Journal of Fluorescence (2019) 29:1113–1123
https://doi.org/10.1007/s10895-019-02422-z

ORIGINAL ARTICLE

Comparison of the Quenching Effects of Two Main Components of *Ziziphi Spinosae Semen* on Serum Albumin Fluorescence

Baoai Wu[1] · Chenhuan Qu[1] · Yaxin Wang[1] · Jinfeng Zhao[1] · Huizhi Du[2]

Received: 25 April 2019 / Accepted: 29 July 2019 / Published online: 9 August 2019

Abstract

Swertisin (6-glucosyl flavonoid) and spinosin (2″-β-O-glucopyranosyl swertisin) are two main components from *Ziziphi Spinosae Semen*, with anti-anxiety and hypnosis effects. The paper aims to compare the differences between the two compounds binding with serum albumins (BSA and HSA). Swertisin and spinosin statically quench intrinsic fluorescence of serum proteins by binding to proteins to form complexes. The fluorescence quenching rates of BSA induced by swertisin or spinosin are faster than those of HSA resulted by swertisin or spinosin, respectively. Each serum protein has only one binding site respectively accessible to the two compounds. Hydrophobic force and hydrogen bond play the important roles during the biding process of swertisin with proteins, but van der Waals force and hydrogen bond are major driving forces for spinosin binding to proteins. Synchronous fluorescence data show that spinosin binds to BSA and HSA and thus changes Tyr and Trp residue microenvironments, and has a greater effect on the latter. Compared with swertisin, spinosin has a stronger effect on the α-helix of proteins. But the distance between swertisin and proteins is slightly closer than spinosin. These findings will contribute to further understand the reaction of *Ziziphi Spinosae Semen* in the liver phase I oxidation, intestinal hydrolysis and deparaffin metabolism.

Keywords Swertisin · Spinosin · Human serum albumin · Bovine serum albumin · Fluorescence quenching

Introduction

Serum proteins, the most abundant carrier proteins in plasma, are synthesized in the liver and can combine with many drugs [1]. The ability of serum proteins to bind to drugs is closely related to the free concentration of drugs in the blood, so, when the degree of binding is strong the free concentration of the compound in the plasma is reduced. Serum protein not only plays an important role in the metabolism of drugs, but also maintains blood pH and osmotic pressure [2]. As an important part of human protein, human serum albumin (HSA) accounts for about 60% total proteins. HSA, a monomeric globulin with 585 amino acid, is synthesized in human liver and presents in the interstitial fluid of the interstitial space [3]. There are three domains (I, II, III) in HSA, wherein domain I consists of amino acid residues at position 1–195, domain II contains residues 196–383, and domain III consists of amino acid residues at 384–585. And each domain includes two subunits (A and B) that are connected by a long extended loop. The molecular weight of HSA is 66 kDa, and the overall structure forms a heart-shaped stable structure protein through 17 pairs of disulfide bonds and 1 free cysteine residue [4]. The tertiary structure of HSA is composed of α-helix and a loop, forming pockets of binding sites at domains I-II and II-III to strongly bind with various endogenous and exogenous substances [5]. Because the combination of drugs and HSA will affect the transportation and distribution of drugs in the human body it is important to study their interactions to understand the action mechanism and pharmacokinetics of drugs. Bovine serum albumin (BSA) is widely used in the study of the interaction of compounds with serum proteins [6]. Because BSA is a homologous protein with HSA, its domain is very similar to HSA, and it is also a large globulin. BSA consists of 583 amino acid residues in a spiral structure with 20 tyrosine (Tyr) residues and 2 tryptophan (Trp-134, Trp-213) residues

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10895-019-02422-z) contains supplementary material, which is available to authorized users.

✉ Huizhi Du
duhuizhi@sxu.edu.cn

[1] School of Physical Education, Shanxi University, Taiyuan 030006, China

[2] Institute of Molecular Science, Shanxi University, Taiyuan 030006, China

[7]. Its structure resembles a heart shape. Three specific homologous domains (I-III) are composed, each of which is further divided into two subdomains (A and B). There are major binding sites in the hydrophobic regions of subdomains IIA and IIIA of BSA [8].

Ziziphi Spinosae Semen (ZSS), the semen of *Ziziphus jujuba* Mill. var. spinose (Bunge) Huex, is used for nourishing the heart, soothing the nerves and helping sleep as a traditional Chinese medicine. Its main bioactive ingredient is jujube seed flavonoid, including swertisin and spinosin [9] whose structures shown in Fig. 1. In the herb seed, the content of spinosin is high, while the content of swertisin is low. When taking orally into the human body, the content of spinosin decreases sharply and the content of swertisin increases significantly, which may be due to that spinosin being metabolized into swertisin [10, 11]. Using HPLC-ESI-MS/MS combined technique, Zhang et al. found that spinosin can penetrate the blood-brain barrier and is widely distributed in the hippocampus, striatum, cerebral cortex and cerebellum [12]. Studies have confirmed that spinosin has good anti-anxiety and hypnosis effects [13]. Wang et al. discovered that spinosin can effectively reduce sleep latency and increase sleep time [14]. Modern pharmacological researches found that swertisin also has many functions, such as anti-inflammatory, hypoglycemic and cognitive effects [15]. Lee et al. found that swertisin can improve Alzheimer's disease by improving cognitive improvement and memory [16]. Abhay group believed that swertisin can effectively induce pancreatic secretion of insulin by pancreatic cells, which can effectively lower blood sugar and treat diabetes [17].

The interaction information that includes binding mode, binding site and binding constant between proteins and compounds is very important to know in detail the pharmacodynamics and pharmacokinetics of compounds in biological, pharmacological and clinical applications. Because of many advantages, for example high accuracy, sensitivity and rapidity, the spectroscopic methods are usually used to study the protein-compound interactions along with other technologies [18, 19]. In the paper the interactions between two main components in *Ziziphi Spinosae Semen* and two kinds of serum proteins were studied, and the relationship between the functional groups and the binding constants was also discussed.

Materials and Methods

Materials and Solution Preparation

Swertisin and spinosin (98.6%) that were purchased from Must Bio-Technology (Chengdu, China) were prepared stock solution in DMSO. BSA and HSA that were purchased from Solarbio (Beijing, China) were made stock solution with Tris buffer of pH 7.4. All other reagents are analytical grade.

Fluorescence Measurements

The spectroscopic spectra of BSA and HSA were measured in quartz cuvette of 1 cm path length with the fluorescence spectrophotometer (Fluromax-4, HORIBA Scientific, USA) equipped with a temperature holder at 27 ± 0.5 °C except where designated. The experiments were performed in Tris buffer of pH 7.4. 5 min after adding the compound the fluorescence was recorded in 290–500 nm at exciting wavelength of 280 nm, with fixed excitation and emission slit width of 10 nm each.

