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SYNTHESIS, CHEMICAL CHARACTERIZATION, AND BIOLOGICAL INVESTIGATION OF NATURALLY ISOLATED HESPERIDIN AND ITS METAL COMPLEXES

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ABSTRACT. This study was conducted to investigate the ability of hesperidin (HES) to form metal-complexes (HES-M) by chelation with transition metals such iron(III), copper(II), zinc(II), and silver(I), in addition to screen the antioxidant activity (DPPH free radical scavenging and phosphomolybdenum reduction potential methods PRP), anti-Alzheimer activity [(acetylcholinesterase (AChE)] inhibition assay) and coagulant activity (prothrombin time PT assay). The results indicated that HES could form a 1:1 complex with the studied metals in methanolic solution. The complex has been synthesized and characterized by physicochemical methods. Complexing of hesperidin with metals leads to increase in the antioxidant activity by 15.4-33%. Also, the total antioxidant capacities of HES-M complexes were increased by 13.8-251%. Cu-complex revealed the most increasing antioxidant activity by 33% for DPPH scavenging activity and 251% for PRP activity. The inhibition capacity of the HES, as well as of the HES-M complexes on the enzyme AChE, was revealed that only HES-Zn complex has higher anti-Alzheimer activity (IC_{50} 1.18 mg/mL). HES and HES-M complexes led to accelerate the clotting time by 28.9-67.3%, where HES-Zn complex was the most accelerating clotting time by 67.3%. The high increase in total antioxidant activity of hesperidin after chelating with copper (251%) opens the door for further research.

KEY WORDS: Hesperidin, Metal ions, Complexation, Antioxidant, Anti-Alzheimer, Coagulant activity

INTRODUCTION

Flavonoids are plant-based natural products that are very abundant and have multiple therapeutic benefits and biological activities. This diverse group of compounds exerts antihyperglycemic, antihyperlipidemic, anticarcinogenic, antihyperammonemia, nephroprotective, and hepatoprotective activities as we reported previously [1-3].

The ability of flavonoids to neutralize reactive radicals is usually linked to their antioxidant properties. However, other mechanisms can be involved too. One of them is the ability to sequestrate metal ions, such as iron and copper ions that participate in the generation of hydroxyl radicals in Fenton [Fe(II)] or Fenton-like [Cu(II)] reactions [4]. The chelation takes place via hydroxyl groups or their carbonyl moiety if present [5]. Quercetin, a widespread flavonol, is one of the best investigated flavonoids. Its ability to chelate a wide range of metal ions, such as Al(III), Co(II), Cr(III), Cu(II), Fe(II), Fe(III), Mo(VI), Pb(II), Tb(III), and Zn(II) has been shown in several studies. Similar data have been obtained for rutin (quercetin-3-*O*-beta-rutinoside) or catechin (flavan-3-ol) towards Cu(II), Fe(II), and Zn(II) ions [6].

Hesperidin (3′,5,7-trihydroxy-4′-methoxy-flavanone-7-rhamnoglucoside, hesperetin-7-rutinoside (Figure 1) is the most abundant flavanone glycoside present in citrus peels, being a major component in rind tissues and it is characteristic of oranges. Its consumption may be associated with health benefits and in the prevention of many diseases. It exhibits biological properties such as analgesic, anti-inflammatory, anti-hypercholesterolemic, antihypertensive, diuretic, neuroprotective and cytotoxic against HEP-G2 cancer cells, among others [7-9].

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Literatures revealed that the peels of citrus fruits are one of the most abundant sources of flavonoids, e.g., hesperidin, naringenin, and quercetin, etc., which are well known throughout ages for their beneficial pharmacological activities as antioxidants, anti-tumors and anti-inflammatory agents [10-11]. Citrus fruits are widely used all over the world as food ingredients and in traditional medicines.

