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Interaction of human serum albumin with volatiles and polyphenols from some berries

Rajamohamed Beema Shafreen [a], Tomasz Dymerski [b], Jacek Namieśnik [b], Zenon Jastrzębski [c], Suchada Vearasilp [d], Shela Gorinstein [e],

[a] Molecular Nanomedicine Research Unit, Centre for Nanoscience and Nanotechnology, Sathyabama University, Chennai 600119, TN, India
[b] Gdańsk University of Technology, Chemical Faculty, Department of Analytical Chemistry, Gdańsk 80 952, Poland
[c] Department of Pharmacology, National Medicines Institute, 00-725 Warsaw, Poland
[d] Postharvest Technology Research Institute/Postharvest Technology Innovation Center, Chiang Mai University, Thailand
[e] Institute for Drug Research, School of Pharmacy, Hadassah Medical School, The Hebrew University, Jerusalem 91120, Israel

a b s t r a c t

Headspace solid-phase microextraction coupled with comprehensive two-dimensional gas chromatog-raphy with time-of-flight mass spectrometry (HS-SPME/GC × GCeTOFMS) identified 19 and quantified 6 monoterpenes in gooseberries, blueberries and cranberries. As a protein model human serum albumin (HSA) was used in interaction with terpenes. Specific binding of terpenes to HSA under the physiological conditions was a result of the formation of a complex. In order to determine the synergism of poly-phenols and volatiles in some berries binding properties with HSA were evaluated by 3D-fluorometry and molecular docking. Extracts of polyphenols and monoterpenes, using various ethanol mixtures with water, and their antioxidant properties were determined by two radical scavenging assays (ABTS and CUPRAC). The studied extracts interacted with HSA with different binding affinities which were directly related to their antioxidant properties. The highest binding abilities were in blueberries and γ-terpinene. Among the six terpenes, the highest scoring compound was identified as γ-terpinene. This study pro-vides useful information for the future designing of health food on the basic of polyphenols and volatile substances.

Keywords: Volatile substances, Bioactivity, Three-dimensional fluorescence, Binding properties, Molecular docking, Synergism

1. Introduction

Drug interaction with albumins significantly affects *in vivo* drug transport and biological metabolism. To gain insight into the binding mechanisms of tyrosine-kinase inhibitor to human serum albumin (HSA) steady-state fluorescence quenching and molecular modeling was adopted (Yan et al., 2016). The binding modes of honokiol (HK) and magnolol (MG) with HSA have been established under imitated physiological condition, which was very important to understand the pharmacokinetics and toxicity of HK or MG (Cheng, 2012) or rosmarinic acid (Peng, Wang, Qi, Su, & He, 2016). Polyphenol interactions with whey protein isolate and whey protein isolate epectin coacervates were studied (Thongkaew, Gibis, Hinrichs, & Weiss, 2014). Epigallocatechin-3-gallate (EGCG), which is the major and the most biologically active catechin of green tea, has the highest binding affinity to whey proteins due to galloyl functional group (Al-Hanish et al., 2016). To understand the pharmacokinetic characteristics of caffeic acid phenethyl ester (CAPE), the binding interaction between CAPE and HSA was investigated *in vitro* using multiple spectroscopic methods and molecular docking (Li et al., 2016). HSA binding as a transport carrier for a vast variety of natural compounds and pharmaceutical drugs, was presented by Shahsavani et al. (2016), where two structurally related binuclear Pt (II) complexes were used. From the revised literature and authors recent publications it can be concluded that HSA and polyphenol extracts have relatively high binding properties. But another question was erased by the authors if the volatiles play also the same role of antioxidants as polyphenols and possess the capacity to bind the proteins. The volatile substances (Chmiel, Kupska, Wardencki, & Namieśnik, 2017; Dymerski et al., 2015, 2016) have been determined in berries. Volatile compounds play a key role in the formation of the well-

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recognized and widely appreciated berry aroma. Studies on the isolation and identification of volatile compounds in raspberry fruit (*Rubus idaeus* L.) are reviewed with a focus on aroma-related compounds (Aprea, Biasioli, & Gasperi, 2015). The essential oil of juniper berries (*Juniperus communis* L., Cupressaceae) is traditionally used for medicinal and flavoring purposes (Höferl et al., 2014). Cytotoxic and antimicrobial potential of the berries and leaves of *Juniperus excelsa* were investigated (Topçu et al., 2005). Most of scientific papers concern the qualitative or semi-quantitative analysis of aroma-active terpenes in liquid food matrices. The antibacterial activity and antioxidant effect of the compounds α-terpineol, linalool, eucalyptol and α-pinene obtained from essential oils (EOs), against pathogenic and spoilage forming bacteria were determined (Zengin & Baysal, 2014). Six conjugate agents of the moribund antibiotic sulfamethoxazole (SMZ) joined to 6 individual monoterpenes (Swain, Paidesetty, & Padhy, 2017). Puerarin is a widely used compound in Chinese traditional medicine and exhibits many pharmacological activities, and binding of puerarin to HSA was investigated (He et al., 2008). As can be seen the side of interaction of terpenes with HSA is not investigated widely, therefore in this study polyphenols, volatiles and their synergism in berries was partially characterized.

