Characterization of Bioactive Ligands with Antioxidant Properties of Kiwifruit and Persimmon Cultivars Using In Vitro and in Silico Studies

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Received: 21 May 2020; Accepted: 15 June 2020; Published: 19 June 2020

Abstract: The current study attempted to understand the interaction profiles of phytoconstituents in new and traditionally used fruit cultivars with human serum albumin (HSA) in the context of predicting the biological role under in vivo conditions. Therefore, polyphenols, flavonoids, flavanols, tannins, vitamin C, secondary metabolites and their antioxidant capacities of organic kiwifruit Actinidia (A.) eriantha cv. Bidan (AEB) and A. arguta cv. Cheongsan (AAC), as new cultivars grown in Korea, and widely consumed A. deliciosa cv. Hayward (ADH) and Diospyros kaki Thunb. cv. Fuyu (DKF) were determined and compared. All investigated fruits showed relatively high antioxidant capacities. To complement the bioactivity of these fruits, the binding properties between extracted polyphenols and HSA were determined by 3D-fluorescence spectroscopy and docking studies. The most bioactive was AEB with the highest percentage of binding, following by AAC, ADH and DKF. Our study for the first time unveils the differential binding properties of kiwifruit and persimmon phytoconstituents with HSA. Although cultivars possess virtually the same phytoconstituents, presence of one unique compound significantly alters the binding properties of HSA. The results of fluorescence quenching and molecular docking showed that these fruits possess multiple properties, which have a great potential to be used in industry with emphasis on the formulation of functional foods and medicinal applications.

Keywords: kiwifruit; persimmon; polyphenols; antioxidants; human serum albumin; binding; molecular docking

1. Introduction

All fruits, including Actinidia (A.) deliciosa cv. Hayward (ADH), A. eriantha cv. Bidan (AEB), A. arguta cv. Cheongsan (AAC) and Diospyros kaki Thunb. cv. Fuyu (DKF), contain a variable amount of bioactive substances, depending on cultivar, genotype, growing place and degree of maturity [1–5]. It was found that in addition to the mentioned kiwifruit cultivars, golden kiwifruit (Actinidia chinensis) peel contains higher contents of polyphenols and exerts stronger antioxidant activity than its flesh. Fleshes
with peels of two kiwifruits significantly reduced total cholesterol and triglycerides, and increased the high-density lipoprotein levels in rats [6]. The kiwifruit flour was used for food formulations, and the contents of free phenolics and antioxidant capacities were significantly higher than those of other investigated samples [7]. It was reported that kiwifruit is consumed in many forms such as fresh, flour, juice, vinegar, dried slices, jam, wine, yogurt, and jelly [6–10]. Despite limited absorption and digestion, dietary polyphenols have shown many health-promoting effects [11]. The investigations proved that kiwifruits with high amounts of health-beneficial metabolites, including polyphenols and vitamin C, which are the main compounds, also possess pharmacological properties [6,8,12]. Persimmon possesses high nutritional properties, containing organic acids, phenolic compounds, carotenoids, and tannins as its main nutrients [4,5]. Such composition determines antioxidant, cytotoxic, and antidiabetic activities, where some of them, related to human health, are attributed directly to tannins and gallic acid. The indices of recovery and bioaccessibility of phenols and flavonoids during gastrointestinal digestion in samples of persimmon flour were evaluated in vitro [13–16]. Persimmon in the same way as kiwifruit is used in different forms such as fresh, dry and flours [8,17]. As it was mentioned previously, kiwifruit ‘Hayward’ is one of the most popular in the international scale, as well as persimmon ‘Fuyu’. These traditional cultivars are main income fruits in Korea, and consumption of these fruits is consistently higher compared to pear, peach and grapes [1,2,18]. Kiwifruit ‘Bidan’ and ‘Cheongsan’ are new cultivars, introduced recently in Korea [3,19,20]. As it was cited in the recent literature there are many reports showing the properties of kiwifruit and persimmon, but two new kiwifruit cultivars are rarely compared by their nutritional and functional properties. ADH and DKF [19,20] are the most popular cultivars in comparison with the new ones AAC and AEB [3]. The comparison of the overall properties of the most popular kiwifruit and persimmon cultivars with two new ones will advance their industrialization. There are few reports about the biophysical interaction of polyphenols in the investigated fruits, as well as the correlation between phenolic, antioxidant and binding properties [16,21]. In spite of such wide scientific information it is a lack of medicinal properties of the investigated fruits, based on interaction of polyphenols with human serum albumin (HSA), the main carrier of drugs in human metabolism. These fruits have already been reported for several health benefits. Nonetheless, the study with their bioactive ingredients/ligands is very scanty. Therefore, the aim of the study is to reveal the potential use of bioactive ingredients from four different cultivars of kiwifruit and persimmon in the formulation of functional foods and medicinal application. Hence, we have determined chemical composition and validated their binding efficiency through in silico studies.