Studies have proven that the biological activity of flavonoids increases when they are bonded (covalently or coordinately) with ions of transition elements or heavy metals [12-13]. Consequently, in continuation of our phytochemical and pharmacological research [14-18], this study aims to benefit from the peel of citrus fruits such as *Citrus maxima* or *Citrus aurantium* in the commercial isolation of biologically active compounds such as naringenin and hesperidin. Additionally, trying to increase the biological activity of these compounds by complexing with transition metals such as iron(III), copper(II), zinc(II), and silver(I) and screening their pharmacological properties.

EXPERIMENTAL

Apparatus and reagents

All chemicals, reagents, and solvents used in this study were analytical grade and purchased from Sigma-Aldrich (USA). Metal salts; silver nitrate ($AgNO_3$, 99%), copper chloride dihydrate ($CuCl_2.2H_2O$, 98%), ferric chloride ($FeCl_3$, dry, > 97%), zinc chloride ($ZnCl_2$, anhydrous, ≥ 98%), and standard hesperidin purchased from Sigma-Aldrich (USA). Authentic (standard) hesperidin (98%) was purchased from Roth, Germany. All chemicals and reagents used for bioassay were purchased by BPTL-Alex team.

Extraction, isolation, and characterization of hesperidin

The *Citrus maxima* fresh fruits were purchased from a local market in Alexandria-Egypt, in March 2022, and they originated from Borg El-Arab City (Egypt). Extraction and isolation of hesperidin were conducted according to method C of Victor *et al.* [19]. The pomelo peels were hand-sliced from the pulp and the albedo (white interior layer) was separated from the flavedo (orange exterior layer). The fresh albedo layer was cut into small pieces using a food processor, then immediately, 30 g of fresh albedo was added to 300 mL of methanol in an Erlenmeyer flask. The mixture was heated for 3.5 h at 55 ºC, then the solvent was filtered off, and one more 100 mL of methanol was added, and the new extraction continued for 3 h at 55 ºC. The organic solvents were combined, and the methanol was distilled off under vacuum at 45 ºC. The obtained extract was suspended with 20 mL distilled water and sequentially the mixture was stirred at 60–70 ºC for 30 min, then transferred into a separating funnel. Dichloromethane (3 mL) was added, and the mixture was transferred to a stoppered flask and left for 4 days at room temperature. The organic layer was removed and the hesperidin crystals [239 mg, 0.70% yield or 2.27% yield based on the estimated dry weight of albedo powder (11 g)] were collected by filtration through filter paper and dried in a vacuum desiccator. The extraction process was repeated many times to get the requested hesperidin weight.

Identification of the isolated hesperidin was established by direct comparison of the R_f with an authentic sample using a solvent system chloroform-methanol-water (30:60:5, v/v/v) and examination of the spot under UV light before and after exposure to ammonia. The structure of hesperidin was confirmed based on spectral analysis UV, IR, ^{1}H and ^{13}C-NMR.

Hesperidin. Pale yellow powder, m.p. 252–254 ºC, $C_{28}H_{34}O_{15}$ (610.565 g.mol^{-1}); UV (λ_{max}, nm): 230, 285 and 325; FT-IR (KBr disc, cm^{-1}): 3450 (OH), 3419.41, 1644.52, 1603.71, 1518.22, 1504.30, 1442.07, 1274.01, 1204.45, 1181.63, 1128.23, 1093.77, 1063.24. ^{1}H-NMR (DMSO-d6, 400 MHz): δ (ppm) 12.01 (s, 1H, OH-5), 9.08 (br. s., 1H, OH-3′), 6.96–6.88 (m, 3H, H-2′, H-

