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INNOVATIVE SYNTHESIS OF ZINC AND SELENIUM COMPLEXES WITH GALLIC ACID: EXPLORING THEIR ANTIOXIDANT POTENTIAL

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INNOVATIVE SYNTHESIS OF ZINC AND SELENIUM COMPLEXES WITH GALLIC ACID: EXPLORING THEIR ANTIOXIDANT POTENTIAL (Abstract): Polyphenols are naturally occurring compounds found largely in fruits, vegetables, cereals and beverages. Fruits like grapes, apple, pear, cherries and berries contain up to 200–300 mg polyphenols per 100 grams fresh weight. Among these compounds, gallic acid, (GA), a trihydroxybenzoic acid, stands out for its selective cytotoxicity against various tumor cell lines. This study focuses on the synthesis and analysis of a new complex of Zn(II) with GA, but also Se(IV) with gallic acid, exploring its comparative antioxidant potential. **Materials and methods:** The complex, synthesized in a 1:2 molar ratio of GA to zinc acetate dihydrate, respectively sodium selenite pentahydrate, showed various promising therapeutic roles in vitro. GA and zinc both possess antioxidant properties, and their coordination in the complex increases these attributes depending on the concentration. **Results:** GA, known for its powerful antioxidant effects, outperforms popular antioxidants like ascorbic acid in various tests. The study evaluates the ability of GA to remove reactive species, showing its superior efficiency. In addition, GA and its ester derivatives demonstrate protective effects against lipid peroxidation, a important process in cellular damage. **Conclusions:** The synthesized Zn(II) complex, with its structure-activity relationship suggesting Zn(O4) coordination, opens avenues for understanding its antioxidant potential. Overall, this research reveals the multifaceted pharmacological potential of GA and its complexes, providing insights into their antioxidant effects. **Keywords:** METAL-ION-COMPLEX, GALLIC ACID-ZINC-COMPLEX, ANTIOXIDANT CAPACITIES.

Polyphenols, also known as phenolics, are ubiquitous in nature and represent a structurally diverse group of compounds, particularly prevalent in plant species (1, 2). Among them, condensed tannins, the most abundant polyphenols, are found across various plant families, including lower organisms such as mushrooms (3-6).

Certain polyphenols, such as phytoalexins produced by plants in response to pathogen intrusion, exhibit a hypersensitive reaction (7-10).

The presence of polyphenols can contribute to environmental contamination in proximity to processing facilities engaged in the production of olive oil, coffee, paper, and within the carpet industry. Conversely, numerous polyphenols are renowned for their antioxidative (11, 12), anti-inflammatory (13), and anticancer properties (14, 15), warranting attention in the realm of drug development, particularly for combating cancer (16, 17, 18, 19).

One prominent polyphenol of note is GA, (3,4,5-trihydroxybenzoic acid) (fig. 1), which holds significant relevance due to its selective cytotoxicity against various tumor cell lines.

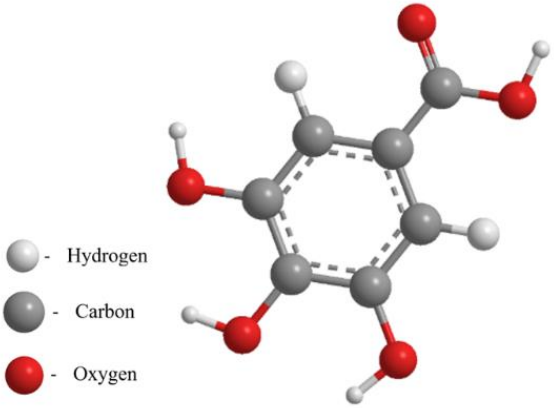


Fig. 1. 3D rendering of the GA molecule (made by using Chem3D)

GA is an organic compound belonging to the class of polyphenols. It is marketed as a crystalline powder, either white or pale yellow. It is a trihydroxybenzoic acid with a benzene ring in its structure, to which a carboxylic group providing acidity to the molecule (-COOH) is attached, along with three hydroxyl groups (-OH) located at positions 3, 4, and 5 of the ring (20, 21).