Synchronous Fluorescence Measurements

The synchronous fluorescence experiments were performed in Tris buffer of pH 7.4 on the fluorescence spectrophotometer (Fluromax-2500, Hitachi, Japan), with the initial excitation wavelength set as 220 nm and scanned up to 500 nm. The difference ($\triangle\lambda$) between excitation and emission wavelength was set at 15 nm for Tyr residues or at 60 nm for Trp residues [20, 21]. Both excitation and emission slit widths were set at 10 nm. The recording was repeated two times.

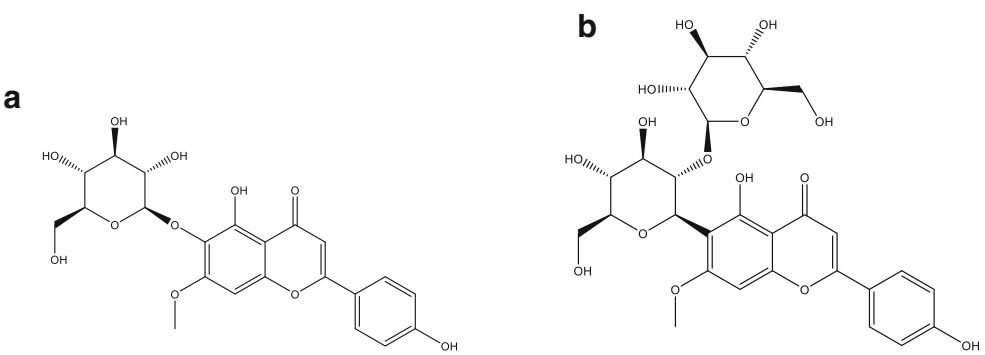

Fig. 1 The structures of swertisin (**a**) and spinosin (**b**)

CD Measurements

Circular dichroism (CD) measurements were conducted in Tris buffer of pH 7.4 with a spectropolarimeter (Applied Photophysics, Chirascan, UK) collocated with a Quantum Northwest temperature controller. Each spectrum is the average of two scans.

UV-Visible Absorption Studies

UV-visible absorption spectra were recorded between 200 and 350 nm with UV-visible spectrophotometer (Cary 50, Varian, USA). All absorbance spectra were corrected by the blank.

Data Analysis

All data were expressed as means ± S.D. Each experiment was conducted three times.

Results and Discussion

Fluorescence Quenching of BSA and HSA Induced by Swertisin and Spinosin

Fluorescence quenching is one of the most convenient methods to examine the interaction between compound and protein [22]. When fixing the excitation wavelength at 280 nm, BSA and HSA exhibit maximum emission wavelength at about 345 nm that is the characteristic emission wavelength of Trp [23], while both compounds show no intrinsic fluorescence emission at 345 nm. Fluorescence quenching of BSA and HSA was studied by addition of swertisin or spinosin to serum protein solution. The concentrations of proteins were kept at 1 μM, while the concentrations of compounds were 2, 4, 6....14 μM, 19, 24, 29...54 μM. As shown in Fig. 2, as the concentration of swertisin and spinosin increase the fluorescence intensities of BSA and HSA gradually decrease until almost vanish, indicating that the compounds interact with the serum proteins.

Though no obvious shift of the fluorescence peak was observed, the fluorescence quenching rate of BSA induced by swertisin is faster than that of HSA, and the fluorescence quenching rate of BSA caused by swertisin is faster than spinosin (Fig. S1). In a word, the interaction between swertisin and BSA is the strongest.

The Synchronous Fluorescence Study of Interaction of Swertisin and Spinosin with BSA and HSA

Synchronous fluorescence spectroscopy can be used to study the effect of small molecules on protein conformation [20]. Δλ of 15 and 60 nm between the excitation wavelength and the emission wavelength represent the spectral characteristics of Tyr and Trp, respectively [24]. Similar to recording of fluorescence quenching, we successively added compounds to serum protein solution to measure synchronous fluorescence, with the concentrations of proteins of 1 μM and the concentrations of compounds of 2, 4, 6....12 μM. Figure 3 shows the effects of different concentrations of swertisin on the Tyr and Trp in BSA and HSA. The fluorescence intensities were regularly reduced with the increase of the compound concentration, which further confirmed the fluorescence quenching data; and there was no significant shift in the maximum emission peak. The fluorescence intensities of Trp residues decreased more, indicating that Trp residues were more affected than Tyr residues when the compound bound to serum proteins. No shift was observed in maximum emission wavelength, which was consistent with the fluorescence quenching results.

The UV–vis Study of Swertisin and Spinosin on BSA and HSA

Firstly UV-visible absorption spectra of the compounds (16 μM) and serum proteins (1 μM) alone were recorded, shown in Fig. S2. The UV absorption peaks of BSA and HSA are around 280 nm, and the UV absorption peaks of swertisin and spinosin are around 220, 270 and 340 nm from one carbonyl conjugated system in common.

To study further the effects of swertisin and spinosin on the structures of BSA and HSA, the UV-visible absorption spectra of BSA and HSA were measured by gradual addition of swertisin and spinosin (2, 4, 6… 14 μM) into protein solution (1 μM). With the increase of swertisin or spinosin concentration, the intensity of the absorption peak gradually increased, suggesting interaction of the hydrophobic benzene ring of the compounds with the amino acid residues in the hydrophobic cavity of proteins (Fig. 4). Fluorescence quenching of the protein consists of the static quenching and the dynamic quenching. Dynamic quenching is a process in which a collision between a protein and a compound causes a decrease in fluorescence intensity, while a static quenching process only has a complex formation between a protein and a compound by weak interaction [25, 26]. The absorption spectrum changes only when static quenching occurs and complex is formed, so we speculate that they are static quenching and complex is formed [27].