2. Materials & methods

2.1. Materials

Three types of fruits, Blueberry (*Vaccinium 4 corymbosum*), Cranberry (*Vaccinium macrocarpon*) and Cape gooseberry (*Physalis peruviana*) were used. All berries were from West Pomerania Province, harvested in late June 2016, in Poland. The berries were washed and homogenized before each analysis. Five replicates were done for the analysis of each type of fruit, and triplicate analysis for each standard was carried out. The berries were homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1min. A weighed portion (50–100 g) was then lyophilized for 48 h (Virtis model 10-324), and the dry weight was determined.

2.2. Chemicals

6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), human serum albumin (HSA), Tris, tris(hydroxymethy1) aminomethane, Folin–Ciocalteu reagent, 2, 2′- Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$), lanthanum (III) chloride heptahydrate; $CuCl_2 \times 2H_2O$; and 2,9-dimethyl-1,10-phenanthroline (neocuproine) were used. Nineteen analytical terpene standards were used to confirm the identity of selected compounds (Sigma–Aldrich, St. Louis, MO, USA). A high purity deionized water from MilliQ A10 Gradient/Elix System (Millipore, Bedford, MA, USA) and GC grade sodium chloride (Sigma–Aldrich, St. Louis, MO, USA) were used throughout the experiment.

2.3. Determination of volatile substances

Volatile compounds from the fruit samples were extracted using headspace solid-phase microextraction (HS-SPME). Prior to extraction process the samples were incubated at 50 °C for 10 min and agitated at 700 rpm. Extraction in the same temperature was carried out for 30 min using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre of 50/30 μm thickness and 2 cm length (Sigma–Aldrich). The GC × GC system was an Agilent 6890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a liquid nitrogen-based dual stage cryogenic modulator and a split/splitless injector, coupled with Pegasus IV time-of-flight mass spectrometer (LECO Corp., St. Joseph, MI, USA). Positive identification of nineteen analytes (β-pinene, camphene, β-myrcene, α-pinene, α-phellandrene, terpinolene, *p*-cymene, eucalyptol, (R)-(+)-limonene, α - ocimene, γ-terpinene, fenchone, (E)-linalool oxide, (R)-(−)-linalool, (−)-camphor, (−)-terpinen-4-ol, α-terpineol, β-cyclocitral, α-ionone) was confirmed by the comparison of retention times (in ^{1}D and ^{2}D) with authentic standards (Dymerski et al., 2016).

2.4. Determination of bioactive compounds and antioxidant activities

The lyophilized samples of berries (1 g) were extracted with ethanol/water (20%:80%; 50%:50%; 100%/0%) at 40° C during 4 h. Ultrasound-assisted extraction was carried out with Ultrasonic Cleaner Delta DC-80H, operating frequency: 40 kHz, output power: 80 W, heater: 45 W. The extracts were filtered through the Buchner funnel. These extracts were submitted for determination of bioactive compounds. The polyphenols were determined by Folin-Ciocalteu method with measurement at 750 nm with spectrophotometer (Hewlett-Packard, model 8452A, Rockville, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW (Singleton, Orthofer, & Lamuela-Raventos, 1999).

The total antioxidant capacity (TAC) was determined by the following assays:

1. Cupric reducing antioxidant capacity (CUPRAC): To the mixture of 1 mL of copper (II)-neocuproine and NH_4Ac buffer solution, acidified and nonacidified ethanol extracts of berry (or standard) solution (x, in mL) andH_2O [(1.1−x) mL] were added to make a final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank (Apak, Guclu, Ozyurek, & Karademir, 2004).
2. 2, 2′- Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS): $ABTS^{\bullet+}$ was generated by the interaction of ABTS (7 mM) and $K_2S_2O_8$ (2.45 mM). This solution was diluted with ethanol until the absorbance in the samples reached 0.7 at 734 nm (Re et al., 1999).

2.5. Fluorometric measurements

Fluorometric measurements were used for the evaluation of binding properties of berries extracts to human serum albumin. Three dimensional (3D-FL) fluorescence measurements were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Japan. The concentrations of berries extracts were ranged from 0 to 1.5 mg/mL, and the total accumulated volume of berries extracts was no greater than 150 μL. The emission wavelength was recorded between 200 and 795 nm for three-dimensional fluorescence spectra. All solutions for protein interactions were prepared in 0.05 mol/L Tris-HCl buffer (pH 7.4), containing 0.1 mol/L NaCl (Ku et al., 2017).