5′and H-6′), 6.13 (d, J = 6.4 Hz, 2H, H-8 and H-6), 5.51 (dd, J = 12.2, 2.6 Hz, 1H, H-2), 5.16 (d, J = 5.5 Hz, 1H, rhm-1), 4.99 (d, J = 7.3 Hz, 1H, glc-1), 4.67-3.80 (m, rhm. and glc. protons), 4.51 (s, 1H, H-1), 3.80 (br. s., 1H, glc-4), 3.76 (s, 3H, MeO-4′), 3.09–3.20, 3.11 (1H, dd, *J* = 17.0, 11.0 Hz, H-3_{eq}), 2.78 (dd, J = 17.0, 2.6 Hz, 1H, H-3_{ax}), 1.09 (d, J = 6.1 Hz, 3H, rhm-6). ^{13}C-NMR (DMSO-d6, 100 MHz): δ (ppm) 197.70 (C-4), 165.17 (C-7), 163.08 (C-5), 162.58 (C-10), 147.95 (C-4′), 146.48 (C-3′), 130.93 (C-1′), 117.83 (C-6′), 114.19 (C-2′), 112.13 (C-5′), 103.38 (C-9), 100.64 (C-1‴), 99.48 (C-1″), 96.42 (C-6), 95.59 (C-8), 78.48 (C-2), 76.31 (C-5″), 75.56(C-3″), 73.02 (C-4‴), 72.11 (C-2″), 70.71 (C-4″), 70.31 (C-3‴), 69.64 (C-2‴), 68.36 (C-5‴), 66.07 (C-6″), 55.72 (MeO-4′), 42.31 (C-3), 17.88 (C-6‴).

Synthesis and characterization of hesperidin-metal complexes

The hesperidin-metal complexes were synthesized in general by refluxing a mixture of hesperidin solution (0.61 g, 1 mmol in 5 mL methanol) and equimolar amounts of the metal salt solution (i.e., 1 mmol in 10 mL MeOH, 0.162, 0.136, 0.171, 0.170 g of either ferric chloride, zinc chloride, copper chloride dihydrate, and silver nitrate) for 3–6 h. The solution was concentrated and kept at room temperature overnight to precipitate. The obtained precipitate was filtered and washed with cold ethanol, then dried in the vacuum desiccators over anhydrous $CaCl_2$ to give 0.53 g (58.4%), 0.49 g (66.6%), 0.39 g (54.9%), and 0.35 g (48.8%) for HES-Fe, HES-Cu, HES-Zn, and HES-Ag complexes, respectively. All complexing reactions were performed in triplicate. The structure of obtained complexes was characterized by UV–Vis, and FT-IR.

HES-Fe complex. Yield: 58.4%, 0.53 g pure, m.p. 283–284 ºC. UV–Vis (MeOH) λ_{max}, nm: 305, 285, 275, 235, 215 and 205. FT-IR (KBr) ν, cm^{-1}: 3522.61, 3408.94, 2938.21, 2917.33, 1621.94, 1603.31, 584.75 and 418.50.

HES-Cu complex. Yield: 66.6%, 0.49 g pure, m.p. 290–293 ºC. UV–Vis (MeOH) λ_{max}, nm: 315, 305, 280, 270, 250, 235 and 222. FT-IR (KBr) ν, cm^{-1}: 3524.70, 3473.54, 2937.70, 2917.71, 1619.33, 1602.85 and 585.46.

HES-Zn complex. Yield: 54.9%, 0.39 g pure, m.p. >300 ºC. UV–Vis (MeOH) λ_{max}, nm: 325, 285, 230, 220 and 210. FT-IR (KBr) ν, cm^{-1}: 3524.52, 2938.30, 2918.38, 1624.11, 1603.31 and 610.80.

HES-Ag complex. Yield: 48.8%, 0.35 g pure. UV–Vis (MeOH) λ_{max}, nm: 305, 285, 275, 235, 215 and 205. FT-IR (KBr) ν, cm^{-1}: 3533.44, 3420.20, 2937.73, 2918.12, 1633.65, 1604.11 and 586.13.

Antioxidant activity

The antioxidant activity was conducted at BPTL-Alex by using DPPH free radical scavenging and phosphomolybdenum reduction potential (PRP) methods.