GA is an endogenous plant polyphenol abundant in tea, grapes, berries, and other fruits, as well as in certain hardwood species like oak (*Quercus robur*) and chestnut (*Castanea sativa* L.). It manifests as yellowish-white crystals, possesses a molecular mass of 170.12 g/mol, a melting point of 250°C, and exhibits water solubility of 1.1% at 20°C (22, 23).

Known for its profound impact on several pharmacological and biochemical pathways, GA demonstrates potent antioxidant, anti-inflammatory, antimutagenic, and anticancer properties (24, 25, 26). Moreover, GA exhibits pro-oxidant characteristics in a concentration-dependent manner in the presence of metal ions, a trait that has been identified as an inducer of apoptosis in cancer cell lines (27). Studies have also confirmed the protective role of GA against chemically induced carcinogenesis (28, 29).

MATERIALS AND METHODS

For the synthesis and analysis of the studied complex we have used the following reagents and equipment:

- Polyphenol (GA) –analytical grade purity, purchased from Sigma-Aldrich;
- Metalic ion salts (Zinc acetate, Sodium selenite) Sigma-Aldrich;
- Solvents (Distilled water, Methanol, Absolute ethanol, 96% Ethanol) (Sigma-Aldrich);
- Magnetic stirring plate;
- Laboratory grade glassware (Erlenmeyer flasks, beakers, magnet for stirring, thermometer);
- Filter paper;
- Analytical balance Mettler (Mettler Toledo, Greifensee, Switzerland);
- Magnetic stirring FALC-30ST;
- spectrophotometer Specord® 210 PLUS

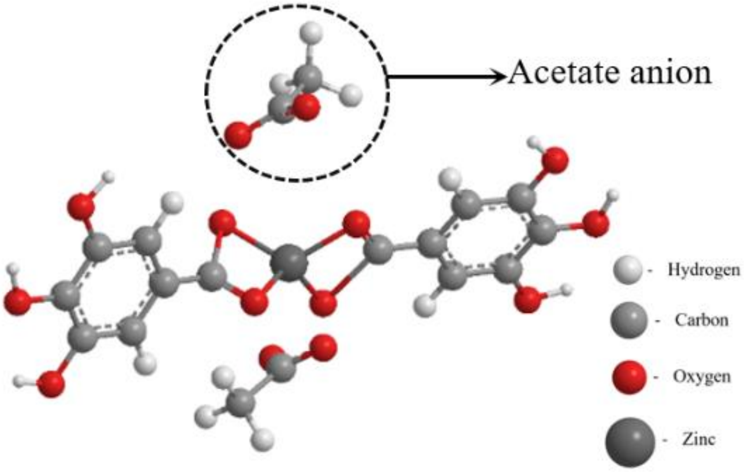


Fig. 2. 3D rendering of the synthesized Gallic acid-Zn(II) complex (made by using Chem3D)

INSTRUMENTATION

Instrumentation for the structural characterization of the formed complex involved Fourier-transform infrared spectroscopy (FTIR). The FTIR spectra were acquired using an FT-IR Bruker Vertex 70 Spectrophotometer in reflexional mode with the attenuated total reflectance (ATR) technique. Spectral analysis was conducted at a scan rate of 40/s within the range of 4000 cm-1 to 380 cm^{-1}, using approximately 1 mg of sample.

A ^{1}H NMR spectrum reveals the chemical shifts corresponding to various protons within the tested samples (30, 31).

The Nuclear Magnetic Resonance spectra ^{1}H-RMN, were registered using a Bruker Vance III apparatus, at 500 MHz in deuterated dimethyl sulfoxide (DMSO-d_6). The displaced chemical signals present in the registered spectra, were represented using δ values in parts per million (ppm), and the coupling constancies J in Hz.

