

# Theoretical and Thermodynamics Studies of Complexes Formation between Natural Flavonoids and Hg (II) Ion

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## ABSTRACT

Mercury, one of the toxic metals, that is highly toxic to humans, it released into the environment as a result of both natural processes as well as due to anthropogenic activities. Chelation therapy is a medical procedure that involves the administration of chelating agents to remove heavy metals from the body. The application of natural chemical chelators is one of the traditional methods of removing toxic metals from the body. Flavonoids are natural products widely distributed in plant and form one of the main classes of secondary metabolites. They can coordinate metal ions and form complexes due to the presence of carbonyl and hydroxyl groups. The review considers the possible role of flavonoids as natural chelating and detoxifying agents. Natural flavonoids such as Quercetin and Catechin used as models to elucidate their impact on against metal toxicity. The equilibrium constants and the thermodynamic parameters for complex formation between natural flavonoids and Hg (II) Ion have been determined by Spectrophotometric measurements in water and ethanol at different temperatures. The stoichiometry of the complexes was determined using Job's method. Both Quercetin-Hg(II) and catechin-Hg (II) complexes presented 1:1 stoichiometry. Thermodynamic parameters ( $\Delta G$ ,  $\Delta H$  and  $\Delta S$ ) of the complexes were obtained from the various Van't Hoff's plots of  $\ln K$  against  $1/T$ . An attempt was made to obtain more information from the quantum chemical calculations about the structures of both free and complex forms of flavonoid. Quantum chemical calculations were performed to ascertain the correlation between their experimental results. The density function theory (DFT) calculation at the B3LYP method with LanL2DZ basis set are used to investigate the optimized geometry parameters of flavonoids and their complexes with Hg (II) metal. Moreover, HOMO - LUMO energies of

**Keywords:** Theoretical and Thermodynamics Studies

Complexes Formation, Natural Flavonoids and Hg (II) Ion

the mentioned compounds have been computed.

## 1. INTRODUCTION

Mercury (Hg), one of the toxic metals that are highly toxic to humans. Mercury is a liquid at room temperature and readily vaporizes into the air around it. Is accomplished by mixing it with mineral ore deposits extracted from the ground to form an amalgam [1]. Mercury poisoning can result from exposure to inhalation of mercury vapour or water-soluble forms of mercury, or eating seafood contaminated with mercury. [2]. Mercury released into the environment as a result of both natural processes as well as due to anthropogenic activities e. g. residential coal burning for heating and cooking and the burning of fossil fuels alternative fuels and waste materials. The technique is globally regarded as the second largest source of atmospheric mercury pollution after coal combustion [3, 4]. Chelation therapy has been in medical use for decades to treat the patients for the intoxication of heavy metals like lead, mercury and arsenic [5]. Chelation therapy has the intent of scavenging toxic metal ions from the organism. It involves the administration of chelating agents, which forms a stable complex with the toxic heavy metal species and prevents them from attacking biological targets [6]. A chelator is a molecule which binds a metal ion to form a stable complex by at least two functional groups known as a chelate [7]. It has a long history of use in clinical toxicology [8]. A metal chelator should possess high enough selectivity to remove the target ion. Selectivity depends on the thermodynamic stability of the complexes formed by the chelating agent with the target metal ion in comparison with the stability of those formed with the essential metal ions [9, 10]. Quantum chemical calculation nowadays helps in choice among different potential structures of the chelating groups around the target metal ion [11]. The ability of chelant to bind metal ion is determined by the stability constants [12]. The larger the stability constant, the stronger the chelation effect and the free metal ion in solution become lesser [13]. Flavonoids can be used as active antidote for heavy metal poisoning *In vivo* [14]. A number of flavonoids have been shown to have chelation with heavy metal ions particularly with copper and iron [15]. Flavonoids are significant classes of natural compounds, which have a polyphenolic structure that, are commonly found in almost all plant species [16]. The major sources of flavonoids being fruits, vegetables, grains, bark roots, stems, flowers, tea and wine [17]. The name of flavonoids is derived from Latin "flavus" which means yellow. Beyond from the physiologic roles in plants; they are secondary plant metabolites, which are noteworthy components of human diet, although they are considered as non-nutritious [18]. The Hungarian Nobel laureate Albert Szent-Gyorgyi discovered flavonoid compounds in 1936 [19]. Flavonoids are considered an essential component in a variety of, dicinal, pharmaceutical and nutraceutical [20]. Their different pharmacological effects are structure dependent. In biological systems the protective effects of flavonoids are ascribed to their capacity to transfer hydrogen or electrons free radical [21]. Flavonoids possess many biochemical properties, but the best described property of almost every group of flavonoids is their capacity to act as antioxidants, which act as free radical scavengers as they are protect from oxidative reactions taking place inside the body [22]. The propensity of a flavonoid to inhibit free-radical mediated events is governed by its

chemical structure. The antioxidant activity of flavonoids is attributed to the capacity of scavenging free radicals, donating hydrogen atoms, electrons, or chelate metal cations [23]. By involving the hydroxyl groups, flavonoids can bind various metal ions, thus resulting in metal-flavonoid chelate complexes. [24]. Flavonoids are metabolites that are constructed at 15 carbon compounds accompanied by two benzene rings, A and B as shown in Fig.1, which are joined by linear three carbon chain [25].

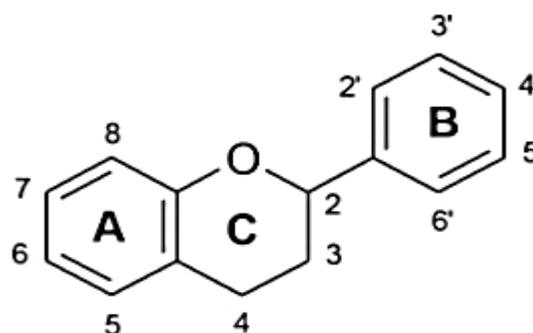


Figure 1. Basic flavonoids structure

According to the saturation of the heterocyclic ring and oxidation status, flavonoids are divided into several groups, which include flavanones, flavones, isoflavones, 3-deoxy flavonoids, flavonols and anthocyanins [26]. Flavonoids structure's can be classified according to type, number and binding position of substitutions binding to aromatic rings cause flavonoids structure's [27]. Flavonoids possess three possible metal-chelating sites that can bind metal ions Fig.2 [28].

