Human serum interactions with phenolic and aroma substances of Kaffir (Citrus hystrix) and Key lime (Citrus aurantifolia) juices

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\textbf{A R T I C L E I N F O}

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Binding
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\textbf{A B S T R A C T}

To understand the therapeutic application of polyphenols extracted from Kaffir (PolKa) and Key (PolKey) limes different analytical methods were applied. Based on quantitative analysis by two dimensional gas chromatography (GC×GC) and time of flight mass spectrometry (TOFMS) it can be observed that the biggest differences in the contents of selected terpenes of Kaffir and Key limes occur in chemical compounds such as limonene, citral and terpinen-4-ol. Limonene concentration is almost 5 times higher in the volatile fraction of Key lime than in Kaffir lime. In the case of citral, the difference in concentration of this compound in Kaffir is 20 µg/g lower than in Key lime. Higher concentration of terpinen-4-ol was noted in Kaffir lime samples and the content was almost 23 times higher. Terpinen-4-ol is the major chemical compound of volatile fraction of Kaffir lime. Among the determined terpenes, potential markers of aroma were selected: terpinen-4-ol and citral for characterization of Kaffir and Key limes. Antioxidant assays revealed the highest bioactivity of Kaffir lime. Fluorescence studies between the interaction of polyphenols with human serum albumin (HSA) showed relatively high binding abilities in comparison with some antiplatelet drugs. The docking results showed that the hydrophobic residues are responsible for the interaction with the phyto-constituents. Citral is the best scored ADMET descriptor. The antioxidant strong affinity to HSA and synergism in bioactivity are the main indices in health application of citrus fruits.

1. Introduction

Juices or real pulps of some commercially grown citrus fruit (Rutaceae), grapefruit (Citrus paradisi), lemon (Citrus limon), lime (Citrus x aurantifolia) and sweet orange (Citrus sinensis) were widely studied, where the phenolics and volatiles were the main antioxidant compounds found in all fruits [1,2]. Four citrus species (C. sinensis, cv. Pera and Lima; C. latifolia Tanaka cv. Tahiti; C. limettoides Tanaka cv. Sweet lime and C. reticulate, cv. Ponkan) were characterized in relation to contents of ascorbic acid, total polyphenols and antioxidant capacity of pulps. The antioxidant capacity of citrus fruit was correlated both to vitamin C and phenolics. Aside from citrus pulps, the peels are also good sources of bioactive compounds and minerals, and can be explored for their health promoting values in food products [3]. There are a number of reports including Kaffir (Citrus hystrix) and Key (Citrus aurantifolia) limes. Citrus aurantifolia is mainly used in daily consumption and in juice production, based on antibacterial, anticancer, antidiabetic, antifungal, antihypertensive, anti-inflammatory, and antilipidemic properties. Antioxidants moreover can protect heart, liver, bone, and prevent urinary diseases. The secondary metabolites are alkaloids, carotenoids, coumarins, essential oils, flavonoids, phenolic acids, and triterpenoids. The other important constituents are apigenin, hesperetin, kaempferol, limonoids, quercetin, naringenin, nobiletin, and rutin, all of these contribute to its remedial properties [4]. The volatiles of limes have a wide application as essential oil microparticles. The use of prebiotic biopolymers can be a good option to add value to encapsulated products, thus promoting health benefits [5]. A combination of bioactive compounds makes all citrus fruits a useful source of everyday diet from a number of diseases [6–8]. As it was mentioned above there are some reports showing the composition of different kinds of limes [1,3,4], but it is a lack of research dealing with bioactive compounds and their interaction with serum proteins. The interactions between polyphenols, especially flavonoids and plasma proteins, have attracted great interest [9–13]. Few papers, however, have focused on...
the structure-affinity relationship of polyphenols and volatiles, on their affinities for plasma proteins, especially on human serum albumin with plant bioactive compounds [14,15] and with citrus fruits particularly [16,17]. The aim of this study was to determine the bioactive and aroma substances and their binding and antioxidant properties, using GC × GC-TOFMS, 2D- and 3D-fluorescence, spectroscopic antioxidant assays measurements and molecular docking.