Synthesis of the gallic acid and Zinc(II) complex

The complex was synthesized using zinc acetate dihydrate and GA in a 1 :2 molar ratio, our study has followed this method with slight alterations. The synthesis started by weighing the reagents and dissolving 376.3 mg of GA monohydrate and 219.51 mg of zinc acetate dihydrate separately in 5 mL of methanol each. After this, the solutions were gradually stirred using a magnetic stirring plate. The complex, Fig. 2, formed as a white, almost milky gelatinous precipitate, which was separated by filtration.

The resulting precipitate was washed multiple times with a 50% methanol solution, dried and stored at standard room temperature in air-tight glass vials.

Synthesis of the gallic acid and Se(VI) complex

The complex was synthesized using sodium selenite and GA, our study has followed the method described in the literature with slight alterations (32). The synthesis started by weighing the reagents and dissolving 376 mg of GA monohydrate and 263 mg of sodium selenite pentahydrate separately in 5 mL of methanol each. After this, the solutions were gradually stirred using a magnetic stirring plate. The complex, formed as a dark grey precipitate which was separated by filtration.

The resulting precipitate was washed multiple times with a 50% methanol solution, dried and stored at standard room temperature in air-tight glass vials.

Zinc(II) exhibits a preferential tetrahedral coordination but can also adopt higher coordination geometries, such as octahedral. It tends to favor ligands that form relatively weak bonds, making zinc less prone to forming highly stable complexes with strongly chelating ligands compared to other transition metals. Zinc complexes are relatively stable and find applications in various pharmaceutical and industrial settings. Due to their moderate stability, zinc complexes are ideal for applications requiring controlled release of the ligand (33, 34).

In the complex with zinc(II) ions, bonds are formed with GA molecules. These bonds can be coordinative, where the oxygen atoms of the functional groups in GA bind to zinc ions. Both zinc and GA possess antioxidant properties. GA, among numerous plant-derived polyphenols, possesses both antioxidative and antidiabetic pharmacological potential (35, 36).

Antioxidant activity

The DPPH scavenging activity of the complex was measured using a modified version of a previously described method by Sanni O *et al.* (37). The assay mixture comprised 75 μl of various concentrations (ranging from 3.75 to 60 μM in the reaction mixture) of the complex, along with its precursor GA, zinc(II) acetate, sodium selenite (IV), or standards such as ascorbic acid, or their respective solvents (control). Additionally, 37.5 μl of a 0.3 mM DPPH solution was added to the mixture in a 96-well plate. Following a 30 minutes incubation period in the dark, absorbance readings were taken at 517 nm using a Specord® 210 PLUS spectrophotometer. Sample blank or sample solvent was utilized as the blank. The following formula was employed to calculate the percentage of DPPH radical scavenging activity:

$$\textbf{\% inhibition} = \textbf{100 x} \ (\mathbf{A_{control}} - \mathbf{A_{final}}) \ / \ \mathbf{A_{control}}$$

where:

$A_{control}$ is the absorbance of DPPH before adding the GA or ots complexes

A_{final} is the absorbance of the DPPH mixture after adding the GA or its complexes and incubating in a dark place.

The evaluation of the antiradical potential of antioxidants commonly employs the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, where DPPH, a stable free radical with an absorbance band at 517 nm, undergoes reduction upon interaction with an antiradical compound (38, 39). The statistical evaluation was performed by determining the mean values of five readings for each sample and for each concentration used. The data included in the graphs represent the average ± the standard deviation.

RESULTS AND DISCUSSION

In our study, the most important assessed parameter was the nature of the solvent used for synthesis. It can be observed in Figure 3, that the superior yield was obtained when using methanol as the solvent for the complexation reactions. The polarity of the solvent and its capacity to form hydrogen bonds has an impact on the interactions between the metallic ions and the ligand used.

The confirmation of complex formation was achieved through ^{1}H-NMR spectroscopy in DMSO-d_6. The absence of the signal corresponding to the proton of the carboxylic group is observed at 13-12 ppm. The region between 10-9 ppm for both complexes undergoes slight modifications, with no change in the region of aromatic protons.

