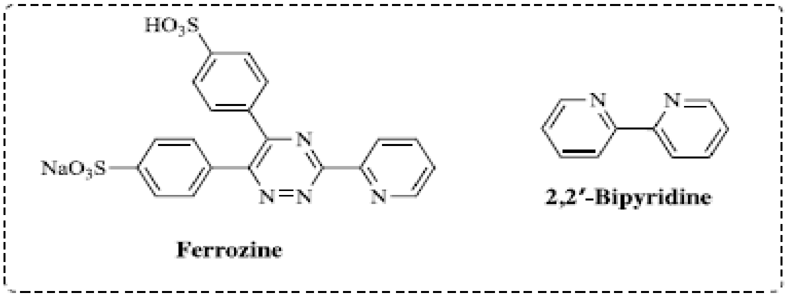


Figure 3 .2,2′-bipyridine's and. Ferrozine chemical structures as metals chelators.

Most common sources of ferrous ions (Fe^{2+}) are $FeCl_2$ and $FeSO_4$. The creation of the metal-antioxidant complex is indicated by absorbance decreasing at 560 nm for ferrozine or 484 nm for 2,2′-bipyridine following transfer of the metal ion to an antioxidant [131]. This method is used to assess chelating of metals capabilities of the metals chelated antioxidants [132]. When antioxidant chelating reagent is added, the quantity of Fe^{2+}-ferrozine or Fe^{2+}-2,2′-bipyridine complexes that are generated decreases because both reagents form complex structures with unbonded Fe^{2+}. Fe^{2+} complexes with 2,2′-bipyridine or ferrozine produce a reddish chromophore with maximal absorbances at 484 and 560 nm, respectively. Despite not being an antioxidant, EDTA is a common metal chelator in antioxidant procedures due to its strong chelating ability. Indeed, EDTA equivalents are frequently used in research to express the metal chelation capabilities of antioxidants or extracts [133]. Constant formation of antioxidants and Fe^{2+} or Fe^{2+} -2,2′-bipyridine complex have an impact on these tests [134]. According to this theory, the Fenton reaction in vivo cannot be stopped by a weak metal chelator. In spite of everything, this reaction might be a good way to assess an antioxidant's capacity to chelate iron.

$$3Ferrozine + Fe(H2O)_6^{+2} \rightarrow Fe - (Ferrozine)^{-4}{}_3 + 6H_2O$$

The capacity to chelate metals is crucial because it lowers their concentration, which catalyzes lipid peroxidation. Furthermore, they lower potential of redox and metals stabilize, metal chelating compounds are regarded as secondary antioxidants. The metal ions that have oxidized [135].

2. Methods of Antioxidants

The antioxidant potential of food, pharmaceutical, medical and biological materials is typically quantified by employing several antioxidant assay methods. The most popular and successful techniques up to this point include linoleic acid autoxidation inhibition. The current study goes into detail about the following: TRAP and ORAC assay [59], acid emulsions (Thiocyanate methods) [136], Fe^{2+} - Fe^{3+} transformation assays [138], ferric ions (Fe^{3+}) reducing assays (FRAP) [108,137], Folin Ciocalteu reducing assay [140], DPPH [141], cupric ions (Cu^{2+}) reducing assay (Cuprak method) [139], ABTS [142], $DMPD^+$ [139], putative Fe^{2+} binding assays , and superoxide anion radical scavenging assays [143]. In most cases, each method utilizes the same basic approach; for example, a synthetic colored compound (or a redox-active compound) is produced, a spectrophotometer measures the ability of biologic sample(s) to decrease the concentration of the redox active compound, based upon a comparison with a reference compound established using a standard [144].

3. Assays of Metals Chelating

3.1. Ferrozine reagents chelating assays

The affinity of binding between metal ions and reagents, such Fe^{2+}, was taken into consideration when developing the spectroscopic techniques outlined. The Dinis method was used to assess Ferrozine's Fe^{2+} chelation [145]. In short, a distinct amount of sample and The standard chemicals were put into a 0.03 ml solution from $FeCl_2$. To provides the interactions between Fe^{2+} and the samples, i.e., the sample chelates the Fe^{2+} ions. added 0.2 ml ferrozine reagents to initiate reaction. The mixture was then swirled and allowed to stand at 25 °C for 10 minutes. The solution's absorbance levels were then measured at 560 nm [146].

3.2. 2,20-Bipyridine reagents chelating assays

Fe^{2+} chelating by 2,20-bipyridine is often carried out using Re et al.'s methodology [147]. In short, varying amounts of the standard and sample chemicals were added to a 0.25 mL $FeSO_4$ (2 mM) solution. Thus, the sample's interaction Fe^{2+} ions are guaranteed. Thus, the sample chelates Fe2+ ions. Their absorbances were measured at 522 nm in comparison to the Tris-HCl buffer blank [148].

3.3. Metals chelating percentage

The following formula is used to calculate the samples' and standards' % chelating ability:

$$Percentage\ chelating\ effect\ \left(\%\right) = [(A0 - A1)/A0)]\ X\ \ 100$$

where the absorbances of the sample and control are denoted by A0 and A1, respectively. FeCl2, ferrozine, and 2,20-bipyridine were not present in the control [149].

3.4. IC50 Value's significance in binding affinity

In biochemistry, the IC50 value is frequently used to compare metal chelating [11]. IC50 values provide a quantitative description of binding affinity. This parameter is a different strategy founded on an appropriate idea and is frequently utilized in biochemical applications. It is not necessary to have additional knowledge of binding constants in order to evaluate half of the binding property. Higher binding affinity and ease of use are indicated by a lower IC50 value. The IC50 value is the most useful method for assessing binding affinities because of all these factors. It typically discusses biological impacts [14,46]. The IC50 is the concentrations needed to construct a dose-response curve and inhibit half of the agonist's maximum biological activity in experiments involving functional antagonists. It can be ascertained by analyzing the impact of varying antagonist concentrations. Although the Cheng–Prusoff equation [150], which is provided below, correlates IC50 and affinity, at least for competitive agonists and antagonists, the IC50 value is not a direct indicator of binding affinity.

$$K_i = IC\,50/1\ +\ \left[S\ /K_i\right]\left(Cheng - Prusoff\ \ equation\right)$$

The Ki can be determined by using the IC50 value for a competitive inhibitor for a single substrate enzymatic reaction. The IC50 indicates the concentration at which there is 50% inhibition of enzyme activity. The IC50 is used in combination with the substrate (S), and the Km is the Michaelis-Menten constant for the substrate, for determining the Ki in enzymatic reactions. Response. The IC50 value of a substance might fluctuate based on experimental settings and characteristics like pressure and temperature, whereas Ki remains a rather steady value [151].

4. CONCLUSION

Several biological processes, including cell growth, development, and proliferation, biomolecule synthesis, the catalysis of several enzyme reactions, and immunology of the body, depend on heavy metals, which play a variety of functional roles in biological systems. body, however excessive metal absorption through various channels is very dangerous and can result in irreparable effects. Furthermore, heavy metals like Fe2+ can make it easier for living systems to produce ROS. The agents' capacity to chelate metals can be highly advantageous for their antioxidant qualities. In order to lessen oxidative damage and the harmful effects of ROS, antioxidants are essential. Metal chelating activity is a frequently employed method in biology, food, and medicinal applications. This review article comprehensively elucidates heavy metals, metals, oxidative stress, reactive oxygen species, the implication of excessive metals exposure, antioxidants, metals chelating capacity, the metal chelation in biological system, in vitro metal chelation assay, and antioxidant methodologies.

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