2. Materials and methods

2.1. Materials

The following chemical compounds: Aromadendrene, Camphene, Citral, Citronellal, Limonene, Linalool, Myrcene, Nerol, Terpinen-4-ol, trans-Geraniol, α-Pinene, α-Terpineol, β-Pinene, γ-Terpine, were used as standards (Sigma-Aldrich, St. Louis, MO, USA). Methanol (Avantor Performance Materials Poland S.A) was used to prepare the calibration solution mixtures. The mixture of n-alkanes from C8 to C20 (Sigma-Aldrich, St. Louis, MO, USA) was utilized for calculation of retention indexes. During the research deionized water of high purity from MilliQ A10 Gradient/ Elix System (Millipore, Bedford, MA, USA) was added to samples. Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid); Folin–Ciocalteu reagent (FCR); Tris, tris (hydroxymethyl)aminomethane; lanthanum (III) chloride heptahydrate; CuCl2 × 2H2O; and 2,9-dimethyl-1,10-phenanthroline (neocuproine), 1,1-diphenyl-2-picrylhydrazyl (DPPH), were obtained from Sigma Chemical Co., St. Louis, MO, USA. All reagents were of analytical grade.

2.2. Sample preparation

The studies were performed on juices of two lime species: Kaffir and Key limes. Kaffir lime (Citrus hystrix, Citrus hystrix) was bought on the floating market in Taling Chan in Bangkok. Kaffir lime samples were transported to Poland in travel fridge and the temperature was between 10 °C and 15 °C. Key lime samples (Citrus aurantifolia) were purchased in local market in Gdansk, where they had been imported from Brazil. During the research for each sample, three repetitions were performed. Before analysis, the fruits were washed and rinsed with distilled water. Next step was to squeeze the juice from the fruit. Samples were prepared in the proportion of 5.0 + 0.1 g of fruit pulp and 1.0 mL of deionized water. Mixtures were then transferred into 20-mL vials. All samples were sealed with caps with 20 mm thick PTFE/silicone membrane [18].

2.3. Methods

2.3.1. Two-dimensional gas chromatography (GC × GC) and time of flight mass spectrometry (TOFMS)

HS-SMPE (Headspace Solidphase Microextraction) extraction was done by the GC × GC-TOFMS procedure. A Gerstel autosampler (MPS autosampler, Gerstel, Mühlheim, Germany) with agitator and SPME fiber conditioning station was used to isolate and to enrich the analytes from citrus samples. Before the extraction, the samples were kept at 40 °C for 5 min. Extraction was carried out at 40 °C for 35 min using a DVB/CAR/PDMS SPME fiber of 50/30-μm thickness and 2-cm length (Sigma-Aldrich, St. Louis, MO, USA). After the extraction, the fiber was removed from the vial and transferred to the injector of a gas chromatograph for thermal desorption of the analytes at 250 °C for 5 min. The GC × GC apparatus Agilent 7890 A (Agilent Technologies, Palo Alto, CA, USA) equipped with liquid nitrogen-based quad-jet cryogenic modulator and an injector in split/splitless mode, coupled with Pegasus 4D time-of-flight mass spectrometer (LECO Corp., St. Joseph, MI, USA), were used for the analysis. Two different capillary columns were used for the analysis. The first non-polar column was Equity-1 (30 m × 0.25 mm i.d. × 0.25 μm film thickness) from Supelco (Bellefonte, PA, USA). The second column with polar stationary phase SolGel-Wax (2 m × 0.1 mm i.d. × 0.1 μm film thickness) was purchased from SGF Analytical Science (Austin, TX, USA). The chromatographic separation was performed using the following temperature program for the primary oven: Initial temperature 40 °C, kept for 3.5 min, ramped at 5 °C/min to 250 °C, and held for 5 min. The secondary oven temperature was programmed from 45 °C, kept for 3.5 min, ramped at 5 °C/min to 255 °C, and held for 5.83 min. The carrier gas was hydrogen (N6.0 class) at a constant flow rate at 1.0 mL/min. Temperature of the MS transfer line and the MS source was 250 °C. The modulation time was 4 s. The mass spectra data acquisition rate was 125 spectra/s. The data were collected over a mass range of 40–400 m/z. The voltage of detector was 1600 V. Analysis of the data obtained after the chromatographic analysis using GC × GC-TOFMS system was done using the algorithm for peak deconvolution implemented in the ChromaTOF software (LECO Corp., version 4.24). Analytes were tentatively identified by comparison of experimental spectra with the NIST 2011 mass spectral library. Analytes were also identified by comparing calculated linear temperature-programmed retention indices (LTPRIs) with literature values [18].