2.6. Molecular docking

The potential interaction of volatile compounds from the different berries with HSA protein was investigated using LigandFit docking program integrated with Discovery Studio 2.5. The ligand structures for volatile substances from the berries (α-pinene, γ-terpinene, terpinolene, (R)-(−)-linalool, (−)-terpinen-4-ol and α-terpineol) were download from Pubchem database. The crystal structure of the HSA was obtained from the RCSB Protein Data Bank (PDB code: 1H9Z). The ligands were prepared using Ligand

preparation module from DS 2.5 package (Shafreen & Pandian, 2013). HSA was prepared by removing the water molecules, adding hydrogen atoms and by assigning partial charges based on the CHARMm force field. All the parameters were set to their default and at the binding site region a spherical cut-off of 8 Å was adjusted for non-bonding interaction. Further the docking protocol was applied to the processed protein and ligand structures. Top 10 best poses were used for evaluation with the scoring algorithm.

2.7. Statistical analysis

To verify the statistical significance, means ± SD of five independent measurements were calculated. One-way analysis on variance (ANOVA) for statistical evaluation of results group's means. P values of <0.05 were considered to be significant.

3. Results and discussion

3.1. Binding properties of terpenes in berries

The concentrations of terpenes in berries, their bioactivity and binding properties are presented in Table 1. The chemical classes that contribute aroma profile of these fruits have a great significance not only for organoleptic profile differences, but also for their bioactive properties. As it was above-mentioned the highest amount of terpenes may suggest that Cape gooseberry can have the highest pro-health value from all three selected fruits (Table 1). It is a well known that terpene compounds have an antioxidant activity. In recent studies (Dymerski et al., 2015, 2016) it was shown that the highest amount of alcohol and ester compounds (85%) was estimated in blueberry; carboxylic acids, ketones and aldehydes were found in cranberry (62%) and terpenes in Cape gooseberry (8%). The method of determination of the terpenes was successfully applied to determine monoterpenes in 27 berry samples of different varieties and 4 berry products. Monoterpenes content is a reliable indicator of fruit maturity and origin (Chmiel et al., 2017). The values of antioxidant activities of terpenes by two assays ABTS and CUPRAC were the highest in γ-terpinene, following by terpinolene, α-terpineol, and with the weakest one (R)-(−)-linalool. The antioxidant activities were correlated with the concentration of polyphenols (Table 1). The presented results are in line with the reviewed data: antioxidant activity of essential oils (EOs) components (terpenes) measured by the FRAP (mmol Trolox mL^{-1}) were the following: eucalyptol (0.58 ± 0.22), α-terpineol (1.23 ± 0.56), linalool (1.20 ± 0.27) and with DPPH (IC50 $\mu L \cdot mL^{-1}$) showed for α-terpineol 433.97 ± 13.69 and for linalool 325.05 ± 20.19 (Zengin & Baysal, 2014). In the present study the values of ABTS and CUPRAC in α-terpineol was 3 times higher than for linalool (Table 1). In basil, The strongest antimicrobial activity of sweet basil was attributed to the eugenol (19%) and linalool (54%) content and a synergistic effect was observed. The juniper berry oil is largely comprised of monoterpene hydrocarbons such as α-pinene (51.4%), myrcene (8.3%), sabinene (5.8%), limonene (5.1%) and β-pinene (5.0%). The antioxidant capacity of the essential oil was evaluated *in vitro* by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzothiazoline-6 sulfonic acid radical cation scavenging assays. The antioxidant activity of the oil attributable to electron transfer made juniper berry essential oil a strong antioxidant; whereas the antioxidant activity attributable to hydrogen atom transfer was lower ((Höferl et al., 2014). The main components in the berries of *Juniperus excelsa* (Topçu et al., 2005), accounting for 56.1% of the oil, were determined as α-pinene (34.0%), cedrol (12.3%), L-verbenol (5.4%), and D-verbenol (4.4%) while in the leaves of *J. excelsa*, accounting for 63.2% of the oil, were found to be α-pinene (29.7%), cedrol (25.3%), α-muurolene (4.4%), and 3-carene (3.8%). GC and GC/MS analyses resulted in the detection of 42 components representing approximately 96.50–99.57% of the essential oil of *Juniperus phoenicea* L. ripe and unripe berries. Major components of the oils were α-pinene (58.61–77.39%), camphene (0.67–9.31%), δ-3-carene (0–10.01%) and trans-verbenol (0–5.24%). The fluorescence studies (Table 1) showed that the fluorescence intensity of measured peaks a and b in HSA during interaction with monoterpenes decreased. The highest decrease was with γ-terpinene and the lowest one appeared with (R)-(−)-linalool.

3.2. Docking studies

Molecular docking is a virtual environment to underpin the details of the ligand-protein interaction complex. According to fluorescence experiments, all the volatile compounds from the berries were able to bind HSA protein. The mode of interaction between the volatiles and the active residues of HSA was investigated using molecular docking studies. Binding site prediction of HSA revealed a hydrophobic binding pocket with PHE134, LEU135, TYR138, LEU139, ILE142, LEU154, ALA158, TYR161 and PHE165 as active site residues. All the six volatiles showed interaction with ALA158 of HSA (Table 2) necessary for maintaining the binding affinity of the ligand with the receptor. Among the six, the highest scoring compound was identified as γ-terpinene with a dock score of 165.52 and binding energy of −86.34 kcal/mol (Fig. 1). Terpinolene is the second highest binding ligand with the receptor which showed hydrogen bonding interaction with TYR138. The volatiles identified from the berries have aromatic structure except linalool from the extracts. The presence of the aromatic ring in the drug often plays a notable role in their recognition by protein-targets. All

Table 1
Antioxidant and binding properties of some monoterpenes in berries.