DPPH free radical scavenging activity

Ascorbic acid solutions (0.5, 2.5, 5, 10, 15, and 20 µg/mL, in methanol) were used as standard reference. Samples solutions were prepared at the final concentration of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL in methanol. DPPH free radical assay was carried out according to the method of Braca *et al.*, [20]. Briefly, 100 µL of freshly prepared DPPH reagent (0.1% in methanol) were added to 100 µL of the samples in 96 wells plate (n = 6), the reaction was incubated at room temperature for 30 min in dark, then the resulting reduction in DPPH color intensity was measured at 517 nm. The radical scavenging activity was calculated from the following equation: Percentage of radical scavenging activity = $[(A_C - A_E)/A_C] \times 100$, where: A_C: The mean of absorbance of negative

control, A_E: The mean of absorbance of extract. The results were recorded using microplate reader FluoStar Omega. Data was analyzed using Microsoft Excel and IC_{50} was calculated from inhibition.

Phosphomolybdenum reduction potential (PRP) method

The total antioxidant capacities of HES and HES-M complexes were assessed using the phosphomolybdenum reduction potential (PRP) method detailed in Prieto *et al.*, [21]. For a 90-minute incubation period at 95 °C, 0.3 mL of extract was combined with 3 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The method's foundation is the extracts' conversion of Mo(VI) to Mo(V), which is followed by the creation of a green phosphate/Mo(V) complex at an acidic pH. The reaction solution was cooled to room temperature before measuring the absorbance at 695 nm in comparison to a blank. The amount of ascorbic acid equivalents used to measure the extract's antioxidant activity.

Acetylcholinesterase (AChE) inhibition activity assay

Acetylcholinesterase (AChE) inhibition activity of HES and HES-M complexes as the mechanism of anti-Alzheimer activity was conducted at BPTL-Alex according to Fale *et al.* [22]. In ELISA plate (Bio Tec., USA), 150 µL of phosphate buffer (0.1 M, pH 8) was directly added in ELISA blank well and 130 µL of phosphate buffer was added in ELISA activity wells. To the blank and activity wells, 5 µL of acetylcholine thioiodide (ACTI) substrate (75 mM in distilled water) was added, then 20 µL brain homogenate supernatant + 20 µL of HES and HES-M solutions (test) or DMSO (control) were added in activity ELISA wells only. The plate was preincubated for 15 min at 37 °C before the addition of the second substrate DTNB (0.32 mM in 10 mL phosphate buffer 0.1 M, pH 8). DTNB (60 µL) was added in both blank and activity wells. Absorbance was measured at 405 nm every two min. Values obtained were analyzed and blank reading was subtracted from sample readings.

AChE inhibition activity of HES and HES-M complexes was estimated from the following formula: AChE inhibition activity (%) = $(\frac{Ac-As}{Ac})$*100, where; A_C: The mean absorbance of negative control, A_S: The mean absorbance of plant extract - the mean absorbance of plant extract blank, 100: Percentage of inhibition. AChE inhibition activity was expressed as IC_{50} value (mg/mL) (the inhibitory concentration at which 50% of acetylcholine esterase are repressed).

Estimation of prothrombin time (PT) assay

The action of the HES and HES-M in the extrinsic pathway was determined by the prothrombin time (PT) assay [23]. Calcium thromboplastin reagent was pre-warmed in a water bath at 37 °C for about 10 min. Human normal pooled plasma (0.1 mL) was pipetted into clotting tubes and incubated in a water bath for 2–3 min at 37 °C. Then, 0.1 mL of DMSO (for control) and 5 mg/mL of HES or HES-M complexes were added. Calcium thromboplastin reagent (0.1 mL) was swiftly added to the mixture, while simultaneously starting a stopwatch. The tubes were gently tilted (angle of 45°) at regular intervals until a clot was formed. The stopwatch instantly stopped, and the time was recorded. The tests were done in triplicate for each compound. The percentage of clotting inhibition was calculated from the equation: % Inhibition of clotting = ((A_C – As)/A_C)*100, where: A_C = absorbance of control and A_S = absorbance of sample.