2. Materials and Methods

2.1. Chemicals and Reagents

The chemicals were bought from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA) and Fluka Chemie GmbH, Buchs, Switzerland. Gallic acid, epicatechin, human serum albumin (HSA), phosphate buffer and Folin-Ciocalteu reagent (FCR), were used for the determination of the amount of polyphenols and their quenching abilities. The chemicals 2,4,6-tripyridyl-s-triazine (TPTZ), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 1,1-diphenyl-2-picrylhydrazyl (DPPH), lanthanum(III) chloride heptahydrate, CuCl₂·2H₂O, 2,9-dimethyl-1,10-phenanthroline (neocuproine), 2,2-azino-bis (3-ethylbenzothiazloine-6-sulphonicacid) (ABTS⁺) radical cation and ferric chloride were used for the determination of antioxidant capacities by four complimentary methods.

2.2. Plant Material

Three batches of organic kiwifruits, including Actinidia (A.) delicosa cv. Hayward (ADH), A. eriantha cv. Bidan (AEB), A. arguta Cheongsan (AAC) and one batch of Diospyros kaki Thunb. cv. Fuyu (DKF) were collected in different commercial orchards from Boseong and Muan counties, Jeonnam and
Wonju-si, Gangwon-do province, South Korea. Each batch was composed of 20 fruits, about two kg of weight [22]. The cultivars that reached commercial maturity stage were harvested in 2018 (Figure 1).

![Image of fruits](image_url)

**Figure 1.** Investigated samples of kiwifruit: (A–C) *Actinidia* (*A.* deliciosa cv.Hayward (ADH), *A.* arguta cv. Cheongsan (AAC), *A. eriantha* cv. Bidan (AEB), and (D) *Diospyros kaki* Thunb. cv. Fuyu (DKF).

The samples were washed with tap water and dried. The fruits were fractionated into edible fraction (pulps), peels and seeds. Only for DKF 5-8 seeds were separated from pulps. Their edible parts were prepared manually without using steel knives. The peeled fruits (pulps) were weighed, chopped and homogenized in liquid nitrogen in a high-speed blender (Silex professional model, Hamilton Beach, Virginia, USA). A weighed portion (50–100 g) was then lyophilized for 48 h (Virtis model 10–324, Midland, ON, Canada), and the dry weight was determined. The samples were ground to pass through a 60-mesh sieve and stored at −20 °C until the bioactive substances were analyzed.