Fluorescence Quenching Mechanism BSA and HSA by Swertisin and Spinosin

In order to clearly know the mechanism of fluorescence quenching of two proteins induced by two compounds, the quenching constants (K_{sv}) and quenching rate constant (k_q) of the interaction of swertisin and spinosin with proteins are compared at different temperatures. Figure 5 is the plots of the

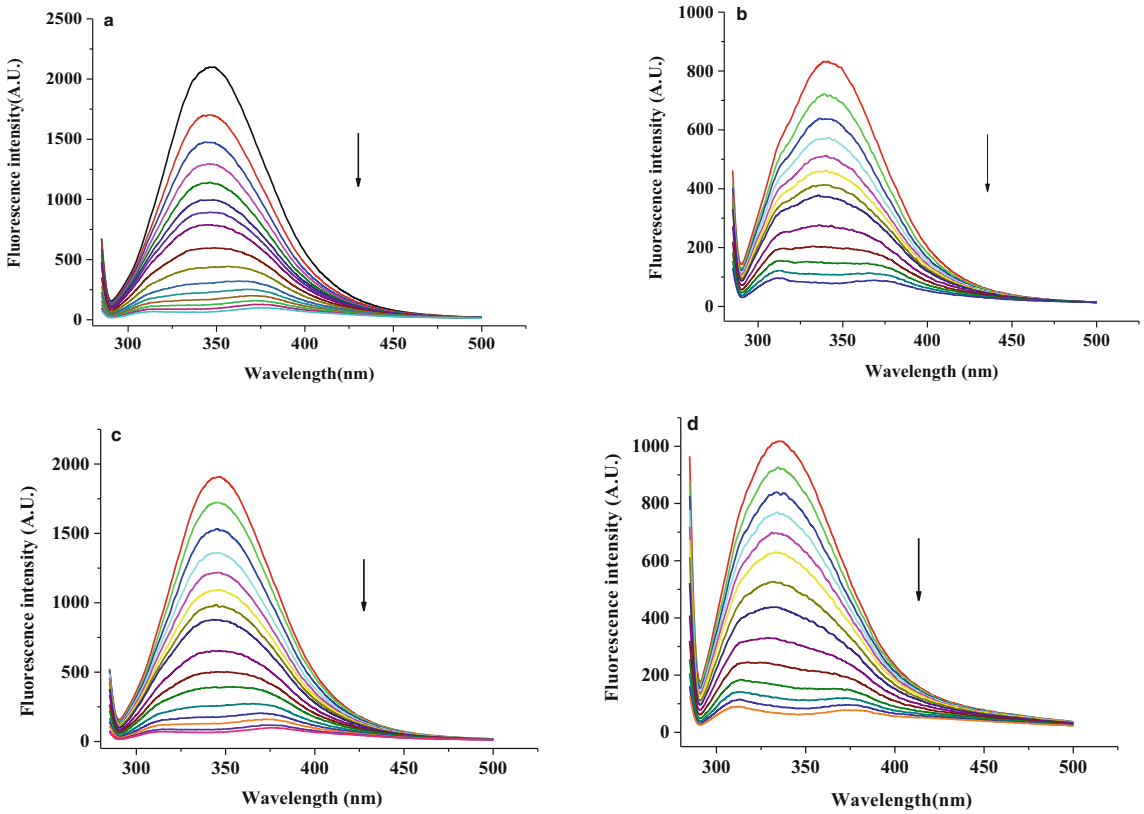

Fig. 2 The changes of fluorescence emission spectra of BSA and HSA induced by swertisin and spinosin with an excitation wavelength of 280 nm. **a** BSA + swertisin; **b** HSA + swertisin; **c** BSA + spinosin; **d** HSA + spinosin

Stern-Volmer equation at different temperature and the values of K_{sv} and k_q are shown in Table 1. The fluorescence quenching data at different temperatures are analyzed by the well-established Stern-Volmer equation:

$$F_0/F = 1 + k_q\tau_0[Q] = 1 + K_{sv}[Q] \quad (1)$$

$$k_q = \frac{K_{sv}}{\tau_0} \quad (2)$$

where F_0 and F are the fluorescence intensities of proteins in the absence and presence of compounds, respectively; k_q is the quenching rate constant of proteins; τ_0 is the average lifetime of proteins without quencher at the average value of 10^{-8} s, K_{sv} is the Stern-Volmer quenching constant and [Q] is concentration of quencher.

There is no significant difference in K_{sv} between swertisin with proteins at 27 °C and 37 °C (1.17 vs. 1.19 ($\times 10^5$ M^{-1}) for swertisin acting with BSA, 8.73 vs. 8.70 ($\times 10^4$ M^{-1}) for swertisin acting with HSA). This phenomenon needs to be reconfirmed by other methods. As the temperature increased, the quenching constants K_{sv} of the interaction between spinosin and proteins decreased, indicating that the effect of spinosin on BSA and HSA was static quenching. The change rules of k_q were similar to those of K_{sv}. The maximum quenching and dispersion collision constant of quencher and biomacromolecule is 2.0×10^{10} $M^{-1}{\cdot}s^{-1}$, while the k_q values of the interaction between swertisin and spinosin with two kinds of proteins are much higher in the present study. These data again demonstrated that swertisin and spinosin causes the fluorescence quenching of BSA and HSA by the static quenching mode, that is to say, swertisin and spinosin were respectively combined BSA and HSA to form complexes.

After knowing that these two compounds bind to BSA and HSA to form complexes, the binding constant (K) and the number of binding sites (n) can be further clarified by the equation:

$$lg\,[(F_0 - F)/F] = lg\,K + n\,lg\,[Q] \quad (3)$$

where F_0 and F are the fluorescence intensities of proteins in the absence and presence of compounds, respectively, [Q] is