Indices	α-pinene	γ-terpinene	(−)-terpinen-4-ol	(R)-(−)-linalool	α-terpineol	Terpinolene
Conc. GB, μg/kg FW	101.8 ± 8.7^{b}	97.1 ± 6.6^{b}	51.5 ± 4.3^{c}	75.5 ± 4.3^{bc}	3.9 ± 0.6^{d}	183.5 ± 11.7^{a}
Conc. CB, μg/kg FW	4.9 ± 0.4^{b}	3.8 ± 0.3^{b}	0.96 ± 0.3^{c}	15.1 ± 2.2^{a}	0.96 ± 0.3^{c}	7.5 ± 0.6^{ab}
Conc. BB, μg/kg FW	18.1 ± 5.6^{b}	19.8 ± 7.6^{ab}	0.94 ± 0.1^{c}	23.3 ± 8.7^{a}	0.94 ± 0.1^{c}	1.9 ± 0.4^{c}
Polyph,mgGAE/g DW	5.72 ± 0.7^{b}	9.05 ± 0.7^{a}	6.61 ± 0.6^{ab}	2.45 ± 0.3^{c}	6.79 ± 0.7^{ab}	6.82 ± 0.6^{ab}
ABTS, μMTE/g DW	39.81 ± 4.4^{b}	62.71 ± 5.2^{a}	46.61 ± 4.6^{ab}	15.31 ± 1.5^{c}	46.22 ± 5.3^{ab}	47.14 ± 4.8^{ab}
CUPRAC, μMTE/g DW	22.14 ± 2.3^{b}	35.31 ± 2.5^{a}	24.28 ± 2.2^{ab}	8.49 ± 0.7^{c}	25.18 ± 2.4^{ab}	26.56 ± 2.7^{ab}
FI (peak a), A.U.	630.02 ± 13.5^{b}	588.05 ± 12.6^{c}	616.61 ± 9.8^{bc}	644.49 ± 17.5^{a}	610.48 ± 9.6^{bc}	604.52 ± 14.4^{bc}
FI (peak b), A.U.	737.14 ± 12.5^{ab}	695.52 ± 8.5^{c}	724.18 ± 6.7^{b}	765.44 ± 7.6^{a}	729.46 ± 6.9^{ab}	735.39 ± 9.6^{ab}
Binding to HSA,%	20.42 ± 2.5^{c}	31.42 ± 2.1^{a}	23.86 ± 2.5^{b}	14.72 ± 1.2^{d}	24.08 ± 2.3^{b}	24.18 ± 2.6^{b}

Values are means ± SD of 5 measurements; Means within a raw with the different superscripts or without superscripts are statistically different ($p < 0.05$; Student's t-test). Abbreviations: Conc., concentration; Polyph, polyphenols; GAE, gallic acid equivalent; ABTS, 2, 2′-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; CUPRAC, Cupric reducing antioxidant capacity; TE, trolox equivalent; FI, fluorescence intensity; A. U., arbitral units; per g dry weight (DW); per kg fresh weight (FW); HSA, human serum albumin; FI of HSA+100% EtOH according to peak a is equal to 708.76 ± 10.2; peak b is equal to 812.41 ± 12.3. All presented extracts are with 100% ethanol.

the volatiles showed hydrophobic interactions with HSA (Fig. 2) such as pi−sigma and pi−Alkyl interactions which is an important factor for rational drug designing (Babine & Bender, 1997; Scrutton and Raine, 1996). Thus γ-terpinene shown with good binding efficiency with HSA can easily be diffused into the blood and retained for longer duration with proper drug action. Therefore from the present investigation it is hypothesized that γ-terpinene being the major volatile component from berries can be used as drug candidate as well as food supplement for various therapeutic approaches. These results are in direct agreement with the fluorescence measurements. The binding properties found by the decrease of fluorescence intensity were slightly different from the ones calculated by the docking (Tables 1 and 2). These results as well were confirmed by others, where using molecular docking against 5 cited bacterial dihydropteroate synthases (DHPSs), effective docking scores of 6 monoterpenes in the specified decreasing order (kcal/mol): −9.72 (eugenol against *Bacillus anthracis*), −9.61 (eugenol against *Streptococcuspneumoniae*), −9. 42 (safrole, against *B. anthracis*), −9.39 (thymol, against *Mycobacterium tuberculosis*), −9.34 (myristicin, against *S. pneumoniae*) and −9.29 (thymol, against *B. anthracis*) were explained. The lowest docking score of sulfamethoxazole SMZ was −8.46 kcal/mol against DHPS (Swain et al., 2017). The lowest docking score was -43.84 kcal/mol for the investigated samples (Table 2).