2.3. Determination of Firmness, Total Soluble Solids (TSS), pH, Total Acidity (TA), and Dry Matter

The fruits were analyzed for firmness by measuring penetration force in kilograms using a fruit-firmness tester (Model KM, Fruit Test Tech, Tokyo, Japan). After peeling, the tester penetrates (punches) the flesh with hand pressing. The mean values of the firmness were expressed in Newtons (N): 1 N is 9.8 kg. The peeled fruits were homogenized and filtered through cheesecloth in order to obtain a clear juice for determination of TSS (Brix), pH, TA, and dry matter. The TSS was measured using a digital refractometer (Atago Co., Ltd., Tokyo, Japan), pH was checked with a pH meter (Model 8100, ETI Co., Ltd., Worthing, West Sussex, UK). The TA was measured in 4 mL of juice, diluted to 20 mL of distilled water and titrated with 0.1 N NaOH. The TA was expressed as a percentage of citric acid. After peeling, dry matter (%) was calculated by the ratio of initial fresh weigh/dry weight [3,19,23,24].

2.4. Sample Extraction

The lyophilized samples of kiwifruit and persimmon cultivars were extracted with deionized water (pH 3) during 60 min at 25 °C. For mass spectrometry (MS) measurements the samples were extracted with ethanol: water mixture (8/2, *v/v*) during 60 min at 25 °C. The proportion of sample to the solvent was 1/10 w/v. The extracts were filtered in a Buchner funnel. After removal of the solvent in a rotary evaporator and the aqueous solution were freeze-dried [25,26].
2.5. Total Contents (T) of Phenolics (TPs), Flavonoids (TFAs), Flavanols (TFLs), Tannins (TNs), Vitamin C (VC) and Main Secondary Metabolites

The TPs content was determined by Folin–Ciocalteu colorimetric method [27], where 0.25 mL of phenolic extract solution (edible fraction) or standard was mixed with 1 mL of Folin-Ciocalteu reagent. In the next step 0.75 mL of 20% sodium carbonate was added and incubated for 6 min in a water bath at 45 °C. The absorbance of the resulting mixture was measured at 750 nm. Quantification of TPs in the samples was performed using a standard curve prepared with gallic acid, and the values were expressed as mg of gallic acid equivalent (GAE) per g dry weight (DW).

The TFA content [28] was measured in the mixture of 0.5 mL of the extract with 2.25 mL of distilled water, followed by the addition of 0.15 mL of 5% (w/v) NaNO$_2$ solution. After 6 min, 0.3 mL of a 10% AlCl$_3$·6H$_2$O solution was added. The reaction was allowed to stand for another 5 min before addition of 1.0 mL of 1 M NaOH. The mixture was mixed well by vortexing, and the absorbance was measured immediately at 510 nm.

TFLs were estimated using the $p$-dimethylaminocinnamaldehyde (DMACA) method, where 0.2 mL of fruit extract was introduced into a 1.5-mL Eppendorf tube, and 1 mL of DMACA solution was added. The mixture was vortexed and allowed to react at room temperature for 10 min. The absorbance at 640 nm was then read against a blank prepared similarly without DMACA. The presence of flavanols on the nuclei subsequent staining with the DMACA reagent resulted in an intense blue coloration in fruit extract [29].

TNs were estimated by spectrophotometric measurements of 0.5 mL fruit extract, where 3 mL of a 4% methanol vanillin solution and 1.5 mL of concentrated hydrochloric acid were added [30]. The mixture was allowed to stand for 15 min. The absorption of samples and blank against water was measured at 500 nm.

A catechin standard was used for the elaboration of the analytical curve and the results of TFAs and TNs contents were expressed as mg catechin equivalent (CE) per g DW. TFLs content was calculated as µg CE per g DW.

VC (mg Asc per g DW) was evaluated in fruit extracts, where 100 mg of freeze-dried fruit sample was extracted with 5 mL water. Then cupric reducing antioxidant capacity (CUPRAC) method was conducted and formed bis (Nc)-copper (I) chelate was determined spectrophotometrically at 450 nm [31].