RESULTS AND DISCUSSION

Hesperidin is a bioflavonoid found in citrus fruits. Structurally, hesperidin is a *β*-7-rutinoside of hesperetin consists of an aglycone, hesperetin and a disaccharide, rutinose. Anticancer and cancer chemopreventive properties of hesperidin and hesperetin are reported in literature [24].

Flavonoids present more than one possible chelating site for metal ions. The complexing of hesperidin with metal was discussed in many studies [25-28]. Metal coordination to the hesperidin can occur via the 5-hydroxyl of the ring A and 4-carbonyl group of the C ring (Figure 1), since the 3-hydroxyl group of the ring B, which plays the most important role in forming bonds between flavones and metals, is not present [29]. In other flavonoids there are more chelating sites, the preferred binding site depends on the flavonoid, the metal ion and on the pH value [28].

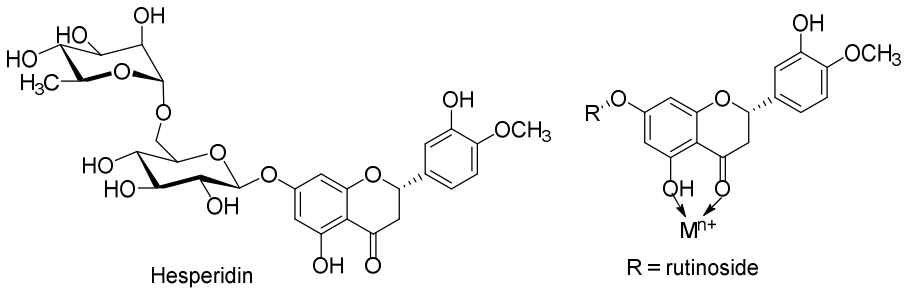

Figure 1. Chemical structure of hesperidin and its possible chelating site with metal.

The chemical structure of the hesperidin complexes was confirmed by comparing the differences in FT-IR, UV and ^{1}H-NMR spectra with the pure hesperidin. In ^{1}H-NMR, the peaks of OH protons [OH-5 (C ring) and OH-3' (B ring)] appeared at δ 12.019 and 12.011 ppm for the pure hesperidin, while for the HES-M complexes only one peak appeared in between 11.998 to 11.955 ppm. ^{13}C-NMR spectra did not give clear evidence for complex formation since no obvious changes were detected in HES-M potential complexes.

In general, the complexation of flavonoids with metals can lead to a bathochromic shift in the UV spectrum. This is because the metal ion can interact with the flavonoid's π-electrons, which can extend the π-system of the flavonoid and lead to a lower energy absorption band. Absorption spectra of HES and HES-M complexes were recorded in the range 200–400 nm against methanol as blank. Free HES exhibits an absorption a very week band at 325 nm, corresponding to the B ring portion (cinnamoyl system, band I) and maximum one in methanol solution at 285 nm, corresponding to the A ring portion (benzoyl system, band II) [26]. The bands II are shifted to higher wavelength in the spectra of the HES-M complexes by 20 nm to record at 305 nm in the HES-Fe, HES-Cu, and HES-Ag complexes.