3.3. Binding properties of phenolics in berries

Three types of berries were extracted with 20, 50 and 100% of ethanol (Table 3). From the presented results it can be seen that the polyphenol extract with 100% ethanol from blueberries was the strongest one with all studied parameters (Table 3, Fig. 3 A). The total polyphenols and antioxidant activities decreased with increasing water content in organic solvent. Therefore the highest amount of polyphenols was determined in all extracts with 100% ethanol in comparison with 20% and 50% (Table 3). The water extract may either contain more nonphenolic compounds or possess phenolic compounds that contain a smaller number of active groups than the other solvents. The polyphenol extracts were consistent with their antioxidant activities. It is clear that 100% ethanol extract gave the highest antioxidant capacity in all *in vitro* assays. Our results are in line with Do et al. (2014), where was shown that the extracts of *Limnophila aromatica* exhibited the highest antioxidant activity and total phenolic content at 100% ethanol.

Antioxidant activities were determined by two different test systems, DPPH and ABTS radical scavenging activities. These results are line with Medini et al. (2011) where was shown that in both systems ripe berries exhibited better activity potential than the unripe ones. The results of binding properties of polyphenols in berries (Table 3) were in line with Thongkaew et al. (2014), where the effects of the interactions between polyphenolic substances (catechin/tannic acid) or plant extracts (grape seed/hibiscus extract), using each in three different concentrations and preheated

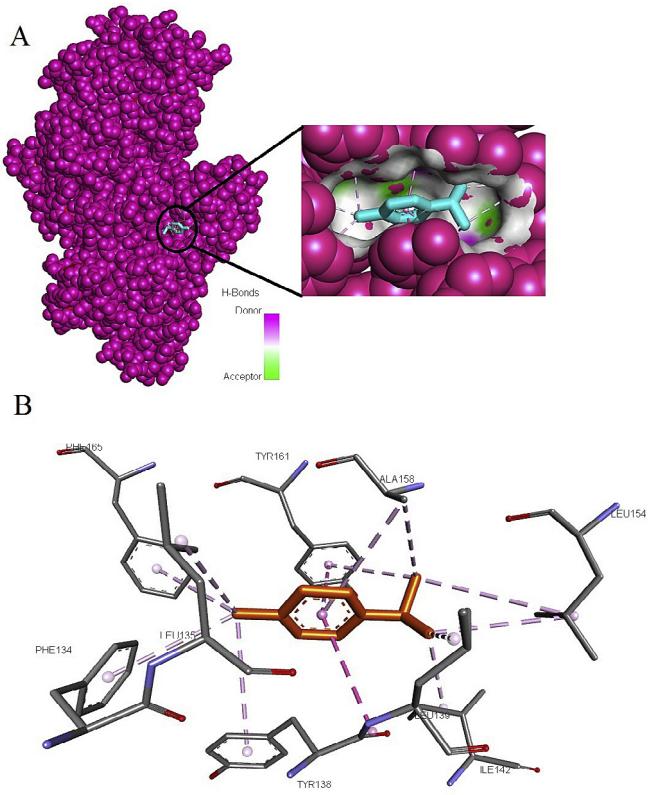

Fig. 1. The docking results of human serum albumin (HSA) with γ-terpinene. (A) Surface diagram of HSA (pink-spheres) and ligand (cyan-stick) is represented. The inset view represents the hydrophobic binding pocket predicted with high binding affinity. B) Detailed interaction view of the γ-terpinene with the active site residue of HSA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

whey protein isolates were discussed. Similar complexes were obtained with interaction of naringin with β-lactoglobulin (β-Lg). To allow the enrichment of clear beverages with these important health promoters, as naringin, native and preheated β -Lg based nanovehicles were used (Shpigelman, Shoham, Israeli-Lev, & Livney, 2014). Binding affinity of epigallocatechin-3-gallate (EGCG) to bovine α-lactalbumin (ALA), as an important Ca-binding protein of milk, determined by fluorescence quenching analysis, was in the range described for complexes of EGCG and other dietary proteins, and lower than affinity of some phenolic compounds to ALA. The docking analysis showed that EGCG binds in the hydrophobic pocket at the entrance of cleft between α-helical and β-sheet rich domains and includes residues of aromatic cluster II. ALA, being of low cost and widely available protein, can serve as suitable delivery system for EGCG, as well as for food fortification with this bioactive catechin (Al-Hanish et al., 2016). The results reveal (Li et al., 2016) that caffeic acid phenethyl ester (CAPE) exhibits a distinctive

Table 2
Molecular docking results of the six volatiles compounds showing interaction with the HSA.

S.No	Ligand name	Pubchem ID	DockScore	Binding Energy (kcal/mol)	Interacting residues
1.	α-pinene	6654	138.21	−53.71	TYR138; LEU139; ILE142; ALA158; TYR161
2.	γ-terpinene	7461	165. 52	−86.34	PHE134; LEU135; TYR138; LEU139; ILE142; LEU154; ALA158; TYR161; PHE165
3.	Terpinolene	11463	153. 57	−63.52	LEU135; ILE142; LEU154; PHE157; ALA158; TYR161
4.	(R)-(−)-linalool	443158	137.31	−43.84	TYR138; LEU139; ALA158; TYR161
5.	(−)-terpinen-4-ol	5325830	151. 64	−62.54	PHE134; LEU135; TYR138; LEU139; ILE142; LEU154; PHE157; ALA158
6.	α-terpineol	17100	153.73	−63.41	PHE134; LEU135; TYR138; ALA158; TYR161; PHE165

Abbreviations: HSA, human serum albumin.