The ethanolic extracts were submitted to MS analysis for main secondary metabolites determination and were processed exactly as described previously [1,32]. A mass spectrometer, a TSQ Quantum Access Max (Thermo Fisher Scientific, Basel, Switzerland) was used. All samples were analyzed by direct infusion in the mass spectrometer by electrospray ionization (ESI) in negative mode, full scan analysis, range of 100–900 m/z. For optimization of the acquisition parameters and for identity confirmation, only a part of standards was employed, not for all compounds that were found in the investigated samples.

2.6. Antioxidant Capacities

Four complementary assays were used for determination of antioxidant capacities:

2. 2,2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt (ABTS$^+$) was generated by the interaction of ABTS (7 mM) and K$_2$S$_2$O$_8$ (2.45 mM). The mixture was kept in the dark at room temperature for 12–16 h before use. This solution was diluted until the absorbance reached 0.7 at 734 nm and equilibrated at 30 °C. After addition of 1.0 mL of diluted ABTS$^+$ solution to 10 µL of extract or Trolox standards, the absorbance reading was taken 1 min after initial mixing and up to 6 min. The percentage decrease of the absorbance was calculated and plotted as a function of the concentration of the extracts and Trolox for the standard reference data [33].

Ferric-reducing/antioxidant power (FRAP) assay based on FRAP reagent (2.5 mL of a 10 mM ferric-tripiridyltriazine solution in 40 mmol HCl plus 2.5 mL of 20 mmol FeCl$_3$·H$_2$O and 25 mL of 0.3 M/L acetate buffer, pH 3.6) of 900 µL was mixed with 90 µL of distilled water and 30 µL of kiwifruit
and persimmon extract samples as the appropriate reagent blank. The absorbance was measured at 595 nm after 30 min [34].

Cupric reducing antioxidant capacity (CUPRAC) is utilizing the copper (II)-neocuproine \([\text{Cu(II)-Nc}]\) reagent as the chromogenic oxidizing agent. To the mixture of 1 mL of \([\text{Cu(II)-Nc}]\) and \(\text{NH}_4\text{Ac}\) buffer solution, acidified and non acidified extracts of fruits (or standard) solution \((x, \text{in mL})\) and \(\text{H}_2\text{O} ((1.1-x) \text{ mL})\) were added to make the final volume of 4.1 mL. The absorbance at 450 nm was recorded against a reagent blank [35].

1-Diphenyl-2-picrylhydrazyl method (DPPH) based on DPPH solution \((3.9 \text{ mL}, 25 \text{ mg/L})\) which was mixed with the samples extracts \((0.1 \text{ mL})\). The reaction progress was monitored at 515 nm until the absorbance was stable [36].

The units for all antioxidant capacities were \(\mu\text{M TE (Trolox equivalent)} \text{ per g DW}\). The absorbances of all investigated resulted mixtures were measured on Hewlett-Packard, model 8452A spectrophotometer.

2.7. Fluorometric Measurements

Two (2D-FL) and three dimensional (3D-FL) fluorescence measurements for all kiwifruit and persimmon extracts at a concentration of 0.01 mg/mL were recorded on a model FP-6500, Jasco spectrofluorometer, serial N261332, Tokyo, Japan, equipped with 1.0 cm quartz cells and a thermostat bath. The 2D-FL measurements were taken at emission wavelengths from 310 to 500 nm and at excitation of 295 nm. The 3D-FL spectra were collected with subsequent scanning emission spectra from 200 to 400 nm at 1.0 nm increments by varying the excitation wavelength from 200 to 500 nm at 10 nm increments. For comparison of the obtained results gallic acid and epicatechin was used [1,10,32]. The solutions for the reaction were in the following concentrations: \(1.0 \times 10^{-5} \text{ mol/L HSA}; 0.05 \text{ mol/L } \text{Tris–HCl buffer with 0.1 mol/L NaCl, pH 7.4.}\)