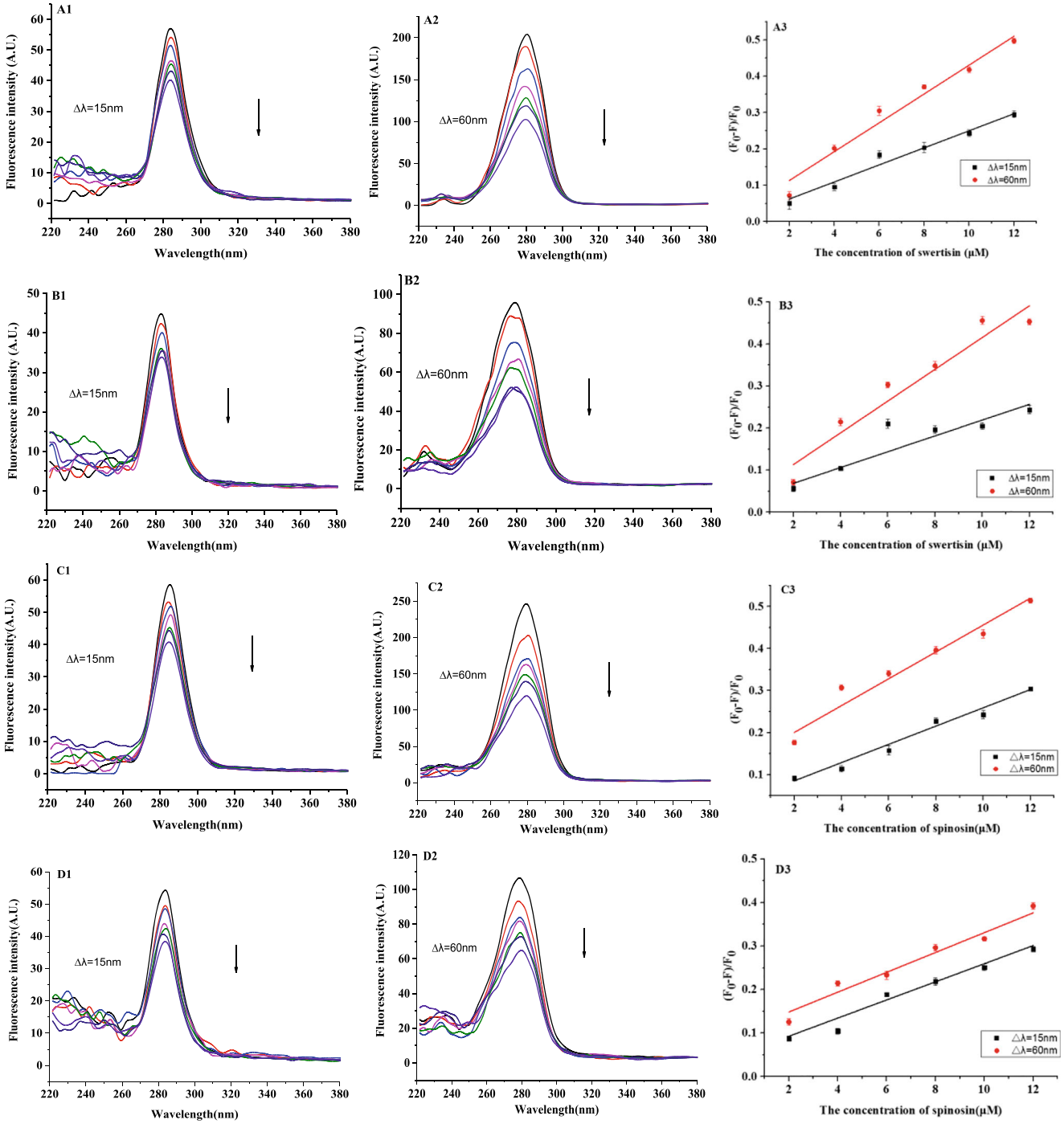

Fig. 3 The effects of swertisin and spinosin on synchronous fluorescence spectra of serum albumins. A1-A3: BSA + swertisin; B1-B3: HSA + swertisin; C1-C3: BSA + spinosin; D1-D3: HSA + spinosin

concentration of compounds, *K* denotes the binding constant of compounds with proteins and *n* is the number of binding sites.

It can be seen from Table 1 that there is only one binding site for interactions of swertisin and spinosin with BSA and HSA. At 27 °C, the binding constant *K* of swertisin with BSA and HSA both is 1.08×10^5 M^{-1}, with no significant difference. The binding constant *K* of spinosin to BSA is 8.15×10^5 M^{-1} and the binding constant to HSA is 2.03×10^6 M^{-1}. The latter is about 2.5 fold of the former, indicating that the interaction between spinosin and HSA is a little stronger than BSA. In addition, the binding constant *K* of spinosin and BSA is about 8 fold, and the binding constant *K* with HSA is about 20 fold, compared with swertisin, indicating that spinosin has

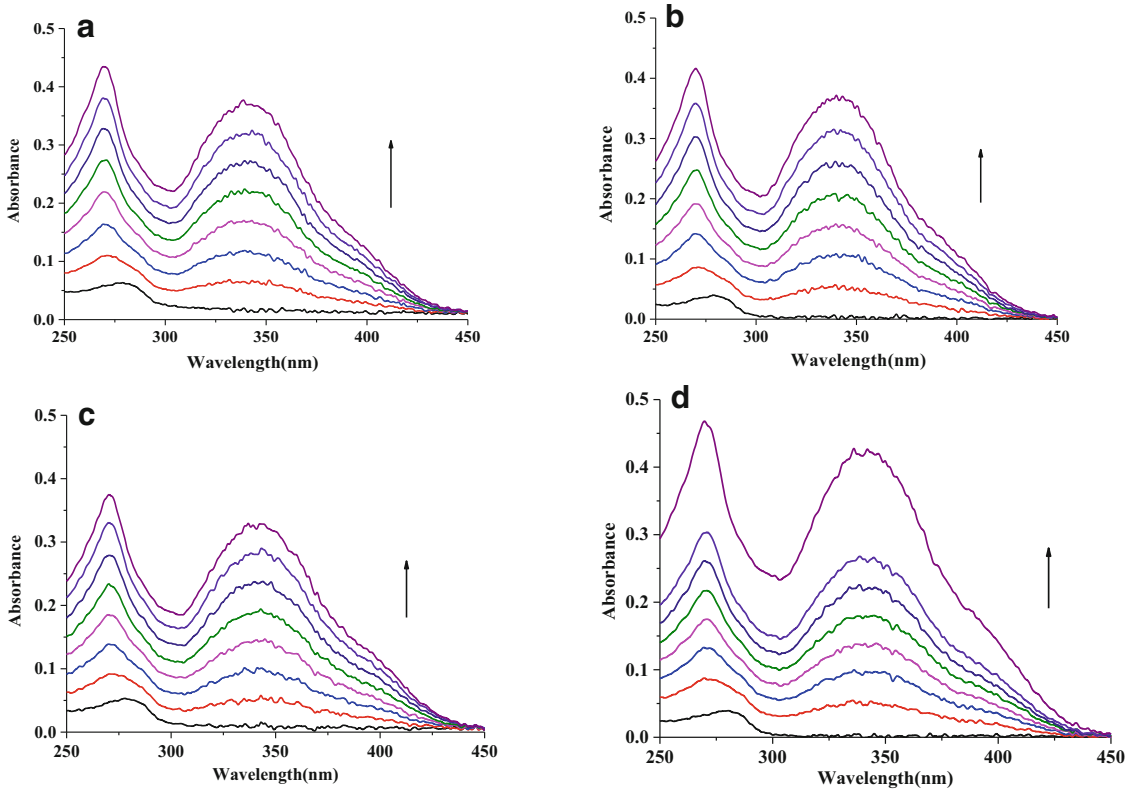

Fig. 4 The UV-vis spectra of swertisin and spinosin effecting on BSA and HSA. **a** BSA-swertisin complexes. **b** HSA-swertisin complexes. **c** BSA-spinosin complexes. **d** HSA-spinosin complexes

stronger binding ability with proteins than swertisin, especially HSA.