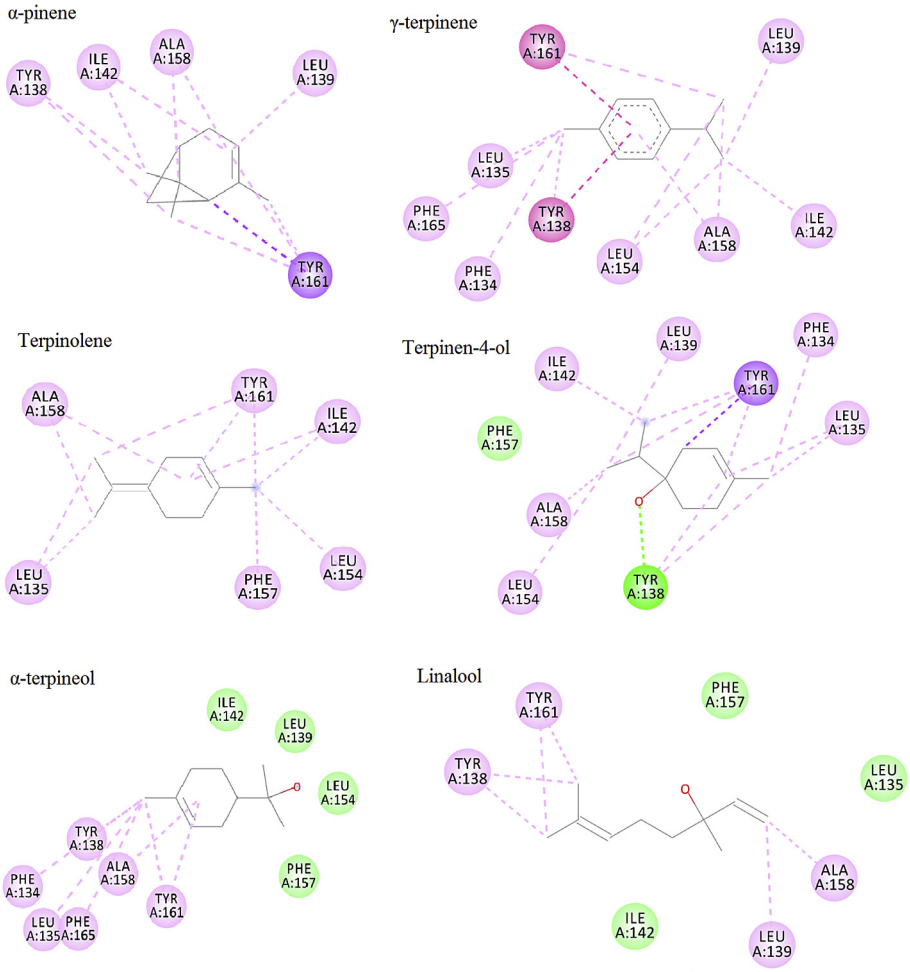

Fig. 2. The detailed 2D view showing the interaction between volatile compounds and the active residues present in the hydrophobic binding pocket of HSA (Dark Pink circles represent Pi-amide interaction, Light pink shows Pi-alkyl and alkyl interaction and Green circles denotes hydrogen bonding). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 3
Bioactive compounds in ethanol-water extracts per g dry weight (DW) of gooseberries (*Physalis peruviana*), cranberries (*Vaccinium macrocarpon*) and blueberries (*Vaccinium corymbosum*).