The structure of GA was confirmed through the ^{1}H-NMR proton spectrum in DMSO-d_6. The region at 13-12 ppm corresponds to the proton of the carboxylic group (-COOH), being the most shielded. The region of 10-9 ppm corresponds to the protons of the hydroxyl groups (-OH), and the region of 7-6.7 ppm is attributed to the aromatic protons in the benzene ring GA.

The characteristic bands of the phenolic hydroxyl group were observed in the FTIR absorption spectrum of GA and its complex in the range of 3200-3400 cm^{-1}, representing stretching vibrations due to intramolecular hydrogen bonds.

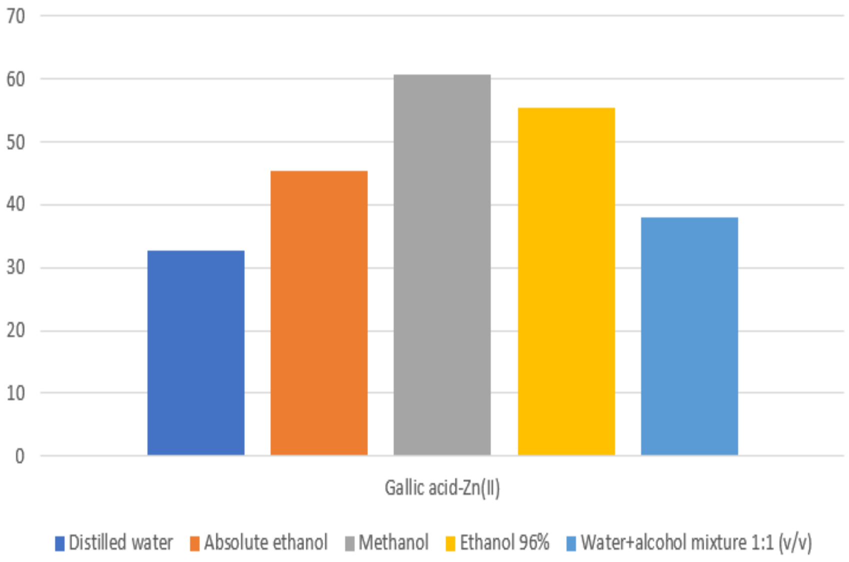


Fig. 3. The yield in relation to the solvent used

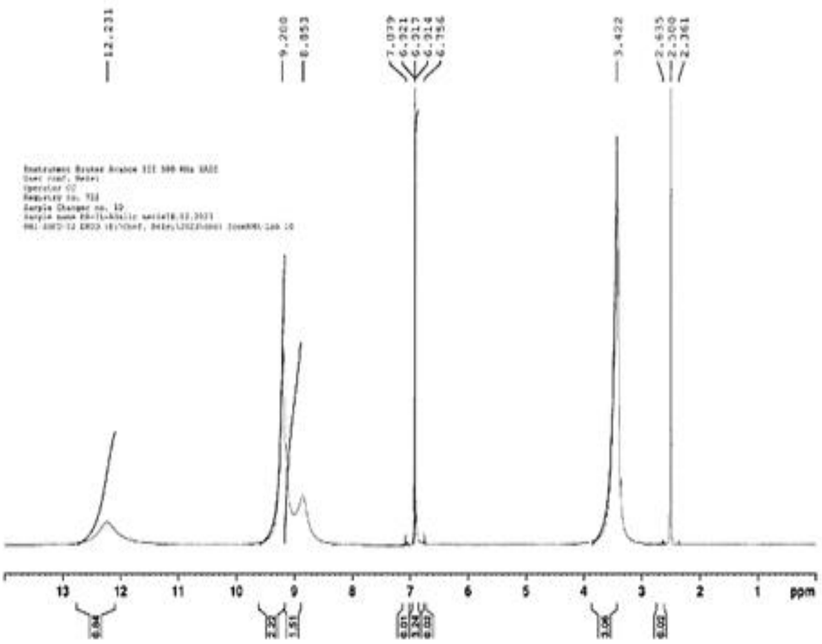


Fig. 4. ^{1}H-NMR spectrum of GA

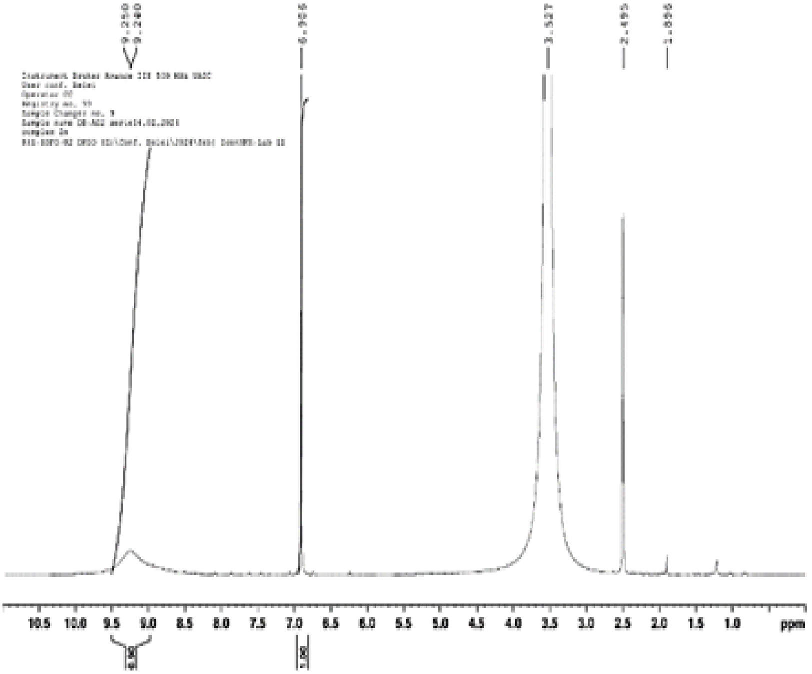


Fig. 5. ^{1}H-NMR spectrum of gallic acid-Zn(II)