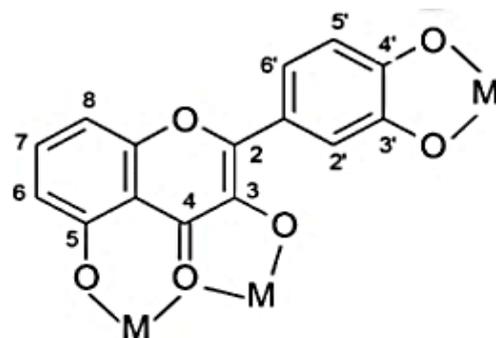


Figure 2. Typical metal (M) chelation sites in flavonoids.

Natural flavonoids are usually oxygenated and bear hydroxyl or methoxyl substituents [29]. The flavonoid compound may be easily identified by their spectra since the majority exhibit a maximum in long UV rang [band I between 325 and 400 nm and a second maximum at shorter wavelength band II between 240 and 295nm [30-32].

Catechins (5, 7, 3', 4'- tetrahydroxyflavan-3-ol) Fig.3 are natural phenols that are also known as flavan-3-ols, it belongs to the group of flavones, part of the chemical family of flavonoids. The Catechin chemical family name originally comes from the word 'catechu', which is the name of the boiled extract (tannic juices) of the *Mimosa catechu plant* [33, 34]. Catechins are phytochemical

compounds found in plant-based foods and beverages, The antioxidant activity of Catechins is most commonly credited with these health benefits [35, 36]. The main sources of Catechin are green and black tea, red wine, chocolate, apricot, apples, peach, red raspberry, and blackberry [37]. Based on their structure, the green tea Catechins are: galliccatechin, Catechin 3-gallate, galliccatechin 3-gallate, epicatechin, pigallocatechin, epicatechin 3-gallate, epigallocatechin 3-gallate [38].

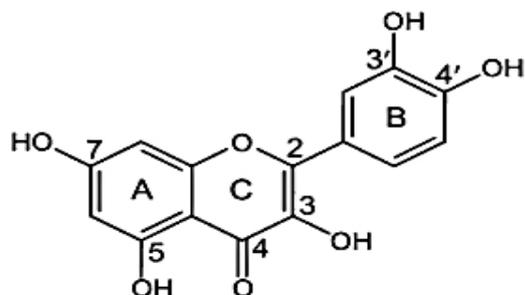


Figure 3. Chemical structure of Catechin

Catechin consists of two benzene rings (called ring A and ring B), ring A is analogous to a resorcinol moiety, whereas ring B is akin to a catechol moiety, as well as a dihydropyran heterocycle (ring C) with a hydroxyl group attached to carbon 3 [39].

Quercetin (3', 3, 4', 5, 7-pentahydroxy flavone) Fig.4 is a flavonol, belonging to a class of naturally occurring flavonoids. It is found in many vegetables, fruits, and flowers [40, 41]. Quercetins have multiple medicinal, biological, and pharmacological properties including anticancer, antiviral, and antithrombotic [42].

Quercetin is a yellow, crystalline solid with a bitter taste, which is insoluble in water, slightly soluble in alcohol, and soluble in glacial acetic acid [43]. Quercetin has potent antioxidant and other activities which contribute beneficial health effects in chronic diseases such as cancer and cardiovascular diseases [44]. Quercetin exerts its antioxidant activity through several protective mechanisms such as reactive oxygen species (ROS) and preventing (ROS) formation with chelating transition metal ions, such as iron and copper [45]. Many studies have investigated the role of quercetin in preventing cardiovascular disease, and interestingly, it has been shown that quercetin has multiple effects in counteracting the different diseases that affect heart and blood vessels. [46].

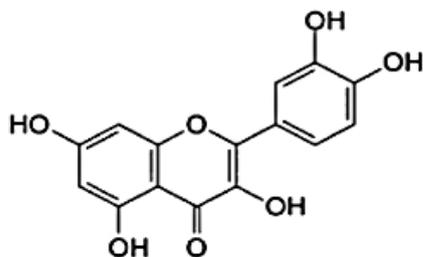


Figure 4. Chemical structure of quercetin

The review considers the possible role of natural flavonoids as natural chelating agents and provides evidence that some natural flavonoids play a protective role against mercury poisoning.

## 2. EXPERIMENTAL SECTION

### 2.1. Chemicals and equipment

The compounds Quercetin and mercuric nitrate  $Hg(NO_3)_2 \cdot H_2O$  were purchased from Sigma Chemicals Co (St. Louis, USA). Catechin was purchased from Aldrich Chemical Co., By a Shimadzu UV-Vis spectrophotometer the absorbance spectra of the reaction mixtures were acquired, model UV-1800 (Shimadzu Corporation, Kyoto, Japan), in the range of 200–800 nm, at different time intervals. All measurements were performed in a 1 cm quartz cuvette.

### 2.2 Procedure

#### 2.2.1. Sample Preparation

A stock solution 0.02M of Quercetin (yellow powder) was prepared by dissolving of 2.1141g in 60% ethanol and 40% distilled. A stock solution 0.02M of Catechin was prepared by dissolving of 1.8141 g of Catechin in distilled water. A stock solution 0.02M of mercuric nitrate was prepared by dissolving of 1.713 g in 5mL nitric acid  $HNO_3$  then with distilled water was completed.

#### 2.2.2. Job's method

In this method, the total molar concentration of the reacting species (metal and ligand) is held constant, but their mole fraction is varied. A series of 1.0 ml volumes of mixture 0.12  $\mu M$  of solution of flavonoids (Quercetin and Catechin) with 0.12  $\mu M$  mercuric nitrate  $Hg(NO_3)_2 \cdot H_2O$  were comprising complementary proportions of the two solutions in various ratios ranging from (0.1: 0.9 to 0.9:0.1ml), absorbance of each solution at wavelength of maximum absorbance  $\lambda_{max}$  is determined. A blank solution consisting of water and 60 % ethanol and 40 % water was used.

#### 2.2.3. Determination of association equilibrium constant $Keq$

Equilibrium constant  $Keq$  values were calculated at four different temperatures on the basis of absorption maxima of complexes. A series of solutions of Quercetin-Hg (II) complexes were prepared by mixing 0.02-0.16  $\mu M$  Quercetin with 0.02-0.16  $\mu M$  Hg (II), and Catechin-Hg (II) complexes were prepared by mixing 0.02-0.16  $\mu M$  Catechin with (0.02-0.16 $\mu M$ ) Hg (II). Then recording the absorption spectrum for them.