Extracts	Polyph, mgGAE	ABTS, μMTE	CUPRAC, μMTE	FI (peak a), A.U.	FI (peak b), A.U.	Binding, %
Gooseberry,100%EtOH	14.42 ± 1.87^{cd}	64.87 ± 4.47^{d}	35.93 ± 2.41^{d}	633.56 ± 22.37^{ab}	727.70 ± 21.25^{b}	$19.83 \pm 1.17c^{d}$
Gooseberry,50%EtOH	12.89 ± 1.33^{cd}	60.89 ± 5.63^{d}	33.14 ± 3.87^{d}	674.26 ± 19.34^{a}	787.52 ± 23.65^{ab}	18.65 ± 1.11^{d}
Gooseberry,20%EtOH	9.37 ± 0.84^{d}	43.95 ± 4.58^{e}	16.09 ± 1.22^{e}	714.41 ± 22.21^{a}	833.04 ± 26.23^{a}	17.12 ± 1.78^{d}
Cranberry, 100%EtOH	27.65 ± 2.24^{bc}	227.89 ± 18.54^{bc}	127.31 ± 11.09^{ab}	604.31 ± 18.12^{b}	708.46 ± 18.13^{c}	26.43 ± 2.15^{bc}
Cranberry, 50%EtOH	24.15 ± 2.58^{bc}	214.65 ± 13.67^{bc}	119.39 ± 11.45^{b}	642.88 ± 24.59^{ab}	769.61 ± 26.69^{ab}	24.93 ± 2.68^{bc}
Cranberry, 20%EtOH	20.42 ± 2.08^{c}	199.33 ± 12.03^{c}	101.11 ± 10.08^{c}	698.84 ± 27.79^{a}	814.25 ± 21.69^{a}	21.18 ± 2.01^{c}
Blueberry, 100%EtOH	50.14 ± 5.07^{a}	348.15 ± 0.05^{a}	204.41 ± 17.05^{a}	523.29 ± 19.06^{c}	677.61 ± 22.01^{c}	41.73 ± 3.06^{a}
Blueberry, 50%EtOH	45.63 ± 4.12^{ab}	326.76 ± 16.04^{ab}	191.83 ± 18.12^{a}	556.69 ± 18.07^{c}	741.12 ± 20.01^{b}	39.73 ± 3.21^{a}
Blueberry, 20%EtOH	40.21 ± 3.37^{b}	300.16 ± 14.83^{b}	161.19 ± 9.54^{ab}	600.18 ± 26.56^{b}	801.24 ± 26.56^{a}	35.09 ± 3.87^{b}

Values are means ± SD of 5 measurements; Means within a column with the different superscripts or without superscripts are statistically different ($p < 0.05$; Student's t-test). Abbreviations: Polyph, polyphenols; GAE, gallic acid equivalent; ABTS, 2, 2′-Azino -bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; FRAP, Ferric-reducing/antioxidant power; CUPRAC, Cupric reducing antioxidant capacity; TE, trolox equivalent; FI, fluorescence intensity; A.U., arbitral units; HSA, human serum albumin; FI of HSA+100% EtOH according to peak a is equal to 705.76 ± 19.2, peak b is equal to 805.34 ± 12.5. FI of HSA+50% EtOH according to peak a is equal to 753.98 ± 11.5, peak b is equal to 856.74 ± 14.8. FI of HSA+20% EtOH according to peak a is equal to 791.17 ± 9.5, peak b is equal to 899.81 ± 12.9.

binding interaction with HSA comparing with other propolis components. The docking and drugs (warfarin and ibupro-fen) competitive results show that CAPE is located in the subdomain IIA (Sudlow's site I, FA7) of HSA, and Gln196 and Lys199 contribute to the hydrogen bonds. Competitive experiments showed a displacement of warfarin by puerarin, which revealed that the binding site was located at the drug site I. Puerarin was about 2.22 nm far from the tryptophan according to the observed fluorescence resonance energy transfer between HSA and puerarin. Molecular docking suggested that the hydrophobic residues such as tyrosine (Tyr) 150, Tyr 148, Tyr 149 and polar residues such as lysine (Lys) 199, Lys 195, arginine 257 and histidine 242 played an important role in the binding reaction (He et al., 2008). The binding process of the tyrosine-kinase inhibitor nilotinib to HSA was mainly