FT-IR spectral scanning was taken in the wavelength region between 400 and 4000 cm^{-1}. The intensity of the band at 3409–3533 cm^{-1}, assigned for the phenolic OH stretching vibrations of HES-M complexes increases in comparison with the band in the spectrum of hesperidin at 3419-5323 cm^{-1}. The other band at 2919 and 2964 cm^{-1} of CH and CH_2 aliphatic groups is observed and shifted to 2917 and 3938 cm^{-1} for metal complexes. The next band at 1644.2 cm^{-1} are specific for the carbonyl group for hesperidin. The intensity of the bands for CO vibrations, carbonyl group are visibly increased and shifted in between 1624-1633 cm^{-1} suggesting the interaction between the hesperidin molecule and metals. The changes in the characteristic bands of pure hesperidin and synthesized compounds confirm the formation of the HES-M complexes. Also, in the IR spectra, the validation of this coordinated bond was detected, a new band in the complexes

appeared at 584–610 cm^{-1}, this band was not found in the free HES and was assigned to C=O-M bond [30-31].

Antioxidant activity

Metal ions, such as iron and copper, are responsible for a significant amount of oxidative stress in all living organisms. These metal ions can generate highly reactive hydroxyl radicals through Fenton reaction. When metal ions bind to a ligand, it can change the speed and energy of the above chemical reactions. This is why flavonoids are considered antioxidants, not only because they can directly scavenge free radicals, but also because they can chelate metal ions, especially iron ions [32] (Equations 1 and 2).

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + OH^- + OH^- \quad (1)$$

$$M^{(n+1)+} + H_2O_2 \rightarrow M^{n+} + HOO^- + OH^+ \quad (2)$$

Several reports have examined the radical scavenging properties of hesperidin using a variety of assay methods. Results from these assays varied considerably, but in most, hesperidin was found to be inactive or only moderately active in comparison with other flavonoids antioxidants. Hesperidin was also found not to inhibit the liberation of reactive oxygen species from stimulated neutrophils. Thus, hesperidin appears not to be a particularly active antioxidant in comparison with most other flavonoids [33].

The antioxidant capacity of hesperidin and its complexes in the present study was conducted by DPPH and PRP methods. The antioxidant capacity of hesperidin in the present study is found to be weak in concordance with other reports that found hesperidin to be inactive or had slow but long-lasting activity [33-35].

According to IC_{50} in Table 1 and Figure 2, the DPPH scavenging activity of HES and its HES-M complexes revealed that complexing of hesperidin with metals lead to increase the antioxidant activity by 15.4-33% in most cases, where Cu-complex was the most potent DPPH scavenging activity (33%) followed by Fe-complex (22%), and finally Zn (15.4%).

Table 1. DPPH radical scavenging activity (IC_{50}), total antioxidant capacity and AChE inhibition activity (IC_{50}) of HES and its HES-M complexes.

Sample	Total antioxidant capacity (mg AAE /g extract) *	DPPH radical scavenging activity IC_{50} value (µg/mL)	AChE inhibition activity (IC_{50}) (mg/mL)
HES	946.25±6.6	277.3	1.59
HES-Ag	701.25±4.1	284.2	4.65
HES-Cu	2380.13±9.7	185.8	4.38
HES-Fe	1179.02±3.1	216.3	2.68
HES-Zn	1076.83±2.3	234.1	1.18
Ascorbic acid	-	6.9	0.48

*Results are expressed as mean ± standard deviation (n = 3); AAE = Ascorbic acid equivalent.

The results of the total antioxidant capacity of HES and its HES-M complexes confirmed the DPPH results. The total antioxidant capacities of HES-M complexes were increased by 251%, 24.6%, and 13.8% in the case of HES-Cu, HES-Fe, and HES-Zn complexes respectively. The chelation of hesperidin and silver metal (HES-Ag complex) led to a decrease in the antioxidant activity of hesperidin. This dramatic increase in the total antioxidant activity of hesperidin after complexing with copper opens the door for further investigation into this type of complex.

