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Binding Parameters of Glucose to Human Serum Albumin in the Presence of Cinnamaldehyde and Cinnamic Acid from Cinnamon Bark (*Cinnamomum verum*)

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Abstract: Eugenol, cinnamic acid and cinnamaldehyde are the main active components of essential oils of cinnamon (*Cinnamomum verum*). This article studies their interaction with human serum albumin (HSA). Binding site number was calculated. Thermodynamic parameters (K_a, $\Delta_r H°$, $\Delta_r G°$ *and* $\Delta_r S°$) were also determined. The binding of glucose to HSA in the presence and absence of cinnamaldehyde, cinnamic acid and eugenol was studied by fluorescence spectroscopy. Cinnamaldehyde possesses the higher binding constant (K_a) values followed by cinnamic acid. The thermodynamic parameters indicated hydrophobic interactions for cinnamaldehyde, van der Waals interaction and hydrogen bonding for cinnamic acid. The values of binding constant K_a and binding site number (n), of glucose to HSA determined by Scatchard method, increased only in the presence of cinnamaldehyde and/or cinnamic acid. Cinnamaldehyde and/or cinnamic acid modify the structure of HSA therefore increasing the binding of glucose to the protein.

Keywords: human serum albumin, glucose, cinnamaldehyde, cinnamic acid, eugenol.

INTRODUCTION

CEYLON cinnamon is the most commonly used spice extracted from the inner bark of the cinnamon tree *Cinnamomum verum,* native to Sri Lanka and India.[1] Many parts of the Ceylon plant are used for medicinal reasons, including the bark, roots, flowers, and leaves. Cinnamon extracts have different biological effects, including antiviral,[2] antimicrobial,[3] antiallergenic,[4] antioxidative,[5] gastroprotective,[6] antiangiogenic,[7] and anti-Alzheimer effects,[8,9] as well as insulin-like activities.[10] Cinnamon extracts contain numerous active components, including essential oils (eugenol, cinnamic acid and cinnamaldehyde).[11,12]

Cinnamaldehyde (C_9H_8O) is the main constituent of essential oils extracted from the bark of cinnamon and gives cinnamon its flavor and smell.[12] Cinnamaldehyde has been reported as an antiproliferative substance,[13] and an antimutagenic agent effective on various cancer cells.[14] Cinnamaldehyde is a candidate for anticancer drug development.[15]

Eugenol ($C_{10}H_{12}O_2$), a phenolic photochemical extracted from leaves, roots and bark of cinnamon;[16,17] has been identified to be effective for the treatment of different diseases including cancer and sepsis.[18]

Cinnamic acid ($C_9H_8O_2$), an aromatic carboxylic acid, has anti-oxidant, anti-fungal, anti-inflammatory, nematicidal, anti-cancer, anti-microbial and anti-malarial properties.[19,20]

Abou-Khalil et al.[21] showed that cinnamaldehyde and/or cinnamic acid decreased the glucose levels in human plasma and in glucose–human serum albumin (HSA) solution, although eugenol was ineffective. The glucose levels were not modified when albumin was absent or had been denatured, suggesting that the native structure of HSA is crucial for the effect of cinnamaldehyde and cinnamic acid.

Human serum albumin, the most abundant protein found in human blood, is implied in the binding of vast variety of exogenous and endogenous ligands.[22,23] HSA contains three homologous domains (I, II and III), and each of these is divided into two subdomains (A and B). HSA

binding capacity depends on the existence of Sudlow's site I and II regions, located in subdomains IIA and IIIA.[24] Glucose binds to sites I and II of HSA, especially by interacting with cysteine, arginine and lysine residues.[25,26] Cinnamic acid binds to site I (sub-domain IIA) of HSA.[27] Cinnamaldehyde interacts mostly with cysteine residues of the protein.[28]

Understanding the interaction between HSA and glucose can provide insights into the mechanisms of glucose transport and metabolism in the body. Additionally, aberrant glucose binding to HSA has been associated with various pathological conditions, such as diabetes and cardiovascular diseases.[29] Therefore, investigating the modulation of glucose binding by ligands isolated from cinnamon, a spice known for its potential health benefits, can offer new possibilities for developing therapeutic interventions. These ligands could potentially influence glucose transport and metabolism, leading to improved glucose regulation and potential treatments for metabolic disorders.