## 3. RESULT AND DISCUSSION

### 3.1. The Optimum Experimental Conditions

Most flavonoids and their metal complexes are poorly soluble in water, so that the complex stability were determined in other solvent mixtures (e.g., in alcoholic solution), it should be noted that the ability of solvent to form hydrogen bonds and its polarity have an impact on the metal ion-ligand interactions. Optimized solvent mixtures (ethanol/water) were obtained by measuring the UV-Vis absorption spectra for Quercetin and Catechin in different solvent mixtures.

Table 1. shows the effect of solvent on absorbance for Quercetin and Catechin in various ethanol-water mixtures

Solvent Ethanol-Water	Catechin at $\lambda_{max} = 279$ nm	Quercetin at $\lambda_{max} = 372$ nm	
		$\lambda_1 = 260$ nm	$\lambda_2 = 372$ nm

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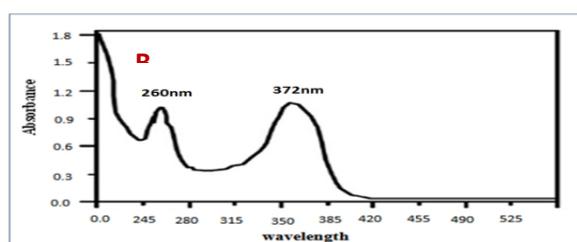
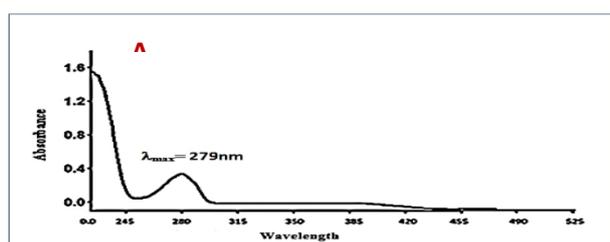
Water 100%	0.382	-	-
20 %	0.032	0.176	<b>0.545</b>
30%	0.012	0.212	<b>0.843</b>
% 40	0.01	0.321	<b>0.954</b>
% 50	0.009	0.932	<b>1.212</b>
% 60	0.00	1.323	<b>1.623</b>
% 70	0.00	1.023	<b>1.122</b>
80 %	0.00	0.624	<b>1.023</b>
90 %	0.00	0.521	<b>0.713</b>

According to these measurements in different solvent mixtures, it is possible to choose water as a solvent for Catechin and % 60 ethanol-water for Quercetin.

### 3.2. Spectral Characteristics of Flavonoids

The UV/Vis properties of Quercetin 0.12  $\mu\text{M}$  versus the

blank solution 60% ethanol and 40% water absorbance spectrum of Catechin displays at  $\lambda_{\text{max}} = 279 \text{ nm}$  and Quercetin displays two bands, the first absorbance occurring around 260 nm and the second at  $\lambda_{\text{max}} = 372 \text{ nm}$ . The full spectrum is shown in Fig. 5.



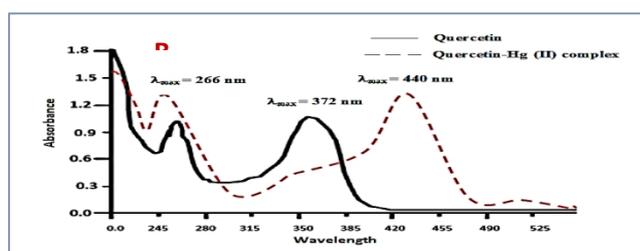
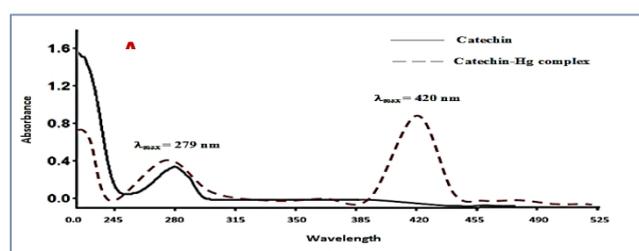
**Figure 5.** (A) UV-Vis absorbance spectrum of Catechin in water  
(B) UV-Vis spectrum of Quercetin in 60% ethanol and 40 % water

### 3.3. UV-Vis Spectra of Flavonoids-Mercury ion Complexes

The Interaction of flavonoids with metal ions can result in chelate formation. The Interaction of flavonoids (Catechin and Quercetin) with Hg (II) yields a highly stable colored complex. The formation of metal complex is indicated by the change in absorbance in the UV-Vis spectroscopy. The formation complex absorbs lights with a maximum absorbance occurring around 420 nm for Catechin-Hg (II) complex and at 440 nm for Quercetin-Hg (II) complex

versus the blank as show in Fig. 6.

The spectrum of the mixture of flavonoids with Hg (II) shows a move in  $\lambda_{\text{max}}$  compared with the free ( $\lambda_{\text{max}}$ ), a change in the absorbance attributed to electronic transitions that occur in the B-ring of flavonoids, through the formation of stable metal complexes, causing changes in the absorption band region of the B-ring of flavonoids, the equilibrium reaction may be written:



**Figure 6.** (A) Absorption spectra of the interaction products of Catechin with Hg (II)  
(B) Absorption spectra of the interaction products of Quercetin with Hg (II)

### 3.4. Determination of Stoichiometry of the flavonoids-Hg (II) Complexes

This method known as Job's method [47] was applied for the determination of stoichiometry of the formation constant of the complexes. In this study two Job's plots with two different total volumes were needed to enable the determination of the formation constant of the

complex. The absorbance's of the resulting solutions were measured at 420 nm for Catechin-Hg (II) complex and at 440 for Quercetin-Hg (II) complex versus the blank as shown in Table 2, The maximum change observed in a plot of absorbance and mole fraction corresponds to stoichiometry ratio of the two species shown in Fig. 8.