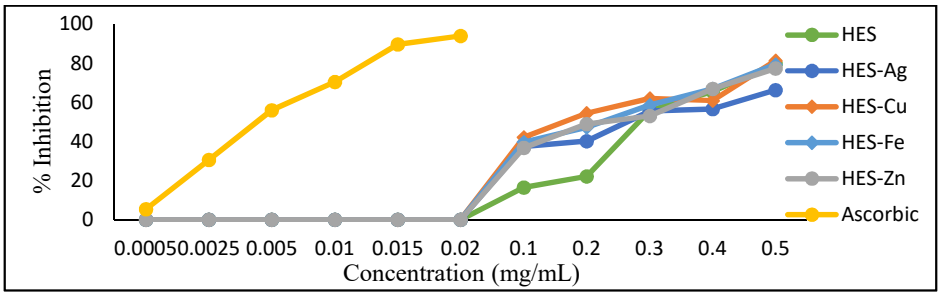

Figure 2. DPPH radical scavenging activities of HES and its HES-M complexes.

The variation of the antioxidant behavior of crude hesperidin extracts in previous reports is due to climate, soil, fruit variety and degree of maturation. These variations lead to changes in the phenolics and vitamins in these extracts in addition to the solvent influence. In polyphenol redox systems the solvents play a fundamental role in the chemical behavior of these compounds, and consequently their antioxidant property. This means that the compounds present different chemical forms related to the environment in which they are solved during the measurement of the antioxidant assay [35].

Anti-Alzheimer activity

Acetylcholinesterase (AChE) is the enzyme that catalyzes the hydrolysis of acetylcholine, a neurotransmitter found in the synaptic gap, and the inhibition of this enzyme could give some explanation for the action of the herbal tea on the nervous system. Nowadays the AChE inhibition is used in the treatment of the Alzheimer's disease (AD). Therefore, the most successful therapy for AD, at present, consists of increasing the levels of acetylcholine through the inhibition of acetylcholinesterase activity [36].

Anti-Alzheimer activity was measured by the AChE inhibition assay, where the HES showed a significant activity with IC_{50} value 1.59 mg/mL. Only complexing of hesperidin with zinc metal led to increasing the anti-Alzheimer activity (HES-Zn complex IC_{50} 1.18 mg/mL), while complexing of hesperidin with the other metal led to decreasing the anti-Alzheimer activity with IC_{50} in between 2.68-4.65 mg/mL as reported in Table 1. AChE enzyme inhibition activities of HES and its HES-M complexes were summarized in Figure 3.

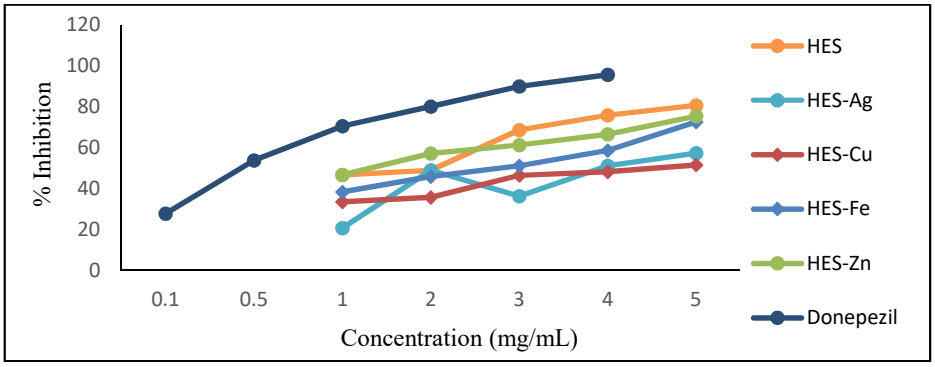

Figure 3. AChE Enzyme inhibition activities of HES and its HES-M complexes. Values expressed as means ($n = 3$) ± SD with *$p < 0.05$ in comparison with Donepezil as control.

It is reported that zinc complexes, improve solubility and bioavailability of zinc, this can enhance zinc's delivery to the brain and its therapeutic effects. Ligands can tailor the complex to interact with specific molecules or processes involved in AD, like Aβ aggregation or tau protein phosphorylation [37].