Thermodynamics Parameters of Interaction of Swertisin and Spinosin with BSA and HSA

Four main types of weak and non-covalent interactions play the important roles in protein-ligand interactions, including van der Waals forces, hydrogen bonds, electrostatic gravitational forces and hydrophobic forces [28]. Thermodynamic parameters enthalpy change (ΔH), free energy change (ΔG), entropy change (ΔS) calculated from the Van der Hoff equation and thermodynamic equation.

$$\mathbf{ln}\ \mathrm{K} = -\mathbf{\Delta}\mathrm{H}/\mathrm{R}\ \mathbf{T} + \mathbf{\Delta}\mathrm{S}/\mathbf{R} \tag{4}$$

$$\mathbf{\Delta}\mathrm{G} = \mathbf{\Delta}\mathrm{H} - \mathrm{T}\mathbf{\Delta}\mathrm{S} = -\mathrm{RT}\ \mathbf{ln}\mathrm{K} \tag{5}$$

where R is the gas constant, T is the temperature and K is the binding constant at corresponding temperature.

From a thermodynamic point of view, when ΔH and ΔS values are positive at the same time, the interaction force is mainly the hydrophobic interaction force. When ΔH and ΔS values are negative at the same time, van der Waals force or hydrogen bond is the main interaction force. When $\Delta H \approx 0$ and $\Delta S > 0$, it is the electrostatic interaction force that plays a major role [24]. It can be seen from Table 1 that the values of ΔG are negative for the binding of two compounds to two proteins, indicating that all these interactions are spontaneous. When swertisin acts with BSA and HSA, both ΔH and ΔS show positive values, which indicates that the main types of forces are hydrophobic force or hydrogen bond. For spinosin, whatever effects on BSA or HSA, all ΔH and ΔS values are less than zero, indicating that the interaction types of spinosin with BSA and HSA are mainly van der Waals forces or hydrogen bonds. The weak interaction forces may help complex to maintain stability of spatial structures such as protein helix, folding and polypeptide chains. Thus, the compounds can be successfully transported to their destinations to play a bioactive function [29, 30].

CD Spectra of Swertisin and Spinosin on BSA and HSA

The secondary structure of a biological macromolecule such as a protein may be get a little bit more insight by measuring its CD spectrum [31]. In order to understand the effects of the two compounds on the secondary structure of BSA and HSA, CD experiments were carried out

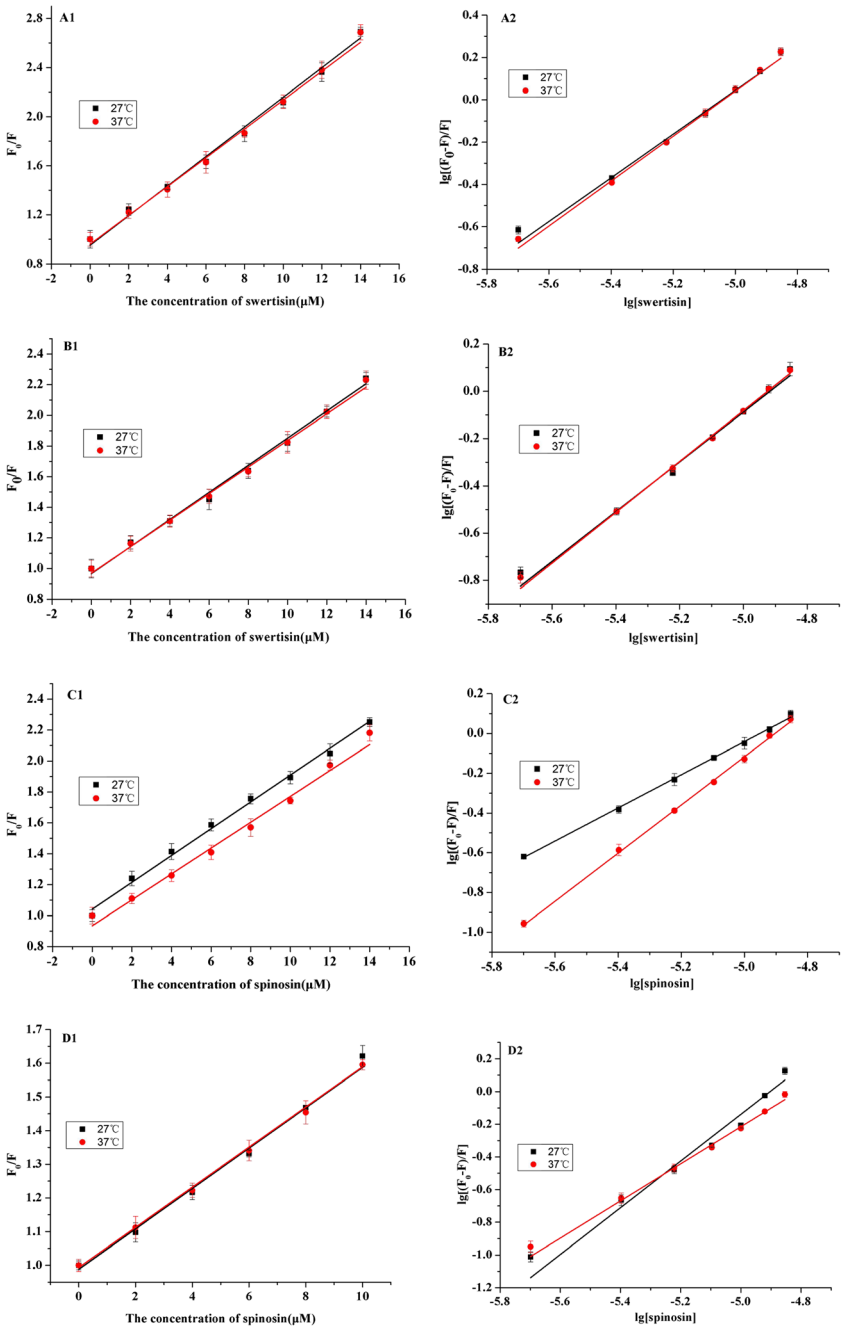

Fig. 5 The Stern-Volmer plots (left) and logerthamic plots (right) for the quenching of proteins by compounds at different temperatures. A1-A2: BSA + swertisin; B1-B2: HSA + swertisin; C1-C2: BSA + spinosin; D1-D2: HSA + spinosin

in Tris buffer, pH of 7.4 (Fig. 6). The compound alone (30 μM) has no absorption peak at 200–260 nm (Fig. S3). BSA and HSA show negative peaks at 208 nm and 222 nm which are characteristic of the α-helix. When adding different concentration of swertisin, the absorption peaks of BSA and HSA increased, however, the position of the peak did not change substantially, indicating that swertisin changed the conformation of BSA and HSA. In addition, the α-helix of BSA increased from 46.63 to 48.34%, and the α-helix of HSA increased from 51.32 to 53.73%, which suggested that swertisin had a greater effect on HSA. After the addition of spinosin to protein solution, the α-helix of BSA increased from 46.64 to 49.01% and the α-helix of HSA increased from 51.33 to 53.92%, indicating that the effect of spinosin on HSA was greater than that of BSA. And comparing with swertisin, spinosin had a greater effect on the α-helix of proteins.