**Table 2.** Summary of result using Job's plot for complexes at 298K.

No	$X_{\text{Hg}}$	Abs of Catechin-Hg (II) Complex at $\lambda_{\text{max}} 420 \text{ nm}$	Abs of Quercetin-Hg (II) Complex at $\lambda_{\text{max}} 440 \text{ nm}$
1	0.1	0.093	0.158
2	0.2	0.192	0.324

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3	0.3	0.248	0.798
4	0.4	0.562	1.023
5	0.5	0.893	1.381
6	0.6	0.793	1.202
7	0.7	0.721	1.091
8	0.8	0.642	0.923
9	0.9	0.541	0.743

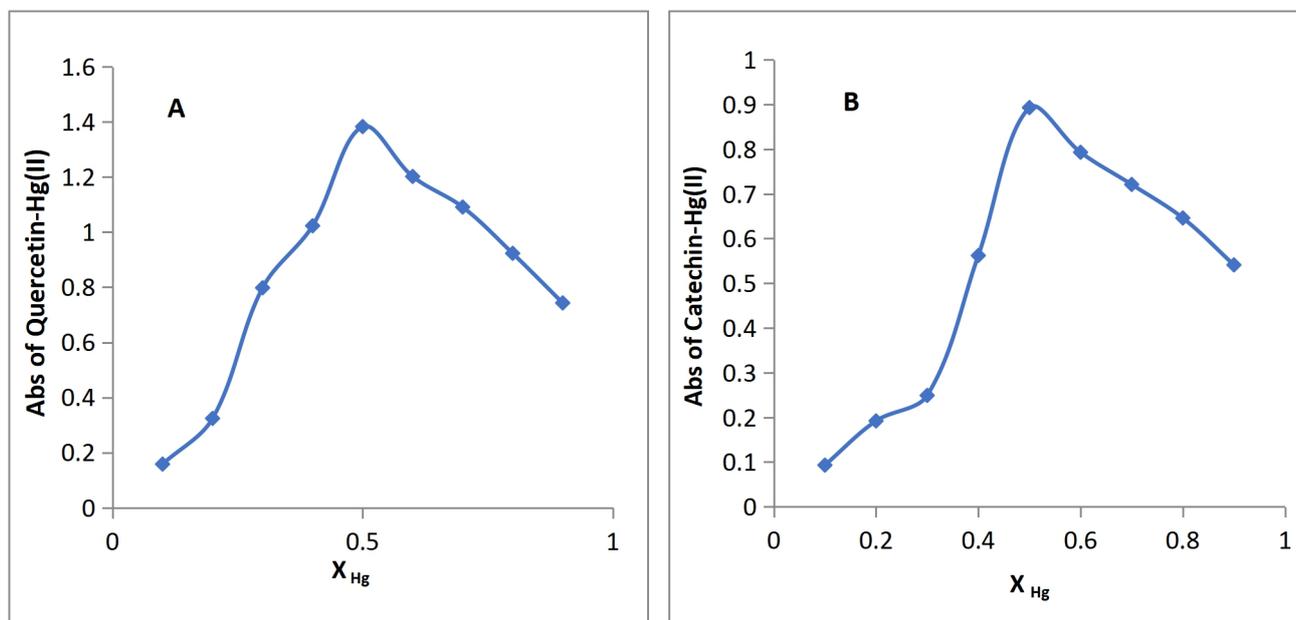
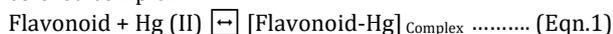


Figure 7. (A) Job's plot for the composition of Catechin-Hg (II) complex at 420 nm (B) Quercetin-Hg (II) complex at 440 nm Job's plot for the composition of

The complexes stoichiometry as being read from Fig. 10 is 1:1 this indicated that only one site is responsible for the formation of the complexes [48].

### 3.5. Calculation of Equilibrium Constant $K_{eq}$

Stability constant is applicable to measure the strength of interactions between the ligands and metal ions that are involved in complex formation in the solution. The determination of association constants for mercuric-flavonoid complexes is important for the estimate the strength of chelation process. The system studied here is the interaction of Flavonoid chelates (Catechin and Quercetin) with metal ion Hg (II) yields a highly stable colored complex.



Based on the concentrations of all the different reaction species at equilibrium, the equilibrium constant  $K_{eq}$  for the reaction in Equation 1 is given as:

$$K_{eq} = \frac{[(\text{Flavonoid} - \text{metal ion})_{\text{complex}}]_{eq}}{[\text{Flavonoid}]_{eq} [\text{metal ion}]_{eq}} \dots\dots\dots (\text{Eqn. 2})$$

The equilibrium concentration of a product or reactant can be determine by used Beer's law if the absorbance is

measured at  $\lambda_{\text{max}}$  specific to that product or reactant.

$$A = \epsilon d c \dots\dots\dots (\text{Eqn.3})$$

Where: A = Absorbance  $\epsilon$  = Molar absorptivity ( $\text{L mol}^{-1}\text{cm}^{-1}$ )  
d = Path length of the cuvette containing the sample (cm)  
C = Concentration of the compound in the solution ( $\text{mol. L}^{-1}$ )

The complex absorbs radiation at 420 nm for Catechin-Hg (II) complex and at 440 for Quercetin-Hg (II) complex. Beer's Law can be rewritten as:

$$A = \epsilon d [(\text{Flavonoid} - \text{metal ion})_{\text{complex}}]_{eq} \dots\dots\dots (\text{Eqn.4})$$

$$[(\text{Flavonoid} - \text{metal ion})_{\text{complex}}]_{eq} = \frac{A}{\epsilon d} \dots\dots\dots (\text{Eqn.5})$$

Equation (2) may be formulated in the following expression:

$$K_{eq} = \frac{\left(\frac{A}{\epsilon d}\right)_{eq}}{\left([\text{Flavonoid}]_0 - \left(\frac{A}{\epsilon d}\right)_{eq}\right) \left([\text{metal ion}]_0 - \left(\frac{A}{\epsilon d}\right)_{eq}\right)} \dots\dots\dots (\text{Eqn.6})$$

By recording the absorbance A of a various concentration of the (1:1) complexes, the molar absorptivity were calculated at various temperatures at 440 for Quercetin-Hg (II) complex and at 420 nm for Catechin-Hg (II) complex versus the blank. A plot of absorbance versus concentration using the data in Table 3,4 given a straight line with the slope equal to ( $\epsilon_{\text{complex}}$ ) Fig. 8, 9.

Table 3. Absorbance of (1:1) Quercetin-Hg (II) complex at 298,303, 308 and 303K.