2.3.2. Determination of bioactive compounds and antioxidant activities

Polyphenols were extracted from lyophilized samples with water (concentration 20 mg/mL) during 1 h in a cooled ultrasonic bath. 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 8452 A, Rockville, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW [19].

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

- Cupric reducing antioxidant capacity (CUPRAC): To the mixture of 1 mL of copper (II)-neocuproine and NH4Ac buffer solution, acidified and non-acidified ethanol extracts of berry (or standard) solution (x, in mL) and H2O [(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 [20].
  1. 1-Diphenyl-2-picrylhydrazyl method (DPHH) solution (3.9 mL, 25 mg/L) in methanol was mixed with the samples extracts (0.1 mL). The reaction progress was monitored at 515 nm until the absorbance was stable. The scavenging rate on DPPH radicals was calculated [21].

2.3.3. Fluorometric measurements and binding properties

Fluorometric measurements were used for the evaluation of binding properties of citrus extracts to human serum albumin. Two dimensional (2D-FL) and three dimensional (3D-FL) fluorescence measurements were recorded on a model FP-6500, Jasco spectrofluorimeter, serial N261332, Japan. The concentrations of citrus extracts were ranged from 0 to 1.5 mg/mL, and the total accumulated volume of citrus extracts was no greater than 150 μL. The corresponding fluorescence emission spectra were then recorded in the range of 300–500 nm upon excitation at 280 nm in each case. The emission wavelength was recorded between 200 and 795 nm for three-dimensional fluorescence spectra. All solutions for protein interaction were prepared in 0.05 mol/L Tris-HCl buffer (pH 7.4), containing 0.1 mol/L NaCl [14,18].

2.3.4. Molecular docking

The Ligand Fit module of Discovery Studio (DS Version 2.5) package was used for molecular docking. The X-ray crystallographic structures of HSA, 1h9z.pdb solved at 2.5 Å and complexed with ligands was retrieved from the protein data bank (PDB) and modified for docking calculations [14]. The co-crystallized ligands and the water molecules were removed from the protein structure. The H atoms were added and side chains were fixed using the protein preparation protocol. Optimization of the atomic charges and the structure minimization was
performed using CHARMM force field. To gain further insight in the interaction of Kaffir and Key lime extracts with HSA proteins, 14 active phyto-constituents detected in lime. Among which the 6 ligands based on the flavoring and aroma of the lime were selected for docking studies (Fig. 1). Two dimensional structures of these phyto-constituents were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The correct protonation states and partial charges were applied using the Ligand preparation module available with DS 2.5. Further the prepared ligands and protein structure were loaded to the docking protocol workspace. Best 10 poses were further processed and calculated [22].

2.3.4.1. In silico ADMET (absorption distribution metabolism excretion and toxicity) studies. Pharmacokinetic properties of the ligands were studied using SwissADME [23] and Molinspiration [24]. ADMET descriptor such as GI absorption, CYP1A2 inhibitor, PSA and MolLogP were predicted. The program requires the input information in SDF/MOL or SMILES file format.

2.3.5. 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 was used, following
by Duncan’s new multiple range tests to assess differences between group’s means. P values of < 0.05 were considered to be significant.