Energy Transfer between the Compounds and Serum Proteins

Next the energy transfer information of the donor-acceptor was explored by Föster's non-radiative energy transfer (FRET) [32]. Whether FRET can be used depends

Table 1 Thermodynamic parameters of interaction of proteins with compounds

Temperature (°C)		Swertisin		Spinosin	
		27	37	27	37
BSA	K_{sv} ($\times 10^4$ M^{-1})	11.71 ± 0.37	11.92 ± 0.33	8.58 ± 0.01	8.45 ± 0.01
	k_q($\times 10^{12}$ $M^{-1}\cdot s^{-1}$)	11.71 ± 0.37	11.92 ± 0.33	8.58 ± 0.01	8.45 ± 0.01
	n	1.00 ± 0.03	1.05 ± 0.03	0.84 ± 0.03	1.21 ± 0.02
	K ($\times 10^4$ M^{-1})	10.83 ± 0.54	20.7 ± 0.19	81.51 ± 4.53	1.44 ± 0.69
	ΔH (kJ/mol)	50.30 ± 4.14		−312.34 ± 6.48	
	ΔS (J·mol^{-1}·k^{-1})	264 ± 17.56		−927.96 ± 36.22	
	ΔG (kJ/mol)	−28.90 ± 1.64	−31.54 ± 3.07	−36.23 ± 2.17	−30.96 ± 1.77
HSA	K_{sv} ($\times 10^4$ M^{-1})	8.73 ± 0.02	8.70 ± 0.03	5.99 ± 0.16	5.65 ± 0.13
	k_q ($\times 10^{12}$ $M^{-1}\cdot s^{-1}$)	8.73 ± 0.02	8.70 ± 0.03	5.99 ± 0.16	5.65 ± 0.13
	n	1.02 ± 0.02	1.04 ± 0.00	1.43 ± 0.10	1.14 ± 0.05
	K ($\times 10^5$ M^{-1})	1.08 ± 1.12	1.31 ± 4.02	20.31 ± 2.28	2.93 ± 2.11
	ΔH (kJ/mol)	15.24 ± 0.74		−275.4 ± 4.88	
	ΔS (J·mol^{-1}·k^{-1})	147.14 ± 13.94		−902.97 ± 22.92	
	ΔG (kJ/mol)	−28.90 ± 2.36	−30.37 ± 1.91	−40.28 ± 2.91	−32.45 ± 1.39

mainly on the following factors: (1) a significant overlap between the donor and the acceptor. (2) the distance (r) between the donor and the acceptor (2–8 nm). (3) the proper oriented transition dipole of donor and acceptor [21].

Figure 7 is the overlap between the fluorescence emission spectrum of BSA/HSA and the UV-vis absorption spectrum of acceptor (swertisin and spinosin). Data processing was carried out according to the calculation method of donor-acceptor energy transfer reported by Li et al. [18]. The average distance between the donor and acceptor was between 2 and 8 nm, and the values also fitted the scale: $0.5R_0 < r < 1.5R_0$. In our study the distances were exactly the same between swertisin and proteins, which reminded us that there was no big different HSA and BSA when combined with swertisin. So did the spinosin (Table 2).

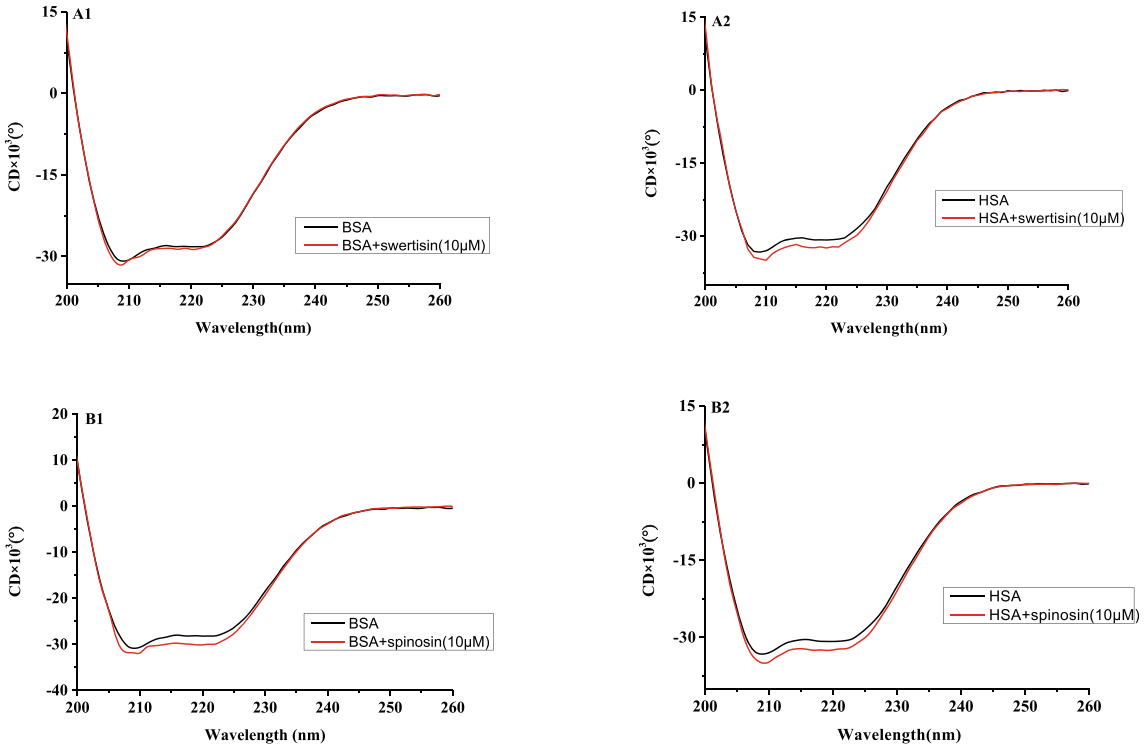

Fig. 6 CD spectra of the BSA-swertisin system (**a**), HSA-swertisin system (**b**), BSA -spinosin system (**c**) and HSA-spinosin system (**d**)