In order to continue our previous work[21] and to understand the mechanisms of cinnamaldehyde and cinnamic acid interactions with albumin, it is important to determine their binding constants, to identify the type of substrate-HSA interactions, as well as the effects of these components on the structure of the protein. This study was performed using fluorescence spectroscopy.

MATERIALS AND METHODS

Materials

Cinnamaldehyde (Purity ≥ 99 %), Cinnamic acid (Purity ≥ 97 %), Eugenol (Purity ≥ 99 %) and HSA (purity ≥ 96 %), were provided by BDH laboratory, England.

Spectra Fluorescence Measurements

A spectrofluorimeter (Thermo Scientific, AMINCO Bowman Series 2, USA), equipped with a data recorder at 25, 30, 37 and 42 ± 0.1 °C, was used to record the fluorescence emission spectra. The slits were adjusted for excitation and emission at 3 and 5 nm, respectively. The sample cuvette pathlength was 1 cm. The HSA solution was excited at 295 nm to measure the intrinsic protein fluorescence. The emission spectra were recorded between 300 and 400 nm. The fluorescence emission is directly related to the single tryptophan residue (subdomain IIA, position 214) of HSA.

Stock Solutions

Cinnamaldehyde, cinnamic acid, and eugenol were prepared in methanol (1 mg mL^{-1}). HSA (1 μmol dm^{-3}) and glucose (100 μmol dm^{-3}) solutions were dissolved in a phosphate buffer (67 mmol dm^{-3}, pH 7.4).

Determination of Binding Constants of the Complex Cinnamaldehyde-HSA, Cinnamic Acid-HSA and Eugenol-HSA

IN THE ABSENCE OF GLUCOSE

Aliquots from cinnamaldehyde, cinnamic acid, and eugenol stock solutions were taken, and methanol was evaporated. 5.0 mL of HSA solution (1 μmol dm^{-3}) was added. After 20 minutes, the fluorescence was measured. The molar ratio of the component to HSA ranged between 0.01 and 4.0.

IN THE PRESENCE OF GLUCOSE

The binding of glucose to HSA was studied in the presence and absence of cinnamaldehyde, cinnamic acid, and eugenol. Aliquots from cinnamaldehyde, cinnamic acid, and eugenol stock solutions were taken, and methanol was evaporated. 5.0 mL of HSA solution was added. The molar ratio of the component to HSA was equal to 1. Aliquots from the glucose stock solution were taken and added to HSA solution containing cinnamaldehyde, cinnamic acid, and/or eugenol. The molar ratio of glucose to HSA varied between 0 and 4.

The Temperature Dependence of the HSA Binding Affinity for Cinnamaldehyde and Cinnamic Acid

Referring to Lehrer,[30] the fluorescence enhancement was studied using the following equation:

$$\log\left(\frac{F_0 - F}{F - F_\infty}\right) = n\log\left[\text{ Component }\right] - n\log K_{\text{diss}} \quad (1)$$

where F_0, F and F_∞ are the fluorescence intensities of the protein alone, the ratio of the component to HSA and the protein saturated with the ligand, respectively. [Component] represents the free component concentration. The number of equivalent binding capacity, n, is determined from the slope of the double logarithm plot (obtained from the experimental data). The value of log [Component] at log $[(F_0 - F)/(F - F_\infty)] = 0$ equals to the logarithm of K_{diss} (the dissociation constant). The reciprocal of K_{diss} is K_a (the binding constant).