In vitro blood coagulation study

Prothrombin plays a crucial role in the coagulation process by influencing both fibrin formation and coagulation time. Its conversion to thrombin is a critical step in the coagulation cascade, and thrombin directly drives the conversion of fibrinogen to fibrin, the main structural component of blood clots. Prothrombin levels directly impact coagulation time, with deficiencies leading to prolonged bleeding and excesses leading to thrombosis [38]. In this study, the *in vitro* anticoagulant activity of the HES and HES-M in the extrinsic pathway was determined by PT assay. In extrinsic coagulation, fibrinogen is formed after adding prothrombin reagent to normal citrate plasma. Time of coagulation was calculated [23]. The PT assay gives a clear picture of the extrinsic pathway factors [39].

HES showed weak anticoagulant activity as it decreased the extrinsic clotting time at concentrations of 5 mg/mL into 18.5 s compared to DEMSO control (26 s). HES-M complexes significantly accelerates the extrinsic clotting time to 16.5, 9.5, 11.5, and 8.5 s at a concentration of 5 mg/mL for silver, copper, iron, and zinc, respectively (Figure 4). All HES-M complexes led to accelerate the clotting time by 36.5-67.3%, where HES-Zn complex was the most accelerating clotting time by 67.3%.

Kuntić *et al.* [40] reported that, the *in vitro* anticoagulant activity of hesperidin complexes with Al(III) and Cu(II) showed significantly prolonged activated partial thromboplastin time (aPTT) and had no effects on prothrombin time (PT) and thrombin time (TT).

The activity of zinc may be due to that zinc is a multi-functional element, it performs catalytic, co-catalytic, and/or structural functions. In 1982, found that a low zinc diet caused poor platelet aggregation and increased bleeding tendency in adult males. This fact drew interest to the role of zinc in blood clotting. It has been shown that hyperzincemia predisposes to increased coagulability, and hypozincemia to poor platelet aggregation and increased bleeding time. It is suggested that Zn^{+2} or Zn-complexes may inhibit the activity of proteins that normally work to prevent blood clotting, such as antithrombin III. This can allow the clotting cascade to proceed more quickly and efficiently [41].

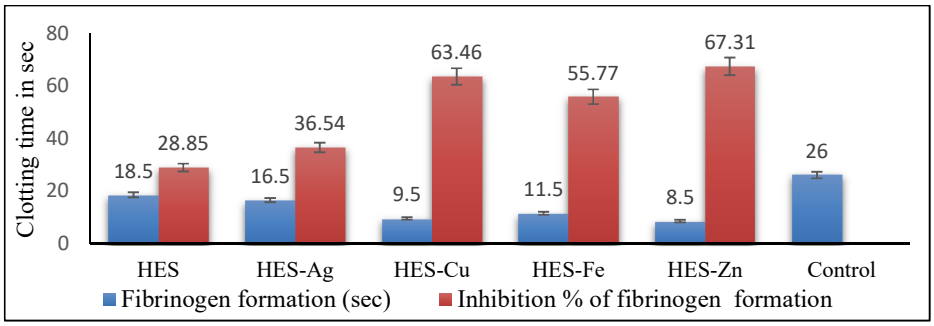

Figure 4. Prothrombin time (PT) of normal human plasma treated with 5 mg/mL of HES and HES-M complexes. Results represent the average of three measurements, SD = standard deviation.

CONCLUSION

The ability of hesperidin to form complexes with four metals; iron, copper, zinc, and silver was investigated. HES was shown to form complexes with the studied metals in methanol. The antioxidant, anti-Alzheimer and coagulant activity of hesperidin and synthesized metal complexes were evaluated by different methods. Complexing hesperidin with metals improved the studied biological activities almost for all metals. Zinc complex revealed the higher anti-Alzheimer and coagulant activities. Copper complex significantly improve the antioxidant activity by 251% than hesperidin, so, this type of complexes should be undergoing advanced further research.

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