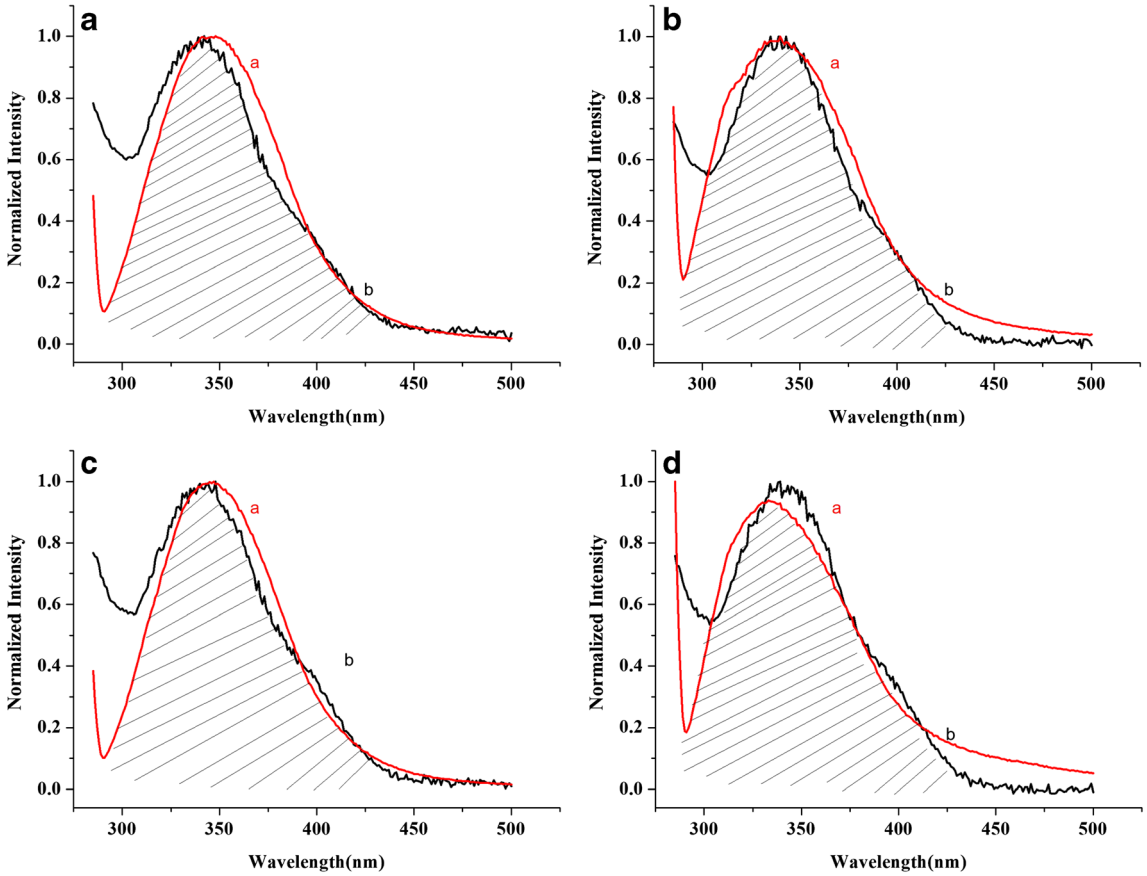

Fig. 7 Overlap of UV absorption (b) of the compound and fluorescence emission spectrum (a) of the protein. **a** BSA-swertisin system; **b** HSA-swertisin system; **c** BSA -spinosin system; **d** HSA-spinosin system. The final concentrations of the compounds and the proteins are all 5 μM

Conclusions

In the paper, the interactions between two main ingredients (swertisin and spinosin) from *Ziziphi Spinosae Semen* and two serum proteins (BSA and HSA) have been studied by spectroscopic techniques. Although the two serum albumin domains exhibit extensive structural homology, their binding modes are not exactly the same. Binding of different types of ligands within a serum albumin can be competitive or cooperative and often is stereospecific. Swertisin and spinosin statically quench intrinsic fluorescence of serum proteins by binding to proteins to form complexes. Each serum protein has only one binding site respectively accessible to the two compounds, and hence the compounds can bind with the two serum protein with stoichiometric ratio of 1:1. Hydrophobic force and hydrogen bond play the important roles during the biding process of swertisin with proteins, but van der Waals force and hydrogen bond are major driving forces for spinosin binding to proteins. We find that swertisin and spinosin change Trp microenvironments by recording synchronous fluorescence, however more methods are needed to reconfirm the binding site. In a word, swertisin and spinosin are the florescence quenchers of serum proteins, whose quenching information is very similar and not exactly the same attributed to the different chemical structures. These findings will contribute to understand the metabolic process of *Ziziphi Spinosae Semen*, for example, the liver phase I oxidation, intestinal hydrolysis and deparaffin metabolism [33, 34].

Table 2 The values of J, E, R_0 and r

Compounds	Proteins	J ($cm^3 \cdot L \cdot mol^{-1}$)	E	R_0 (nm)	r (nm)
Swertisin	BSA	0.92 ± 0.02	0.95 ± 0.02	0.5 3 ± 0.01	0.33 ± 0.01
	HSA	0.94 ± 0.01	0.95 ± 0.01	0.53 ± 0.01	0.33 ± 0.01
Spinosin	BSA	0.91 ± 0.02	0.95 ± 0.01	0.53 ± 0.01	0.33 ± 0.01
	HSA	1.00 ± 0.03	0.95 ± 0.02	0.54 ± 0.01	0.33 ± 0.01

Acknowledgements This work was supported by National Natural Science Foundation of China (No. 81403130), Shanxi Scholarship Council of China (No. 2017-021) and Natural Science Foundation of Shanxi Province (No. 201801D121290).

Compliance with Ethical Standards

Conflict of Interest The authors claim that they have no conflict of interest.