Thermodynamic Parameters of the Complex Cinnamaldehyde-HSA and Cinnamic Acid-HSA

Biomolecule-drug interactions include hydrogen bonds, hydrophobic forces, van der Waals interactions, electrostatic interactions, etc. $\Delta_r G°$ is determined from the slope of the plot: lnK_a versus T^{-1} (where T is the absolute temperature). The value of the enthalpy change ($\Delta_r H°$), if it does not vary considerably with the studied temperature range,

and that of the entropy change ($\Delta_r S°$) can be calculated using Van't Hoff equation:

$$\ln K_a = -\frac{\Delta_r H°}{RT} + \frac{\Delta_r S°}{R} \quad (2)$$

where K_a represents the association constant and R (8.314 J mol^{-1} K^{-1}) is the gas constant. Four temperatures were used 298, 303, 310, and 315 K. $\Delta_r H°$ (enthalpy change) is determined from the slope of Van't Hoff equation. $\Delta_r G°$ (free energy change) is calculated using the following equation:

$$\Delta_r G° = \Delta_r H° - T\Delta_r S° \quad (3)$$

Association Constant and the Binding Capacity for the Interaction of Glucose with HSA in the Presence and Absence of Cinnamaldehyde, Cinnamic Acid, and Eugenol

Fluorescence quenching was determined using Scatchard equation:[31]

$$nK_a - K_a Q = \frac{Q}{L} = \frac{Q}{R - Q}[\text{HSA}]_t \quad (4)$$

where K_a and n are the association constant and the binding capacity, respectively. Q is the fractional quench. [L] represents the ligand concentration. $[HSA]_t$ represents the total concentration of albumin. R is the molar ratio of the ligand to HSA. K_a was calculated from the slope of the plot: Q / [L] versus Q. n was obtained from nK_a (the intercept on the X-axis).

All values are the mean ± standard error of the mean (SEM) of three experiments.

RESULTS AND DISCUSSION

When excited at 295 nm, cinnamaldehyde, cinnamic acid, and eugenol have no intrinsic fluorescence. Adding cinnamaldehyde or cinnamic acid, but not eugenol to the HSA solution increased the fluorescence. Figure 1 shows the fluorescence emission spectra of HSA in the presence and absence of cinnamaldehyde, cinnamic acid, and eugenol (component/HSA molar ratio equals 0.5).

The component concentration was modified, the component–HSA complex was excited at 295 nm and the fluorescence was detected at 350 nm. Figure 2, F / F_0 versus [Component] at 298 K, shows that the fluorescence increased as a function of the concentration of cinnamaldehyde and/or cinnamic acid. HSA fluorescence intensity increased as cinnamaldehyde and/or cinnamic acid concentration varied from 0 to 1 mol dm^{-3}, and then reached a plateau.

Similar results (data not shown) were also observed at 303, 310 and 315 K. The ratio F / F_0, at various temperatures, showed a greater increase in value in presence of cinnamaldehyde than it did with cinnamic acid. Eugenol, even at a molar ratio eugenol/HSA equal to 4, did not produce any modification of the fluorescence intensity of HSA.

HSA fluorescence depends on the presence of the phenylalanine, tyrosine and tryptophan residues. The experimental observations of Sulkowska[32] showed that the intrinsic fluorescence of HSA is almost due to tryptophan alone because the fluorescence of tyrosine is

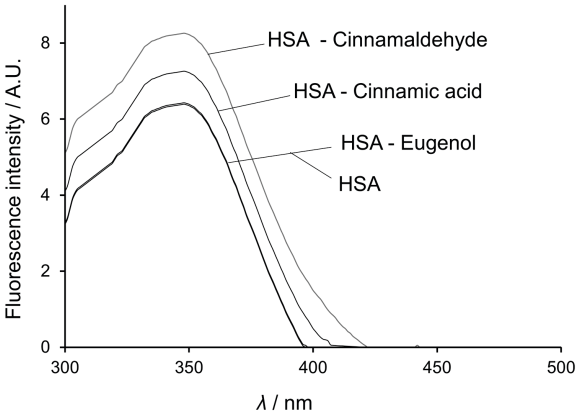

Figure 1. Fluorescence emission spectra of human serum albumin in the presence and absence of cinnamaldehyde, cinnamic acid and eugenol (component/HSA molar ratio equals 0.5).