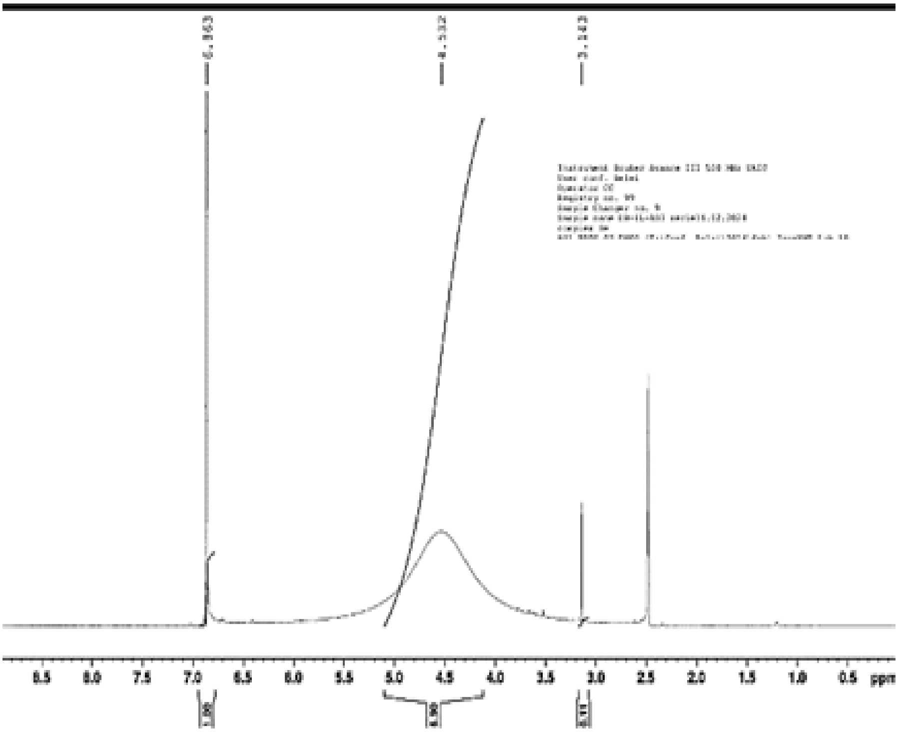


Fig. 6. ^{1}H-NMR spectrum of gallic acid-Se(IV)

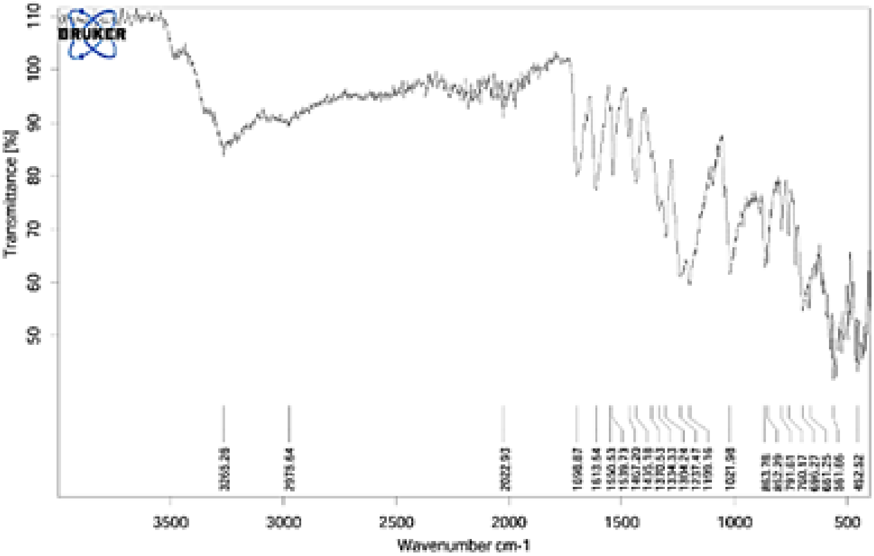


Fig. 7. FTIR absorption spectrum of GA

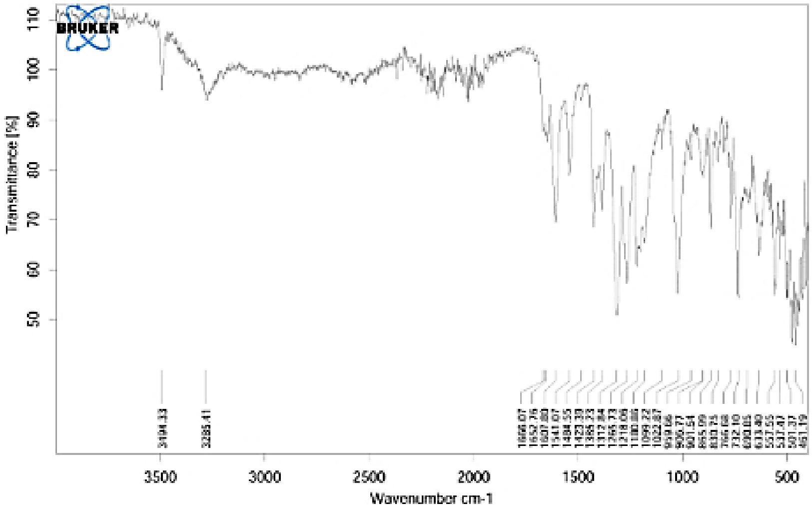


Fig. 8. FTIR absorption spectrum of gallic acid-Zn(II)