[Quercetin- Hg (II)] $\mu\text{M}$	Abs.			
	298K	303K	308K	313K
0.02	0.224	0.242	0.212	0.191
0.04	0.311	0.412	0.251	0.205
0.06	0.431	0.623	0.392	0.253
0.08	0.672	0.768	0.500	0.352
0.10	0.762	1.00	0.612	0.462

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0.12	0.893	1.221	0.751	0.563
0.14	1.152	1.421	0.881	0.642
0.16	1.253	1.568	1.021	0.754

Table 4. Absorbance of (1:1) Catechin-Hg (II) complex at 298,303, 308 and 303K.

[Catechin-Hg (II)] $\mu\text{M}$	Abs.			
	298K	303K	308K	313K
0.02	0.321	0.397	0.200	0.102
0.04	0.511	0.513	0.352	0.157
0.06	0.792	0.895	0.513	0.221
0.08	1.021	1.026	0.674	0.314
0.10	1.231	1.394	0.873	0.429
0.12	1.452	1.632	0.923	0.514
0.14	1.721	1.892	1.074	0.573
0.16	1.923	1.992	1.245	0.624

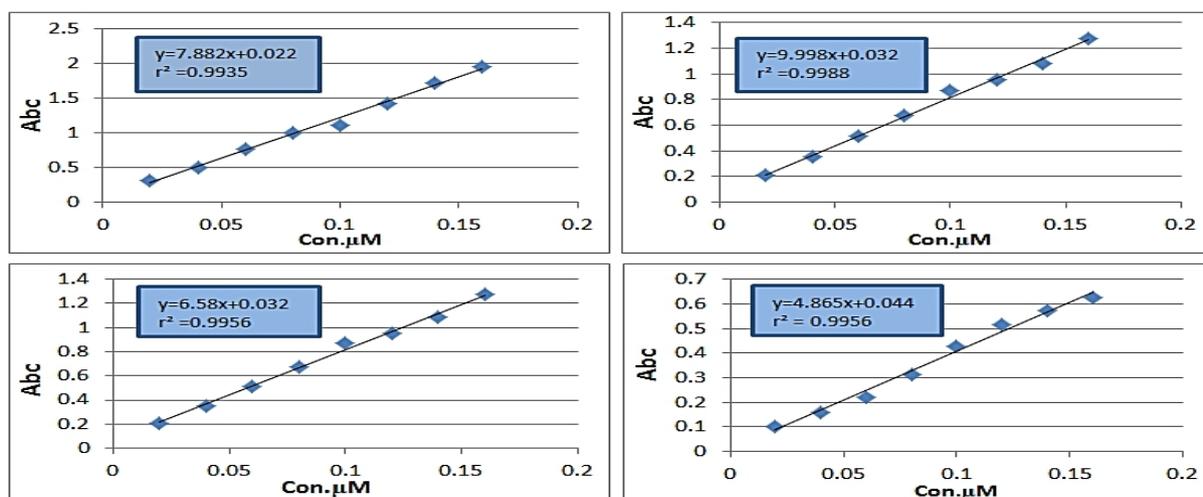


Figure 8. Absorbance vs. concentrations of Quercetin-Hg (II) complex at 298,303, 308 and 303K

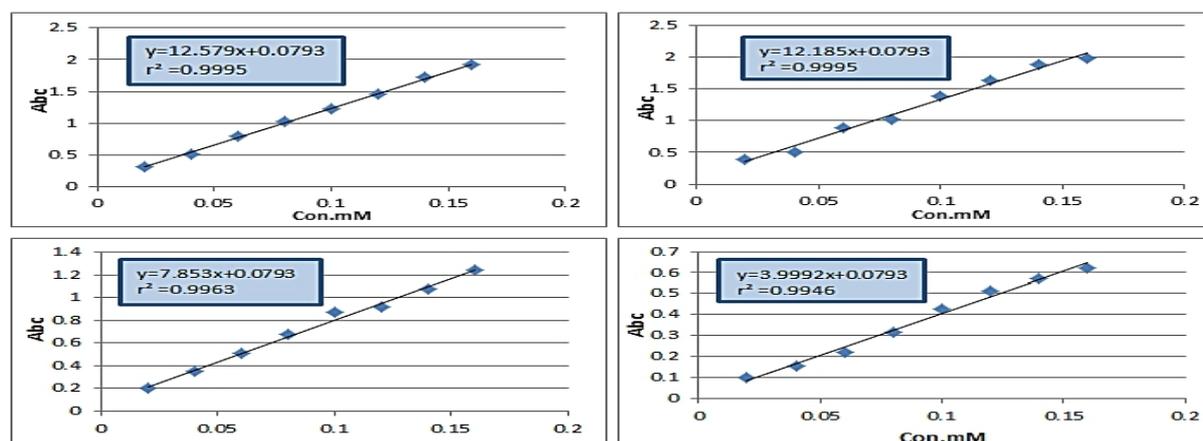


Figure 9. Absorbance vs. concentrations of Catechin-Hg (II) complex at 298,303, 308 and 303K

From the calibration data, molar absorptivity  $\epsilon_{\text{complex}}$  for Quercetin-Hg complex and Catechin-Hg (II) complex were

determined. By the application of equation 6 the Equilibrium constant  $K_{\text{eq}}$  were calculated at four different temperatures and the data are listed in Table 5.

Table 5. The equilibrium constant  $K_{\text{eq}}$  of the flavonoids-Hg (II) complexes at four temperatures

Temp (K)	$K_{\text{eq}}/\text{L.mol}^{-1}$	
	(1:1) Quercetin-Hg(II)	(1:1) Catechin-Hg(II)
298	$8.742 \times 10^6$	$3.00 \times 10^6$
303	$7.572 \times 10^6$	$5.107 \times 10^6$
308	$6.636 \times 10^6$	$7.2954 \times 10^6$
313	$6.157 \times 10^6$	$9.903 \times 10^6$

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The values of the equilibrium constants  $K_{eq}$  for Quercetin-Hg complex showed stability at lowers temperature and showed decreased with increased temperature indicates that the formed complex dissociated easily even with increasing temperature, while the values of the equilibrium constants  $K_{eq}$  for Catechin-Hg complex were low and showed an increase with increase in temperature, that's means the complex is higher stability at higher temperature.