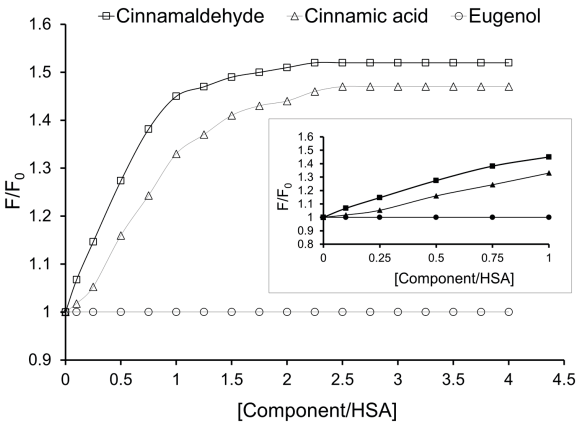

Figure 2. Plots of F / F_0 versus [Component] mol dm^{-3}; the inset represents the linear segment of the plot with [Component] varying from 0 to 1 mol dm^{-3}.

totally quenched (if it is ionized or near a tryptophan, a carboxyl group, or an amino group) and phenylalanine has a low quantum yield. The modification of HSA intrinsic fluorescence intensity is that of the single tryptophan residue when molecules are bound to the protein. Cinnamaldehyde and/or cinnamic acid increased HSA intrinsic fluorescence intensity either after excitation of the intact protein or the single tryptophan.[21] HSA fluorescence, when excited at 295 nm, is due to the single Tryptophan residue (located in subdomain IIA). Ahmad et al.[33] reported an increasement in the tryptophan fluorescence of HSA in response to ligand binding. Our study showed a regular increase in HSA fluorescence intensity with the concentration of cinnamaldehyde and/or cinnamic acid, which explains that these two components can bind to the protein and the binding site on HSA was adjoining to the single tryptophan residue of the albumin.

Binding Parameters of Cinnamaldehyde and Cinnamic Acid to HSA

According to [Eq. (1)], the linear parts of the plots (Figure 2) were used to calculate the binding parameters of cinnamaldehyde and cinnamic acid to HSA. F_∞ was the value of the fluorescence intensity obtained at component/HSA molar ratio equal to 4. Figure 3a represents the graphs of the interaction of cinnamaldehyde and cinnamic acid with HSA at 298 K.

Figure 3b shows those of cinnamic acid with albumin at different temperatures (298, 303, 310, and 315 K).

Referring to the plots shown in Figures 3a and 3b, K_a and n of cinnamaldehyde and cinnamic acid to albumin (Table 1) are calculated.

K_a values were significant and greater for cinnamaldehyde than for cinnamic acid. n values, whatever the temperature, varied between 1.10 and 1.45. In addition, for these two components, the n values were slightly affected with rising temperatures, while K_a values significantly decreased.

Table 1. Association constant and the binding capacity of HSA-cinnamaldehyde and HSA-cinnamic acid complexes at pH 7.40. Values are the means of triplicate experiments ± Standard Deviation (SD).