3. Results and discussion

3.1. Aroma, polyphenols and antioxidant activities in lime samples

Based on Fig. 1a, it can be observed that myrcene and γ-terpinene occur in similar amounts in both types of lime juices. However, the remaining four selected terpenes show more significant differences in concentrations in the tested samples. The essential oil of Citrus aurantifolia [25] possesses important spasmylytic properties, which are due to their major constituents, such as limonene (58.4%), δ-pinene (15.4%), γ-terpinene (8.5%), and citral (4.4%). Limonene and citral are chemical compounds characteristic of Key lime. The concentration of limonene in Key lime is estimated to 50.5 ± 2.1 µg/g and it is higher than in Kaffir lime. In the case of citral, the concentration of this substance is more than 30-fold higher in the volatile fraction in the Key lime than in the Kaffir lime. Terpinen-4-ol and linalool are the main terpenes of the Key lime volatile fraction. The content of these substances was respectively more than 20- and 5-fold higher in Kaffir lime compared to the Key lime. Above-mentioned facts can explain the variation in flavour and aroma of tested fruit species. Among the determined substances, potential indicators of Kaffir and Key limes were selected. As a criterion for qualifying a chemical compound to a group of potential markers was 20-fold difference in concentration. For Kaffir lime, terpinen-4-ol which concentration was estimated at 44.8 ± 1.1 µg/g, was selected as the indicator. This compound is characterized by a woody aroma. In the case of Key lime, the substance with fresh and citrus scent, namely citral with estimated concentration at 20.91 ± 0.68 µg/g, was chosen as a marker (Fig. 1 b). The main constituent of the volatile fraction of Key lime is limonene. This substance was not selected as a potential flavor indicator, because its presence is observed in many citrus juices.

The content of citral in lime up to 5% was found and this refers to essential oil [25,26]. The results of phenolic acids and antioxidant activities are presented in the Table 1. The amount of polyphenols in water extract in Kaffir lime was as much as twice higher than in Key lime. Citral as an indicator of Key lime was 1.5 times higher than terpinen-4-ol for Kaffir lime. The antioxidant activities were in accordance with the amount of polyphenols. There is a lack of data to compare the present results with the refereed literature. Most recent reports are connected with citrus essential oils from peels. Between four different varieties of citrus species peels Citrus aurantifolia contained high amounts of bioactive substances such as phenolics and flavonoids [27]. For estimation of antioxidant activity in peels the same methods as in the present research were used and correlation of total phenolics in various extracts was found [28]. Recent reports were also based on the few reports dealing with antioxidants and terpenes in lime juice and pulp. The values of bioactivity obtained in this report are in line with the recent report [30], where was concluded that the health benefits of Citrus aurantifolia are associated with its high amounts of photochemical and bioactive compounds such as flavonoids and phenols. The phenolic content and antioxidant properties of the manually squeezed lemon juice had higher total phenol content (64.5 GAE mg/L), while lime juice had higher total flavonoid content (29.5 QE mg/L). These results were slightly lower than the found in this research. Both juices exhibited antioxidant activities as typified by their ferric reducing power, and radicals (DPPH, ABTS, OH-, and NO+) scavenging abilities. Lime juices showed higher antioxidant activities than lemon. The inhibition of anti-angiogenesis-1-converting enzyme (ACE) activity in vitro and in vivo hypcholesterolemic effect of the juices could explain the use of the juices in the management of cardiovascular diseases [31]. Comparison of antioxidant activities of water extracts [32] of different plants showed that Phoenix dactylifera and Citrus aurantifolia had a significantly higher total phenol and DPPH scavenging activities than other investigated plants. The water extracts of Phoenix dactylifera and Citrus aurantifolia had the highest protective ability and this probably due to its higher antioxidant activity, total phenol content, and DPPH scavenging activity [32]. Our results are in accordance with reported in [33], where all oils showed the effects on DPPH in the range of 17–76–60%. The oils of Ichang lemon (64.0%, 172.2 mg TE/mL), Tahiti lime (63.2%, 170.2 mg TE/mL), and Eureka lemon (61.8%, 166.2 mg TE/mL) showed stronger radical scavengers than other citrus oils. Citrus volatile components such as geraniol (87.7%, 235.9 mg TE/mL), terpinolene (87.4%, 235.2 mg TE/mL), and γ-terpinene (84.7%, 227.9 mg TE/mL) showed marked scavenging activities on DPPH (p < 0.05). These numbers are in accordance with the results presented in Table 1, where terpinen-4-ol and citral showed relatively high DPPH values. The results of antioxidant activity of citral was similar to [34], where citral isolated from sweet orange possesses antioxidant activity by DPPH radical scavenging activity and cytotoxic properties, and is a potential source of active ingredients for food and pharmaceutical industry. Limonene, linalool and citral are common non-phenolic terpenoid components of essential oils, with attributed controversial antioxidant properties. Results indicate that antioxidant behavior of limonene, linalool and citral occurs by co-oxidation with the substrate, due to very fast self-termination and cross-termination of the oxidative chain [35]. Individual substances such as citral and limonene had the minimum antioxidant activities, but the antioxidant activities of their mixture were higher. The synergistic effects in the antioxidant activity and stability of the main oil components were found [36]. In another report [37] as well were discussed the similarities and differences between the antioxidant activities of some essential oils and their main components such as thymol, estragole, menthol, eugenol, carvacrol, camphor and limonene. The comparison of antioxidant values of the oils and their components shows that the

Table 1

Antioxidant and binding properties of limes and monoterpenes in water extract.