### 3.6. Determination of Thermodynamic Constants of Flavonoids-Mercury ion Complex

The Thermodynamic quantities for ligand -cation complexation, that is,  $\Delta G$ ,  $\Delta H$ , and  $\Delta S$ . are determined in the solution phase from the temperature variation of the stability constant ( $K$ ) The free-energy and entropy changes  $\Delta G^\circ$  and  $\Delta S$  at the specific temperature are to be calculated from the Thermodynamic quantities obtained above according to Eq.7 and Eq.8:

$$\Delta G = -RT \ln K_{eq} \dots\dots (Eqn.7)$$

$$\Delta G = \Delta H^\circ - T\Delta S \dots\dots (Eqn.8)$$

The method for determining thermodynamic quantities is to measure the complex stability constants ( $K_{eq}$ ) at different temperatures. According to Eq. (9), derived from Eq.7 and Eq. 8, the logarithm of the stability constant ( $K_{eq}$ ) is plotted complexation as slope and intercept, respectively.

$$\ln K_{eq} = \left(\frac{\Delta H^\circ}{R}\right) \frac{1}{T} + \left(\frac{\Delta S^\circ}{R}\right) \dots\dots (Eqn.9)$$

$\Delta H$  is the energy change when a reaction occurs with all products and reactants in a standard state and it indicate whether a reaction is endothermic or exothermic. The enthalpy  $\Delta H$  was calculated by van't Hoff equation [49, 50]. Plot between  $\ln K_{eq}$  and temperature for Hg-Catechin and Hg-Quercetin complexes are shown in Figure 10,11 gives a straight line with Slope =  $-\Delta H^\circ/R$  and shows that natural  $\ln K_{eq}$  is linearly dependent on  $(1/T)$ .

Thermodynamic parameters for (1:1) complexation of flavonoids-Hg (II) Ion are present in Table 6, 7.

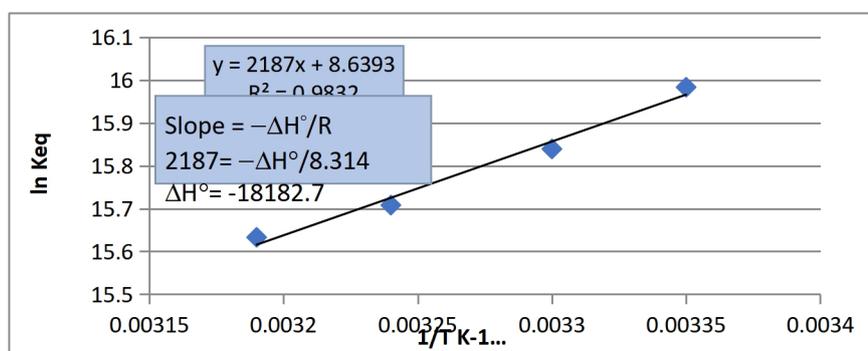


Figure 10. Van't Hoff for Quercetin -Hg (II) complex at 440 nm.

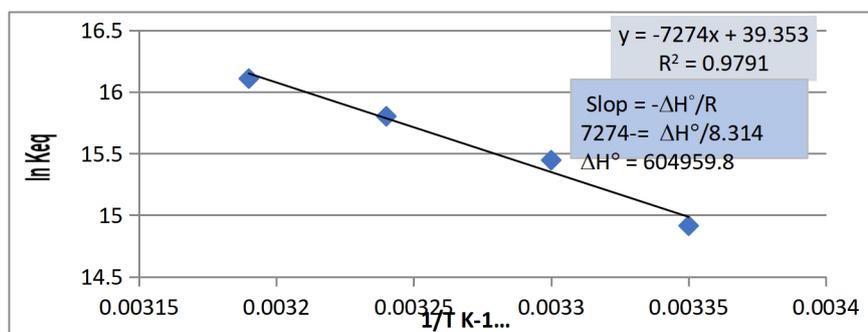


Figure 11. Van't Hoff for Catechin-Hg (II) complex at 420 nm.

Table 6. Thermodynamic parameters for Quercetin -Hg (II) complex calculated at various temperatures.

T(K)	$\Delta G^\circ(\text{J.mole}^{-1})$	$\Delta H^\circ(\text{J.mole}^{-1})$	$\Delta S^\circ(\text{J.mole}^{-1}\text{K}^{-1})$
298	-39600.51	18182.7-	71.8717
303	-39902.95		71.6839
308	-40223.66		71.5615
313	-40681.734		71.8818

Table 7. Thermodynamic parameters for Catechin-Hg II complex calculated at various temperatures.

T(K)	$\Delta G^\circ(\text{J.mole}^{-1})$	$\Delta H^\circ(\text{J.mole}^{-1})$	$\Delta S^\circ(\text{J.mole}^{-1}\text{K}^{-1})$
298	-36950.75	604959.8	326.93
303	-38910.66		328.00
308	-40466.37		327.72
313	-41917.55		327.13

The study of the effect of temperature on interactions process and the evaluation of the thermodynamic

properties of the process provide valuable information about the strength of the interactions between flavonoids

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and Hg (II) Ion. The changes in the standard enthalpy  $\Delta H^\circ$  for Hg-Catechin and Hg-Quercetin complexes were obtained from the slope of the Van't Hoff plots. Plot between  $\ln K_{eq}$  and temperature are shown in Figure 11, 12 gives a straight line with slope =  $-\Delta H^\circ/R$  and shows that natural  $\ln K_{eq}$  is linearly dependent on  $(1/T)$ .

From Equation (8) Change in the Gibbs free energy for the reaction was calculated, and the parameter is related to the spontaneity interactions process. Negative values of  $\Delta G^\circ$  indicate that the Interactions process is spontaneity. A high negative value of  $\Delta G^\circ$  indicates that the position of equilibrium favors the product complex; hence the complex formed will be more stable.

With rise in temperature, the values of free energies of formation of the flavonoids-Hg (II) complexes were become more and more negative, this indicates that the complex formation is a spontaneous process and spontaneity increases with temperature T.

The changes in standard entropy  $\Delta S^\circ$  were calculated from the relationship

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

The experimental values of standard enthalpy ( $\Delta H^\circ$ ) and standard entropy ( $\Delta S^\circ$ ) show that in most cases, the complex is enthalpy stabilized and in all cases entropy stabilized.