T / K	Cinnamaldehyde		Cinnamic acid	
	$10^6 K_a$ / mol^{-1} dm^3	n	$10^6 K_a$ / mol^{-1} dm^3	n
298	4.12 ± 0.02	1.16 ± 0.01	2.37 ± 0.02	1.22 ± 0.02
303	3.47 ± 0.01	1.22 ± 0.01	1.60 ± 0.01	1.32 ± 0.02
310	3.05 ± 0.01	1.11 ± 0.01	1.32 ± 0.02	1.35 ± 0.01
315	2.72 ± 0.01	1.34 ± 0.02	1.07 ± 0.01	1.43 ± 0.02

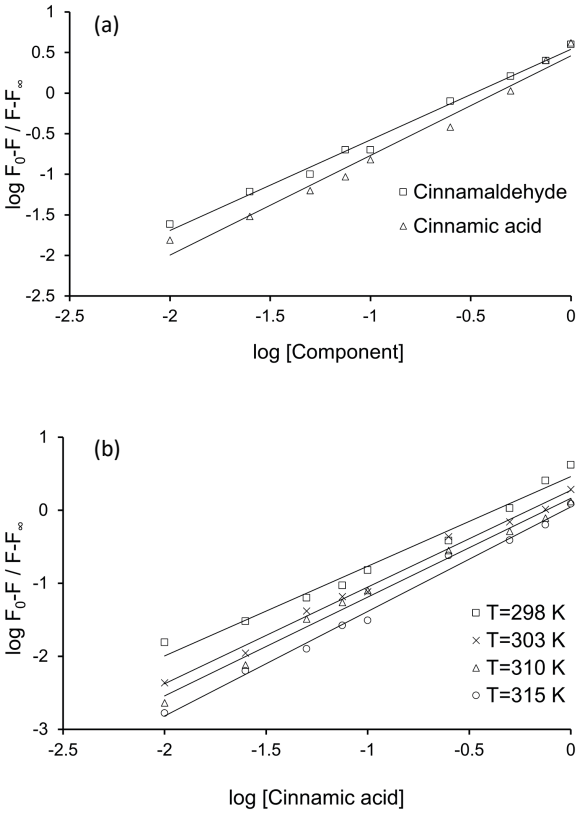

Figure 3. (a) Plots of the interaction of cinnamaldehyde and cinnamic acid with human serum albumin at 298 K. [HSA] = 1 μmol dm^{-3}, pH 7.40, [Component] = 0–1 mol dm^{-3}; (b) Plots of the interaction of cinnamic acid with human serum albumin at 298, 303, 310 and 315 K. [HSA] = 1 μmol dm^{-3}, pH 7.40, [Cinnamic acid] = 0–1 mol dm^{-3}.

Thermodynamic Parameters of HSA-Cinnamaldehyde and HSA-Cinnamic acid Complexes

According to [Eq. (2)] and [Eq. (3)], and in order to get information about the types of non-covalent interactions of HSA–component complexes, the thermodynamic parameters ($\Delta_r G°$, $\Delta_r H°$ and $\Delta_r S°$) for cinnamaldehyde and cinnamic acid were determined. By using the data of Table 1, Figure 4 proves that assumption of near constant $\Delta_r H°$ is justified.

The values of $\Delta_r G°$, $\Delta_r H°$ and $\Delta_r S°$ are shown in Table 2.

The formation of HSA–component complexes was an exothermic reaction with negative entropy and enthalpy values for cinnamic acid, and positive entropy and negative enthalpy values for cinnamaldehyde. $\Delta_r H°$ and $\Delta_r S°$ values are greater for HSA-cinnamaldehyde complex.

$\Delta_r G°$ is negative (Table 2) meaning that the binding process is favorable (spontaneous). Ross et al.[34] have

characterized the sign and magnitude of the thermodynamic parameters associated with various individual kinds of interaction that may take place in protein association processes, as described below. A positive value of $\Delta_r S°$ is often taken as evidence of hydrophobic interaction (from the point of view of water structure). A negative value of $\Delta_r H°$ and a positive $\Delta_r S°$ value characterize electrostatic interactions. Van der Waals forces and hydrogen bonds are characterized by negative enthalpy and entropy changes. Accordingly, the binding of cinnamic acid to HSA might involve hydrogen bonding and van der Waals forces as evidenced by negative $\Delta_r H°$ and $\Delta_r S°$ values. The binding of cinnamaldehyde to the protein might involve hydrophobic interaction as evidenced by positive $\Delta_r S°$ values. Rahman et al.[35] reported that $\Delta_r H°$ values are very small for electrostatic interactions. Therefore, the negative values of $\Delta_r H°$ (−17.23 and −32.20) observed for cinnamaldehyde and cinnamic acid respectively cannot be associated with electrostatic interactions.