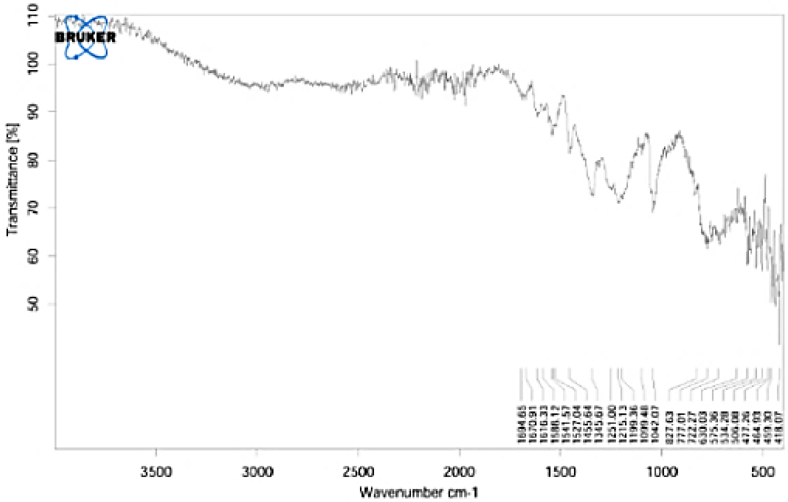


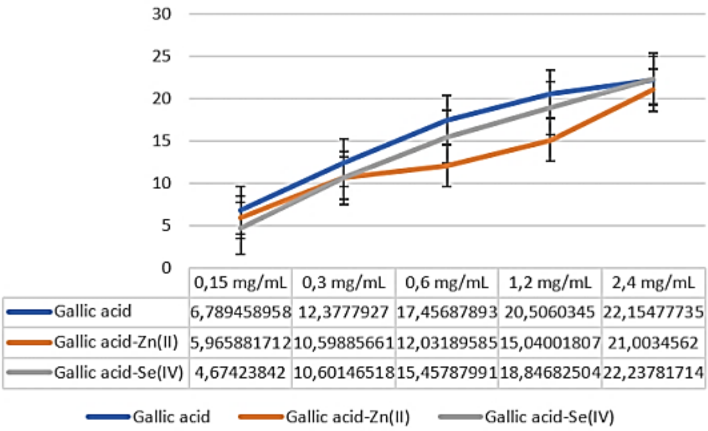
Fig. 9. FTIR absorption spectrum of gallic acid-Se(IV)

The presence and structure of the gallic acid-Zn(II) complex is confirmed by the presence of bands observed in GA at 1378 and 1306 cm^{-1} and in its complex with zinc acetate at 1374, 1336, and 1307 cm^{-1}, representing specific bending vibrations of aromatic nuclei. The band at 3085-3024 cm^{-1} is characteristic of the stretching vibration of the C-H bond in the aromatic ring. In the ranges of 2995-2863 cm^{-1} and 1648-1582 cm^{-1}, bands appear due to stretching vibrations of –CH3 groups and the >C=C< bond, respectively. Bending vibrations of the aromatic CH bond and the –CH3 group occur at

1458 cm^{-1} and 1357 cm^{-1}, respectively. Bands in the ranges of 1247-1000 cm^{-1} and 567-501 cm^{-1} are attributed to in-plane and out-of-plane deformation vibrations of the aromatic ring.

The presence and structure of the gallic acid-Se(IV) complex is confirmed by the presence of bands observed in GA at 1378 and 1306 cm^{-1} and in its complex with sodium selenite at 1345, 1251, and 1215 cm^{-1}, representing specific bending vibrations of aromatic nuclei. The band at 3085-3024 cm^{-1} is characteristic of the stretching vibration of the C-H bond in the aromatic ring, and in the range of 1648-1582 cm^{-1}, bands are present due to the >C=C< bond. Bending vibrations of the aromatic CH bond occur at 1458 cm^{-1} and 1357 cm^{-1}, respectively. Bands in the ranges of 1247-1000 cm^{-1} and 567-501 cm^{-1} are attributed to in-plane and out-of-plane deformation vibrations of the aromatic ring. The confirmation of complex formation can also be supported by the absence of vibration bands at 3263 cm^{-1} corresponding to the -OH groups.