In Table 6, 7. The variation in  $\Delta H$  values for complexes provides clear evidence to the assumption that  $\Delta H$  value is dependent on the chemical structure of the complexes, that is, on strength of the M-L coordination. The negative values of  $\Delta H^\circ$  for Hg-Quercetin complex, show that the chelation process is exothermic, indicating that the complexation reaction are favored at low temperatures and refers to an increase in binding interaction. The obtained positive values of  $\Delta H$  for Catechin-Hg (II) complex show that the chelation process is endothermic which refers to a decrease in binding interaction and mean that enthalpy is not the driving force for the formation of the complexes. The change of entropy ( $\Delta S^\circ$ ) reflects essentially the variation on the disorder a system along a process. The positive values of  $\Delta S$  indicate

increased randomness and that entropy is responsible for the complexing process [51]. Based on the calculated values of thermodynamic parameters, order of stability of the title complexes is found to be [Quercetin-Hg<sup>+2</sup>] > [Catechin-Hg<sup>+2</sup>].

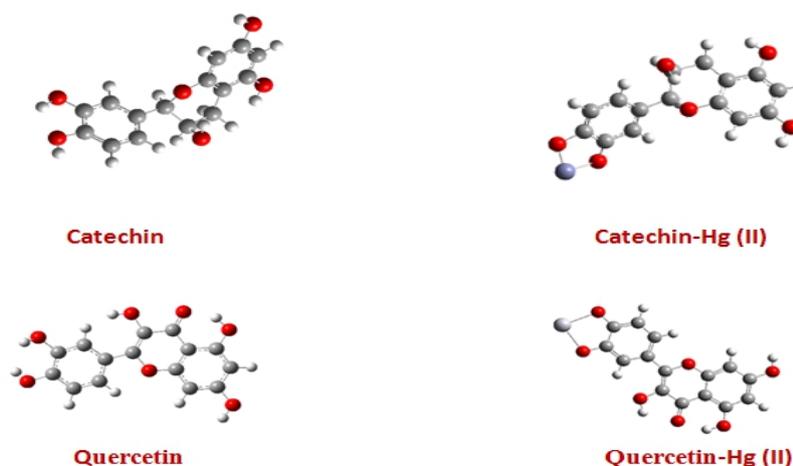
### 4. QUANTUM CHEMICAL CALCULATIONS

The term theoretical chemistry may be defined as the mathematical description of chemistry. Quantum chemical calculations are today performed on a wide range of molecules using advanced computer programmers. Computational theoretical chemistry is concerned with the numerical computation of molecular electronic structures and molecular interactions [52]. Application of computation chemical methods allows rapid progress in estimating reaction pathways or products from theoretically obtained information on the reaction position or bond conditions [53,54]. Density functional theory (DFT) is the most widely used method of modern computational chemistry, It is nowadays one of the most popular methods for ground state electronic structure calculations in quantum chemistry [55]. The basic concept of DFT is to make it possible to perform high-speed calculations of many-electron system by representing the potential as a functional not of the orbitals but of the electron density [56]. DFT calculations are used to compare energy difference between structures and determine energy barriers between transitions [57]. The density functional theory (DFT) calculations were performed using the Gaussian 09 [58]. To calculate optimized geometrical structure of studied compounds the Lee-Yang-Parr Becke's three parameter hybrid functional method (B3LYP) with LanL2DZ basis set was used [59].

#### 4.1. Molecular geometry

The Optimized molecular structure of the flavonoids and its complexes using the Hyper Chem 8.0.10 software and DFT at the B3LYP level, employing the basis set LanL2DZ is represented in Fig. 12.

**Figure 12.** Optimized molecular structure of the minimum energy structure of Catechin, Quercetin, Catechin-Hg (II) and Hg



(II)-Quercetin obtained at B3LYP/ LanL2DZ level.

#### 4.2. HOMO-LUMO analysis by DFT calculations

Molecular orbital and their properties like energy and their frontier electron density used for predicting the most reactive position in  $\pi$ -electron system and also explained several types of reaction in conjugated system [60]. When a chemical reaction occurs, electrons move

out from molecular orbitals of the bonds broken and fill molecular orbitals of new bonds. According to the highest occupied molecular (HOMO), the orbitals that are the highest in energy, and the lowest unoccupied molecular orbitals (LUMO), these which are lower in energy, of the reacting components have the strongest interactions. The

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HOMO and LUMO are termed the frontier orbitals. The reaction occurs because the overlap is maximum between the HOMO and LUMO of the reacting species via a favored transition state. It may be stated that it is the interaction between the occupied-unoccupied orbitals that can produced of the species and results in new bond formation [61]. Stabilizing interaction will arise from interaction of occupied orbitals on one reactant and vacant on the other of these stabilizing occupied-vacant interactions, the interactions of the HUMO of one reactant with the LUMO of the other usually will be the largest terms, because are the orbitals closest in energy. The energy of HOMO characterizes electron donating ability of a molecule while LUMO energy determines the ability to accept an electron [62]. Ionization potential (I) is the minimum energy required to remove an electron from a neutral molecule and is related to the HOMO energy. High ionization energy indicates high stability and chemical inertness and small ionization energy indicates high reactivity of the atoms and molecules [63]. The electronic affinity (A) is defined as the energy released when an electron is attached to neutral system and is related to the LOMO energy. A molecule with high the electronic affinity values tend to take electrons easily. In Table 12 it is reported the values of the electronic energies calculated for all studied compounds in order to describe

their reactivity. Higher values of HOMO indicate better tendency towards the donation of electron, As can be seen in Table 12 that the Quercetin-Hg (II) has the highest HOMO energy ( $E_{\text{HOMO}} = -0.28813$  eV) that allows him to be the best electron donor molecule, and it has the lower LUMO energy ( $E_{\text{LUMO}} = -0.20250$  eV) which indicate that is the most molecules has the ability to accept electrons. Quercetin-Hg (II) has the lowest ionization potential value ( $I = 0.288131\text{eV}$ ), which indicate that it is the best electron donor. The energy gap is very important in determining the chemical reactivity of the molecule and critical parameter to determine molecular electrical transport properties. The energy gap,  $E_{\text{gap}}$ , defined as the difference between HOMO and LUMO energy according to Koopmans' theorem  $E_{\text{gap}} = (E_{\text{LUMO}} - E_{\text{HOMO}}) \approx \text{IP} - \text{EA}$  [64]. The order of stability has been correlated in terms of the height of the energy gap between the highest occupied molecular orbital and lowest occupied molecular orbital. The small energy gap between HOMO and LUMO indicated that charge transfer occurs within the title molecule and the molecule can be easily polarized. While a high value of the energy gap indicates that the molecule shows high chemical stability. The effects of the complexation of each Catechin and Quercetin with the Hg (II) ion on these orbital energies, as well as their gap, are displayed in Table 8.