Our results are not compatible with those published by Sun et al.[36] Sun et al., determined the binding mechanism of cinnamaldehyde and cinnamic acid to HSA via molecular modeling studies and multi-spectroscopic methods.

Table 2. Thermodynamic parameters of the complex HSA-cinnamaldehyde and HSA-cinnamic acid at pH 7.40.

Thermodynamic parameters	*T*	Cinnamaldehyde	Cinnamic acid
$\Delta_r H°$ / kJ mol^{-1}	298 K 303 K 310 K 315 K	−17.23	−32.20
$\Delta_r G°$ / kJ mol^{-1}	298 K	−36.51	−33.99
	303 K	−36.84	−34.02
	310 K	−37.29	−34.06
	315 K	−37.61	−34.09
$\Delta_r S°$ / J mol^{-1} K^{-1}	298 K 303 K 310 K 315 K	64.70	5.99

Binding Parameters of Glucose to HSA in Presence and Absence of Cinnamic Acid, Cinnamaldehyde, and Eugenol

The binding parameters of glucose to HSA were determined in absence and presence of cinnamic acid, cinnamaldehyde and eugenol, and the fluorescence quenching was studied by the Scatchard method. Scatchard plots of the binding of glucose to HSA are shown in Figure 5.

The binding parameters of glucose to HSA in the absence and presence of cinnamic acid, cinnamaldehyde and eugenol are summarized in Table 3.

Table 3. Association constant and the binding capacity for the interaction of glucose with HSA in the absence and presence of cinnamaldehyde, cinnamic acid or eugenol, measured by fluorimetric titration at pH 7.40.

	$10^3 K_a$ / mol^{-1} dm^3	*n*
HSA-Glucose	4.78 ± 0.03	2.9 ± 0.1
HSA-Glucose-Cinnamaldehyde	8.82 ± 0.03	6.8 ± 0.2
HSA-Glucose-Cinnamic acid	6.40 ± 0.01	4.9 ± 0.2
HSA-Glucose-Eugenol	4.79 ± 0.02	2.9 ± 0.1

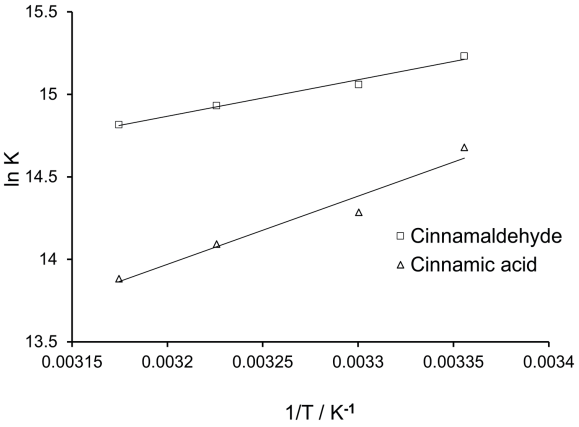

Figure 4. Van't Hoff plots for the interaction of cinnamaldehyde and cinnamic acid with human serum albumin at 298, 303, 310 and 315 K. [HSA] = 1 μmol dm^{-3}, pH 7.40.