<table>
<thead>
<tr>
<th>Indices</th>
<th>Kaffir lime</th>
<th>Key lime</th>
<th>Terpinen-4-ol</th>
<th>Citral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenols, mgGAE/g DW</td>
<td>23.65 ± 2.5a</td>
<td>12.13 ± 1.1ab</td>
<td>5.84 ± 0.6c</td>
<td>8.32 ± 0.8b</td>
</tr>
<tr>
<td>CUPRAC, µTE/g DW</td>
<td>123.45 ± 10.4b</td>
<td>28.34 ± 2.3b</td>
<td>13.43 ± 1.3a</td>
<td>19.54 ± 1.8b</td>
</tr>
<tr>
<td>DPPH, µTE/g DW</td>
<td>33.87 ± 3.6e</td>
<td>17.21 ± 1.6b</td>
<td>8.54 ± 0.8c</td>
<td>12.23 ± 1.7b</td>
</tr>
<tr>
<td>FI (peak a), A.U.</td>
<td>450.69 ± 9.7b</td>
<td>488.90 ± 7.7ab</td>
<td>520.80 ± 11.6a</td>
<td>164.15 ± 10.3c</td>
</tr>
<tr>
<td>FI (peak b), A.U.</td>
<td>709.71 ± 12.4b</td>
<td>730.85 ± 5.2ab</td>
<td>784.42 ± 6.3b</td>
<td>–</td>
</tr>
<tr>
<td>FI (peak c), A.U.</td>
<td>168.32 ± 6.3b</td>
<td>83.64 ± 2.5a</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Binding to HSA, peak a</td>
<td>20.96 ± 1.3b</td>
<td>19.04 ± 4.6a</td>
<td>8.67 ± 0.9c</td>
<td>28.79 ± 2.4a</td>
</tr>
<tr>
<td>Binding to HSA, peak b</td>
<td>16.74 ± 1.2a</td>
<td>14.26 ± 0.5ab</td>
<td>7.66 ± 0.7b</td>
<td>–</td>
</tr>
</tbody>
</table>

Values are means ± SD of 5 measurements; Means within a row with the different superscripts are statistically different (p < 0.05; Student’s t-test). Abbreviations: GAE, gallic acid equivalent; CUPRAC, Cupric reducing antioxidant capacity; DPPH, 1, 1-Diphenyl-2-picrylhydrazyl method; TE, trolox equivalent; FI, fluorescence intensity; A. U., arbitral units; per g dry weight (DW); HSA, human serum albumin; FI of HSA in water according to peak a is equal to 570.21 ± 9.2; peak b is equal to 852.40 ± 11.3.
antioxidant properties of essential oil do not always depend on the antioxidant activity of its main component, and that they can be modulated by their other components. The obtained results in this study support the conclusion that when comparing the antioxidant properties of essential oils and their main components, the concepts of synergism, antagonism and additivity are very relevant.

3.2. Fluorescence studies

The fluorescence properties of lime extracts and standards are shown in Table 1 and Fig. 2. The highest binding properties were in Kaffir lime (Table 1 and Fig. 2B, in two peaks). Lower binding properties in comparison with Kaffir were in Key lime (Table 1, Fig. 2C). The binding properties of the polyphenols extracted from limes were relatively high showing the correlation between the antioxidant and quenching properties of polyphenols towards human serum albumin (Table 1). Peak b mainly reveals the spectral behavior of Trp and Tyr residues. Peak a mainly exhibits the fluorescence spectral behavior of polypeptide back-bone structures, and its intensity relates to the secondary structure of the protein. Both fluorescence peaks of HSA had been quenched by limes, but to different extents (Table 1). Peak c, which was detected only after interaction of lime samples with HSA, did not influence the quenching and remained in the same position and with the same fluorescence intensities in two samples (Fig. 2B, C).