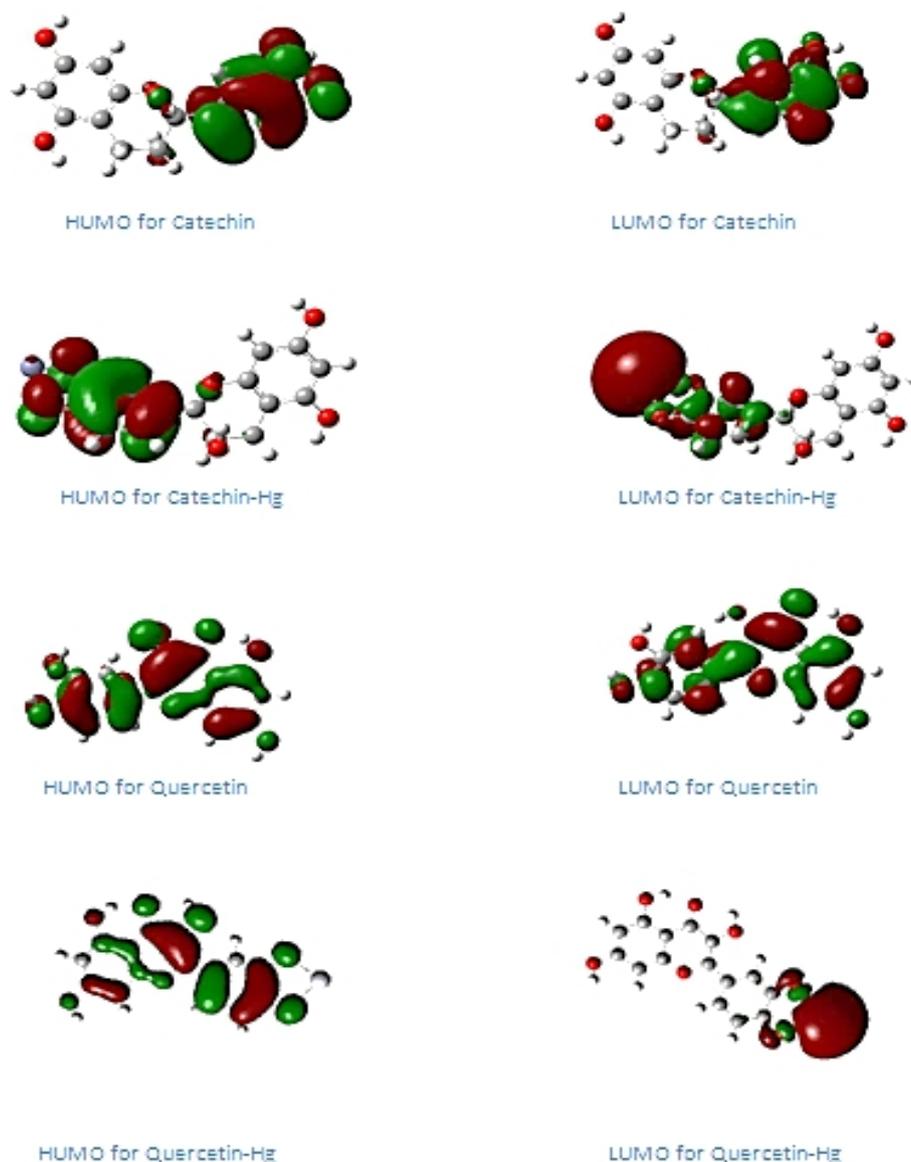
**Table 8.** Changes in orbital energies resulting from complexation with Hg (II). The shorter LUMO-HOMO gap is considered as the high reactivity.

Compounds	$E_{\text{HOMO}}$ eV	$E_{\text{LUMO}}$ eV	$E_{\text{gap}}$ eV	Ionization potential (I) eV	Electron affinity (A) eV
Catechin	-0.22785	-0.02290	0.20495	0.22785	0.02290
Quercetin	-0.22977	-0.08775	0.14202	0.22977	0.08775
Catechin-Hg (II)	-0.31681	-0.16677	0.15004	0.31681	0.16677
Quercetin-Hg(II)	-0.28813	-0.20250	0.08563	0.28813	0.20250

Density functional theory (DFT) calculation showed that the complexes had low energy gap and higher tendency to interact with electron-accepting species. Quercetin-Hg (II) complex has the smallest energy gap ( $\Delta E_{\text{gap}} = 0.08563$  eV) so it is the most polarizable. It has the highest chemical reactivity and the lowest kinetic stability. In contrast Catechin-Hg (II) complex has the largest value of the energy gap of 0.15004 eV it is therefore the least polarizable, with low chemical

reactivity and high kinetic stability. From Table 8 it is clear that Quercetin-Hg (II) is the best reactive. These results are in good agreement with the available experimental and theoretical predicted findings. Computational analysis of energy levels of HOMO and LUMO orbitals was calculated and the frontier orbitals (HOMO, LUMO) of free flavonoids and complex forms of flavonoid are plotted in Fig. 13 respectively.

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**Figure 13.** Highest occupied molecular orbital HOMO and lowest unoccupied molecular orbital LUMO energy levels of flavonoids and flavonoids-Hg at LanL2DZ level of theory.

### CONCLUSION

In the present review, we give an update of the appropriate use of chelation agents in the treatment of intoxications by metals. This study provides evidence that some natural flavonoids, Quercetin and Catechin specifically as natural chelating in the treatment of mercury toxicity. Chelating activity of flavonoids were determined by the measurement of its association equilibrium constant  $K_{eq}$  with Hg (II) in water and ethanol at different temperatures. by the use of optical spectrophotometry. Thermodynamic parameters of complexation were determined from the temperature dependence of the formation constant. The negative values of  $\Delta G$  show the ability of the studied flavonoids to form stable complexes and the process tend to proceed spontaneously. Theoretically, we have calculated the geometrical parameters and frontier orbitals with DFT method and it found that these theoretical results are in good agreement with the experimental data.

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