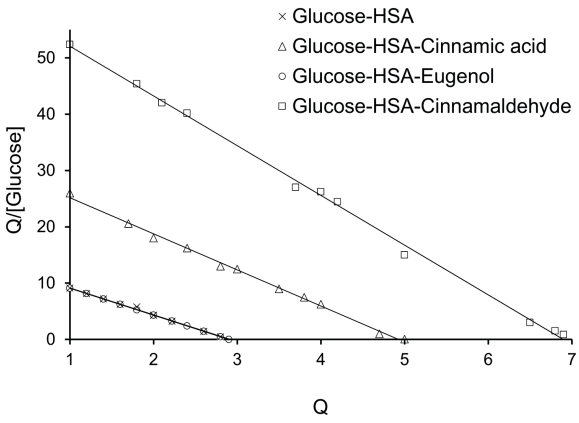

Figure 5. Scatchard plots for the interaction of glucose with human serum albumin in the presence and absence of cinnamaldehyde, cinnamic acid or eugenol. [HSA] = 1 μmol dm^{-3}, pH 7.40, [Component] = 1 mol dm^{-3}.

Figure 5 shows that cinnamaldehyde and cinnamic acid increase K_a values of glucose (8.82×10^3 and 6.40×10^3 (mol dm^{-3})$^{-1}$, respectively) compared to control (4.78×10^3 (mol dm^{-3})$^{-1}$) determined in the absence of any component. An important increase in binding site number, *n*, is noted from cinnamaldehyde and cinnamic acid. Eugenol did affect neither the binding constant nor the binding site number of glucose. The binding constant and the number of binding sites of glucose to HSA obtained in our study are very close to those reported in the literature (K_a, 4.78×10^3 (mol dm^{-3})$^{-1}$ and *n*, 2.9 compared to 4.95×10^3 (mol dm^{-3})$^{-1}$ and 3 respectively).[37]

Cinnamaldehyde and cinnamic acid have been found to increase the number of glucose binding sites on HSA. It is believed that these compounds enhance the affinity of HSA for glucose, potentially by forming specific interactions with the protein or altering its conformation in a way that promotes glucose binding. The modulation of glucose binding sites on HSA by cinnamaldehyde and cinnamic acid highlights the intricate interplay between cinnamon compounds and the protein, suggesting their potential role in regulating glucose metabolism and potentially influencing the management of metabolic disorders. Further research is needed to elucidate the exact mechanisms underlying these interactions and their therapeutic implications.

In addition, the increase in the fluorescence intensity of HSA-glucose complex in the presence of cinnamaldehyde and cinnamic acid (Figure 5) suggests that HSA might produce a rearrangement in its structure, allowing glucose to bind with greater affinity to the protein. Therefore, cinnamaldehyde and cinnamic acid, but not eugenol, modify the albumin structure which allows glucose to better bind to the protein.

These findings highlight the potential of both cinnamaldehyde and cinnamic acid as modulators of HSA conformation and provide insights into their potential applications in various biomedical and pharmaceutical fields. However, it is important to note that further studies are required to fully understand the mechanisms underlying these observations and to assess the stability and long-term effects of these compounds on HSA's structure and function. Additional research will provide valuable insights into the potential applications of cinnamaldehyde and cinnamic acid in various fields, including biomedicine and pharmaceuticals.

CONCLUSIONS

In this study, the interaction of cinnamaldehyde, cinnamic acid and eugenol with human serum albumin has been studied using fluorescence spectroscopy. The results showed that cinnamaldehyde and cinnamic acid, but not eugenol, are strongly bound to HSA. The acting forces are mainly hydrogen bonding and van der Waals interaction for cinnamic acid, and hydrophobic interactions for cinnamaldehyde. Displacement experiments show that cinnamic acid and cinnamaldehyde increased the binding of glucose to albumin. In conclusion, cinnamaldehyde and cinnamic acid are the active components present in the inner bark of the cinnamon tree *Cinnamomum verum,* these components interact with HSA producing rearrangement in its structure which allows glucose to better bind to the protein.

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