The results of 2D-fluorescence showed the changes in the fluorescence intensity (FI) according only to one peak, and the results of
binding (%) were the following: with catechin (13.21, Fig. 2F, line 3 from the top), Key lime (14.26, Fig. 2F, line 4 from the top) and Kaffir lime (16.74, Fig. 2F, line 5 from the top).

The present results are in agreement with [38], where the effects of three kinds of flavonoids, quercetin, rutin and baicalin, on the binding of ticagrelor to HSA were investigated using fluorescence. According to the data in [38] the binding properties of ticagrelor, a new antiplatelet drug, is about 28.8%. The addition of Kaffir lime to HSA showed increased binding of 37.7%, and with Key lime was slightly lower of 33.3%. As it was described above volatile and polyphenol substances have numerous pharmacological activities, including cardiovascular effects, antioxidant, anti-inflammatory, antiallergic, antimicrobial, antithrombotic, antiviral, antidiabetic, estrogenic and anticarcinogenic activities. Our results of interaction of volatile substances with human serum albumin are in line with other research reports. It was shown that the volatiles possess antioxidant activity similar to polyphenols, then their binding properties can be compared with polyphenols [39–41]. The present results showed that polyphenols extracted from limes quenched HSA (Table 1) similar to the drugs shown in the cited above reports.

Fig. 3. (a) The docking images of the chemotypes. A. Binding ligands- citral/myrcene from the Chemotype I. B. Binding of the ligand - citral/limonene from chemotype II. Roman numerals represent: I-citral; II-myrcene; III-limonene. (b) 2D images showing the active residues in the binding site involved in the interaction of the ligands with the receptor protein (HSA).
The binding energy of citral was 1.44 times higher than for terpinen-4-ol. This result substantiates that isomerisation of the citral from trans-geraniol to nerol can reduce the binding energy of the ligand. The LOQ is also higher for these compounds compared to the other monoterpenes. The docking results have also shown that binding energy of the trans-geraniol and nerol is less compared to citral. The binding sites were predicted in HSA. Citral and myrcene from chemotype I, citral/limonene and Chemotype II; citral/limonene and Chemotype-III; limonene/linalool/citronellal. Chemotypes I and II have been vastly reported with essential oil of lime such as Chemotype-I; citral/myrcene, Chemotype-II; citral/limonene and Chemotype-III; limonene/linalool/citronellal. The ligands myrcene and limonene the membrane permeability was most widely-used descriptors for predicting membrane permeability. The ligands myrcene and limonene showed interaction with binding site 2 of HSA. Both the compounds bind to site-2 with poor compared to the other scoring compounds of lime. Citral was detected with TPSA of 17.07. The GI absorption is used to reveal the absorption of the drug across the intestine. Intestine has a surface rich in microvilli and covers about 1000-fold of the stomach thus GI absorption of the drugs is efficient pharmacokinetic properties. Therefore among the top scoring compounds (citral, limonene and myrcene) it was observed that citral is the best scored ADMET descriptors (Table 2b).

### 3.3. Molecular docking

Based on the flavoring profile, high concentration and LOQ the phyto-constituents from lime were selected and the ligands were subjected to docking with HSA. Citral, myrcene, linalool, nerol and trans-Geraniol showed good dock score compared to the other monoterpenes (Fig. 3a, b). The LOQ is also high for these compounds compared to the other volatiles. Citral at low pH and under oxidative stress can isomerize from geraniol to nerol that leads to the degradation of the citrus aroma [42]. The docking results have also shown that binding energy of the trans-geraniol and nerol is less compared to citral. The binding energy of citral was 1.44 times higher than for terpinen-4-ol [39]. This is in direct correlation with the binding properties determined by fluorescence (Table 1), because the binding of citral was 1.76 times higher than for terpinen-4-ol. This result substantiates that isomerisation of the citral from trans-geraniol to nerol can reduce the binding efficiency. Citral and limonene are major compounds available at high concentration in Key lime which gives the unique lemon odor and also makes the difference from the Kaffir lime. There are three different chemotypes reported with essential oil of lime such as Chemotype-I; citral/myrcene, Chemotype-II; citral/limonene and Chemotype-III; limonene/linalool/citronellal. Chemotypes I and II have been vastly studied as antitumor [43], antibacterial, antifungal [44] and anti-depressant [45]. Citral, limonene and myrcene are the main chemical constituents of the three chemotypes. From the Ligandfit module, 24 binding sites were predicted in HSA. Citral and myrcene from chemotype-I have similar dock score and binding energy. Interestingly, citral showed interaction with amino acid residues in binding site 20 of HSA and myrcene interacts with binding site 2 of HSA. Citral/limonene under the chemotype II demonstrates interaction with the same amino acids in the binding site 2 of HSA. However, in chemotype III citronellal and linalool showed interaction with binding site 2 and limonene have interaction with residues from binding site 20 (Table 2a).