References

1. Li H, Wu F, Tan J, Wang K, Zhang C, Zheng H, Hu F (2016) Caffeic acid phenethyl ester exhibiting distinctive binding interaction with human serum albumin implies the pharmacokinetic basis of propolis bioactive components. J Pharm Biomed Anal 122:21–28
2. Liu X, Ling Z, Zhou X, Ahmad F, Zhou Y (2016) Comprehensive spectroscopic probing the interaction and conformation impairment of bovine serum albumin (BSA) by herbicide butachlor. J Photochem Photobiol B 162:332–339
3. Leboffe L, Di MA, Trezza V, Polticelli F, Ascenzi P (2017) Human serum albumin: a modulator of cannabinoid drugs. IUBMB Life 69(11):834–840
4. Hekmat A, Hajebrahimi Z, Motamedzade A (2017) Structural changes of human serum albumin (HSA) in simulated microgravity. Protein Pept Lett 24(11):1030–1039
5. Ishima Y, Maruyama T (2016) Human serum albumin as carrier in drug delivery systems. Yakuqaku Zasshi 136(1):39–47
6. Shahabadi N, Maghsudi M, Kiani Z, Pourfoulad M (2011) Multispectroscopic studies on the interaction of 2- tert - butylhydroquinone (TBHQ), a food additive, with bovine serum albumin. Food Chem 124(3):1063–1068
7. Sekula B, Zielinski K, Bujacz A (2013) Crystallographic studies of the complexes of bovine and equine serum albumin with 3,5-diiodosalicylic acid. Int J Biol Macromol 60(6):316–324
8. Suryawanshi VD, Walekar LS, Gore AH, Anbhule PV, Kolekar GB (2016) Spectroscopic analysis on the binding interaction of biologically active pyrimidine derivative with bovine serum albumin. J Pharm Anal 6(1):56–63
9. Li En W, Xue Qiong Z, Yan Qi Y, Yong He Z (2012) Augmentative effect of spinosin on pentobarbital-induced loss of righting reflex in mice associated with presynaptic 5-HT1A receptor. J Pharm Pharmacol 64(2):277–282
10. Yan Y, Li Q, Du CH, Jia JP, Feng HX, Qin XM (2017) Investigation of the potentially effective components of semen Ziziphi Spinosae based on "in vitro to in vivo" translation approach. Yaoxuexuebao 52(02):283–290
11. Yan Y, Zhang M, Cui XF, Zhang FS, Gao XX, Guo XD, Du CH, Qin XM (2019) Discussion on research ideas for process *in vivo* of chemical compositions from *Ziziphi Spinosae Semen* and its quality marker. Chin Tradit Herb Drugs 50(2):299–309
12. Zhang Y, Zhang T, Wang F, Xie J (2015) Brain tissue distribution of Spinosin in rats determined by a new high-performance liquid chromatography–electrospray ionization–mass/mass spectrometry method. J Chromatogr Sci 53(1):97–103
13. Liu J, Zhai WM, Yang YX, Shi JL, Liu QT, Liu GL, Fang N, Li J, Guo JY (2015) GABA and 5-HT systems are implicated in the anxiolytic-like effect of spinosin in mice. Pharmacol Biochem Behav 128:41–49
14. Wang LE, Bai YJ, Shi XR, Cui XY, Cui SY, Zhang F, Zhang QY, Zhao YY, Zhang YH (2008) Spinosin, a -glycoside flavonoid from semen Zizhiphi Spinozae, potentiated pentobarbital-induced sleep via the serotonergic system. Pharmacol Biochem Behav 90(3):399–403
15. Oh HK, Jeon SJ, Lee S, Lee HE, Kim E, Park SJ, Kim HN, Jung WY, Cheong JH, Jang DS (2016) Swertisin ameliorates pre-pulse inhibition deficits and cognitive impairment induced by MK-801 in mice. J Psychopharmacol 31(2):250–259
16. Lee HE, Jeon SJ, Ryu B, Park SJ, Ko SY, Lee Y, Kim E, Lee S, Kim H, Jang DS (2016) Swertisin, a C-glucosylflavone, ameliorates scopolamine-induced memory impairment in mice with its adenosine A1 receptor antagonistic property. Behav Brain Res 306:137–145
17. Srivastava A, Dadheech N, Vakani M, Gupta S (2018) Swertisin ameliorates diabetes by triggering pancreatic progenitors for islet neogenesis in Streptozotocin treated BALB/c mice. Biomed Pharmacother 100:221–225
18. Li Y, Guo Q, Yan Y, Chen T, Du C, Du H (2019) Different effects of Forsythia suspensa metabolites on bovine serum albumin (BSA). Spectrochim Acta A Mol Biomol Spectrosc 214:309–319
19. Yan X, Chen T, Zhang L, Du H (2018) Study of the interactions of forsythiaside and rutin with acetylcholinesterase (AChE). Int J Biol Macromol 119:1344–1152
20. Miller JN, Fell AF (1980) The characterization of proteins by synchronous and derivative luminescence spectroscopy. J Pharm Pharmacol 32:70P
21. Siddiqui GA, Siddiqi MK, Khan RH, Naeem A (2018) Probing the binding of phenolic aldehyde vanillin with bovine serum albumin: evidence from spectroscopic and docking approach. Spectrochim Acta A Mol Biomol Spectrosc 203:40–47
22. Ashoka S, Seetharamappa J, Kandagal PB, Shaikh SMT (2006) Investigation of the interaction between trazodone hydrochloride and bovine serum albumin. J Lumin 121(1):179–186
23. Yousefi R, Jamshidi M, Shahsavani MB, Nabavizadeh SM, Haghighi MG, Rashidi M, Taheri-Kafrani A, Niazi A, Keshavarz F, Alavinamehr MM (2016) Study on the interaction of three structurally related cationic Pt(II) complexes with human serum albumin: importance of binding affinity and denaturing properties. J Iran Chem Soc 13(4):617–630
24. Tang J, Luan F, Chen X (2006) Binding analysis of glycyrrhetinic acid to human serum albumin: fluorescence spectroscopy, FTIR, and molecular modeling. Bioorg Med Chem 14(9):3210–3217
25. Ross PD, Subramanian S (1981) Thermodynamics of protein association reactions: forces contributing to stability. Biochemistry 20(11):3096–3102
26. Anand U, Jash C, Mukherjee S (2010) Spectroscopic probing of the microenvironment in a protein-surfactant assembly. J Phys Chem B 114(48):15839–15845
27. Khade BS, Mathe VL, Dongre PM (2017) α-Amylase binding to thermal plasma synthesized zinc oxide nanosheets: a fluorescence study. J Lumin 187:449–456
28. Klotz IM (2010) Physiochemical aspects of drug-protein interactions: a general perspective. Ann N Y Acad Sci 226(1):18–35
29. Desfrançois C, Carles S, Schermann JP (2000) Weakly bound clusters of biological interest. Chem Rev 100(11):3943–3962
30. Pitera JW, Gunsteren WF (2001) The importance of solute−solvent van der Waals interactions with interior atoms of biopolymers. J Am Chem Soc 123(13):3163–3164

31. Zhang YZ, Zhou B, Liu YX, Zhou CX, Ding XL, Liu Y (2008) Fluorescence study on the interaction of bovine serum albumin with P-Aminoazobenzene. J Fluoresc 18(1):109–118
32. Shi JH, Chen J, Wang J, Zhu YY, Wang Q (2015) Binding interaction of sorafenib with bovine serum albumin: spectroscopic methodologies and molecular docking. Spectrochim Acta A Mol Biomol Spectrosc 149:630–637
33. Jiao L, Li Y, Zhang Y, Liu J, Xie J, Zhang K, Zhou A (2017) Degradation kinetics of 6‴-p-coumaroylspinosin and identification of its metabolites by rat intestinal flora. J Agric Food Chem 65(22): 4449–4455
34. Li Q, Du CH, Zhang M, Yan Y, Gao Y, Qin XM (2017) Investigation of effective components screening of *Ziziphi Spinosae Semen* based on serum pharmacochemistry and network pharmacology. Chin Tradit Herb Drugs 52(02):283–290