The complex combinations of GA with zinc(II), selenium(IV), as well as free GA, were subjected to examination, and their antioxidant activity was tested through recorded absorbance.



	0,15 mg/mL	0,3 mg/mL	0,6 mg/mL	1,2 mg/mL	2,4 mg/mL
Gallic acid	6,789458958	12,3777927	17,45687893	20,5060345	22,15477735
Gallic acid-Zn(II)	5,965881712	10,59885661	12,03189585	15,04001807	21,0034562
Gallic acid-Se(IV)	4,67423842	10,60146518	15,45787991	18,8468250	22,23781714

Fig. 10. DPPH• % inhibition of gallic acid and its complexes with Zn(II) and Se(IV)

GA complexed with selenium ions exhibited the highest antioxidant activity at the highest concentration. However, it can be observed that at different concentrations, GA demonstrates stronger antioxidant action. The action also depends on the molar ratio used in synthesis, in this case, GA: zinc salt = 1:2. Referring to the structure of GA, the newly formed bonds resulted in a decreased antioxidant activity.

Comparing our results to the literature, the findings were confirmed by Samy M. and colleagues (40), who also obtained lower absorbance for free GA at maximum concentration and higher absorbance for the gallic acid-selenium(IV) complex. In another study conducted by Denice M. Motloung and colleagues (41), the gallic acid-zinc(II) complex recorded a higher inhibition percentage at concentrations of 15, 30, and 60 µM compared to free GA and ascorbic acid.

Under these conditions, chelating GA with selenium(IV) enhances the antioxidant activity of the polyphenolic acid, depending on the molar ratio, solvent, and the concentration of the tested complex. The antioxidant properties of zinc acetate, used for synthesizing the complex with GA, were also tested, but the results obtained were very small, almost irrelevant for its use as an antioxidant.

An important aspect is that these metal ions can be used in combination with polyphenols, improving the antioxidant activity of the complex.

Studies by Abdelwahed *et al* (42) reveal that GA and 1,2,3,4,6-pentagalloylglucose (PGA) employ a hydrogen-donating mechanism to scavenge DPPH radicals, proving more effective than Vitamin E. The antioxidant activity order, ranks GA above caffeic acid, ascorbic acid, Trolox, sinapinic acid, and isoeugenol (43).

Beyond the DPPH assay, GA excels as a potent antioxidant in various assays such as Trolox equivalent antioxidant capacity, total radical-trapping antioxidant parameter (TRAP), photochemiluminescence (PCL), and ferric reducing ability of plasma (FRAP). It outperforms popular antioxidants like ascorbic acid, Trolox, and uric acid. Computational studies further confirm GA's superior antioxidant efficiency compared to various counterparts. At a concentration of 4.17 mM, GA exhibits significant scavenging effects on DPPH and H_2O_2 radicals. GA and n-alkyl gallates effectively scavenge other reactive species, HOCl, at low concentrations, protecting a1-antiproteinase and reducing ox brain phospholipid peroxidation (44, 45).

Moreover, GA and its ester derivatives showcase antioxidant capacity against various reactive oxygen and nitrogen species, including peroxyl, azide, and hydroperoxyl radicals (46, 47).

CONCLUSIONS

The complex produced by the combination of zinc acetate and GA resulted in good yield when methanol was used as solvent and NaOH as co-regulator. The same mechanism was in the case of the reaction between GA and sodium selenite.

Various modern techniques have confirmed the formation of complex combinations. Moreover, the antioxidant capacity did not show very large variations in the case of the tested compounds, the activity being dependent on the concentration of the sample to be analyzed.

It can be concluded that the antioxidant action is much better for the complex with zinc or selenium ions compared to pure salt.

Other antioxidant tests are needed to describe a more complex mechanism of the complex combination.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest and no funding regarding this scientific research.

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