### 3.3.1. In silico ADMET studies

In ADMET descriptors, total polar surface area (TPSA) is one of the most widely-used descriptors for predicting membrane permeability. The ligands myrcene and limonene the membrane permeability was poor compared to the other flavoring compounds of lime. Citral was detected with TPSA of 17.07. The GI absorption is used to reveal the absorption of the drug across the intestine. Intestine has a surface rich in microvilli and covers about 1000-fold of the stomach thus GI absorption of the drugs is efficient pharmacokinetic properties. Therefore among the top scoring compounds (citral, limonene and myrcene) it was observed that citral is the best scored ADMET descriptors (Table 2b).

### 4. Conclusions

Citral, myrcene and limonene are well known as plant-derived natural products finding their use in therapeutic applications in recent decades. The present investigation provides an insight into the binding properties of HSA with these pharmacologically important molecules. Chemotype II showed similar binding characteristics, with the latter have a stronger affinity to HSA. Both the compounds bind to site-2 with hydrophobic residues enclosed in the binding pocket of HSA. The data from present study can be useful in the establishment of their pharmacokinetic profiles in the process of future health food development. The binding properties of polyphenols from citrus fruits to HSA were relatively high in comparison with other plants, and it was a correlation between the binding properties and their bioactivities. This study gives evaluation of the bioactive interaction with human physiological system since HSA is the most important serum protein.

### Table 2a

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phyto-constituents of lime</th>
<th>Flavor profile</th>
<th>Docking Fitness</th>
<th>LOQ</th>
<th>Binding Energy (kcal/mol)</th>
<th>Interacting residues from HSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Citral</td>
<td>Lemon</td>
<td>240.900</td>
<td>1.64</td>
<td>-89.92</td>
<td>VAL576; LEU575; VAL547; ALA528; PHE507; LEU532; PHE551</td>
</tr>
<tr>
<td>2</td>
<td>Nerol</td>
<td>Floral, Fruit</td>
<td>236.001</td>
<td>1.67</td>
<td>-9.36</td>
<td>CYS90; CYS101</td>
</tr>
<tr>
<td>3</td>
<td>Geraniol</td>
<td>Geranium, Lemon Peel, Passion Fruit, Peach, Rose</td>
<td>238.232</td>
<td>1.53</td>
<td>-66.26</td>
<td>LEU139; ALA158; LEU154; PHE157; ILE142; TYR161</td>
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<td>4</td>
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<td>Balsamic, Fruit, Geranium, Herb, Must</td>
<td>246.248</td>
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<td>-63.75</td>
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<td>1.22</td>
<td>-56.22</td>
<td>VAL576; PHE551; LEU532; LEU575; PHE509; PHE507; HIS535; LEU585; PHE502</td>
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<td>6</td>
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<td>Citrus, Leaf</td>
<td>135.074</td>
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<td>Coriander, Floral, Lavender, Lemon, Rose</td>
<td>138.067</td>
<td>1.69</td>
<td>-61.94</td>
<td>TYR138; LEU135; LEU139; PHE157; ILE142; LEU154; TYR161; PHE134</td>
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### Table 2b

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<th>S.no</th>
<th>Phyto-constituents of lime</th>
<th>TPSA</th>
<th>LogP&lt;sub&gt;o/w&lt;/sub&gt;</th>
<th>GI absorption</th>
<th>MolLogP &gt; 4.15</th>
<th>Drug likeness (Lipinski)</th>
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</table>
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