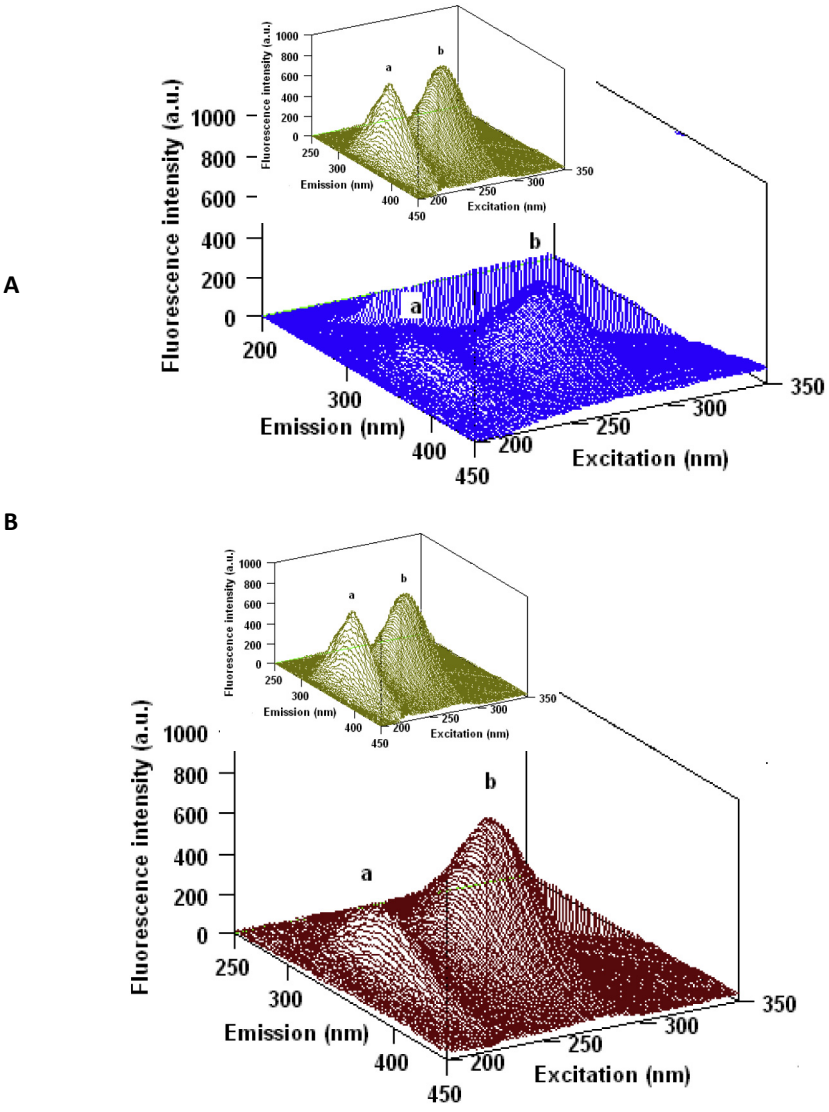

Fig. 3. Synergistic effect of volatiles and polyphenols on binding properties with HSA. Changes in the fluorescence intensity of peaks a and b in HSA after interaction: A, with polyphenols of blueberries, insert, HSA in 100% ethanol; B, with γ-terpinene, insert, HSA in 100% ethanol. The values of the peaks in the investigated samples and their binding properties are given in Tables 1 and 3.

driven by hydrogen bonds and van der Waals forces. Molecular docking simulation was also used to establish a molecular binding model, and findings were consistent with those of displacement and the saturation-transfer difference NMR spectroscopy (1H STD-NMR) experiments (Yan et al., 2016). Tea polyphenol (TP) made egg white (ovalbumin (OVA) and lysozyme (LYZ)) easier for digestion in the pepsin solution at pH 1.2 and inhibited OVA/LYZ digestion in pancreatin solution at pH 7.5. The conformational and second structural change of proteins (substrate) might be a reason for promoting and inhibiting digestion of OVA/LYZ affected by TP (Shen et al., 2014). The experimental results proved that the fluorescence of HSA was quenched by honokiol (HK) or magnolol (MG) through a static quenching procedure. The binding process was a spontaneous molecular interaction procedure, in which the hydrophobic interaction played a major role in the formation of the HK–HSA complex, whereas, the binding interaction between MG and HSA might involve the hydrophobic interaction strongly and electrostatic interaction (Cheng, 2012). The fluorescence emission of HSA was quenched by rosmarinic acid (RA) through a combined static and dynamic quenching mechanism, but the static quenching was the major constituent (Peng et al., 2016). Fluorescence experiments suggested that RA was bound to HSA with moderately strong binding affinity through hydrophobic interaction. The probable binding location of RA was located near site I of HSA. Ponceau 4R (P4R) and bovine serum albumin (BSA) may interact changing food properties. Fluorescence data pointed to the formation of a complex where P4R was bound on site I or II of BSA, with a stoichiometry around one (Lelis et al., 2017). Juliani, Koroch, and Simon (2009) showed the importance of the hydroxyl group (–OH) of phenols by the higher antimicrobial and antioxidant activities of eugenol in relation to methyleugenol (–O-Me). Interestingly, phenolic monoterpenes and phenylpropanoids (typically showing strong antimicrobial activities) in combination with other components were found to increase the bioactivities of these mixtures (Wang and Arntfield, 2016). Such synergism appeared when the binding properties of the whole berries extracts were determined (Tables 1 and 3, Fig. 3A, B). The binding properties of blueberries increased by the participation of monoterpenes and polyphenols (Table 3, Fig. 3A). Most of the studies have focused on the interaction of phenolic monoterpenes (thymol, carvacrol) and phenylpropanoids

(eugenol) with other groups of components, particularly with other phenols, phenylpropanoids and monoterpenes alcohols, while monoterpenes and sesquiterpenes hydrocarbons were used to a lesser extent (Zengin & Baysal, 2014). This is in the line with the present results. The combination of phenolics with monoterpene alcohols produced synergistic effects on several microorganisms, in particular, the combination of phenolics (thymol with carvacrol, and both components with eugenol) were synergistically active against *E. coli* strains, though other reports have observed additive and antagonism effects (Gallucci et al., 2009) shown in Table 3 about the antioxidant activities of the whole berries extracts.

4. Conclusions

Volatile compounds play a key role in the aroma of berries, but the nutritional role of these compounds is still controversial. Therefore the interaction with HSA of main volatile monoterpenes in berries was determined by fluorescence and molecular docking. The docking results of terpene group, which was found in the recent studies as the main group of compounds present in volatile fraction of berries, showed the highest binding properties of γ-terpinene. Binding parameters showed that the berries extracts bind to HSA with different binding affinities which are related to their antioxidant properties and the changes in the secondary structure. Therefore from the present investigation it is hypothesized that γ-terpinene being the major volatile component from berries can be used as drug candidate as well as food supplement for various therapeutic approaches. These results indicate that berries can be used in dietary applications with a potential to reduce oxidative stress.

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