2.8. Protein and Ligand Preparation for Molecular Docking Studies

The potential interaction of ligands from the different varieties of kiwifruit and persimmon with HSA was investigated using Autodock docking program version 4.2 [37]. The crystal structure of the HSA was obtained from the RCSB Protein Data Bank (PDB code: 1H9Z). Prior to docking studies, the ligands were downloaded as 2D structure (.sdf format) from PubChem database and converted to 3D structure (.pdb format) (Supplementary Table S1). The receptor protein HSA was prepared by removing the water molecules, adding hydrogen atoms and by assigning partial charges based on the CHARMM force field. The ligand structures were minimized by applying MMFF94 Force Field and other parameters were set to their default. Furthermore, the binding cavity region of the receptor was set to spherical cut-off of 8 Å for non-bonding interactions. Finally, the docking protocol was applied to the processed protein and ligand structures. The resulting best poses were extracted and evaluated using the scoring algorithm and visualized through BIOVIA Discovery Studio 4.5 software (Dassault Systemes BIOVIA Corporate, San Deigo, CA, USA) [38].

2.9. Statistical Analysis

All obtained data were calculated on the basis of statistical analysis of Duncan’s multiple range test. Values are means ± SD per gram dry weight (DW) of 25 measurements, representing commercial maturity status of fruits and their replicates. Five replications of five extracts from each cultivar were performed. To determine the statistical significance as 95% interval of reliability, ANOVA, one-way analysis on variance, was used.

3. Results and Discussion

3.1. Determination of Harvest Parameters and Physicochemical Properties of Kiwifruit and Persimmon

The properties of investigated fruits, including ripeness indices were evaluated and compared. At commercial maturity stage the following results of harvest parameters were obtained: firmness
The evaluation of 62 consumed fruits [18] showed that persimmon with 112.09 mg GAE for Actinidia (Newton (N)) for Actinidia (A.) delicosa cv.Hayward (ADH) was 40.32 ± 0.32; followed by A. eriantha cv. Bidan (AEB) of 38.52 ± 0.28; Diospyros kaki Thunb. cv. Fuyu (DKF) of 32.61 ± 0.43 and A. arguta Cheongsan (AAC) of 29.64 ± 0.21. Total soluble solids (TSS, Brix) showed the following values: DKF (11.94 ± 0.15) > AAC (8.14 ± 0.13) > ADH (7.81 ± 0.09) > AEB (7.34 ± 0.04). Acidity (%) was estimated in the following order: ADH (1.47 ± 0.07) > AAC (1.45 ± 0.09) > AEB (1.40 ± 0.08) > DKF (1.21 ± 0.04). The measurements of pH were the following: DKF (5.41 ± 0.34) > ADH (3.22 ± 0.16) > AEB (3.20 ± 0.12) > AAC (3.04 ± 0.09). In the investigated samples the dry matter (%) was calculated and presented in the following order: DKF (18.69 ± 0.94) > ADH (16.66 ± 0.73) > AEB (16.19 ± 0.68) > AAC (15.72 ± 0.49). The most important indices for the ripening of the investigated fruits were firmness and TSS, which showed the ranges of 40–30 N and 12–7 Brix, respectively. Based on these results the harvest was done. The determined ripening data, acidity, pH and dry matter were in the ranges similar to other recent reports [3,19,23,24].

3.2. Biologically Active Compounds and Secondary Metabolites in Investigated Fruits

The contents of total phenolics (TPs), flavonoids (TFAs), flavanols (TFLs), tannins (TNs) and vitamin C (VC) are presented in Table 1. The main active compounds in the fruits were polyphenols and vitamin C and their values were ranging from 32.37 ± 1.34 to 4.31 ± 0.21 mg GAE/g DW and from 36.51 ± 1.65 to 2.31 ± 0.23 mg AA/g DW, respectively (Table 1), showing the highest values for kiwifruit cultivar ‘Bidan’. The obtained results differ from previously presented data [1,5,32,39], because of different conditions of extraction, including various solvents, temperature and time of the process. In the present research, as mentioned above, the extraction of bioactive substances was performed in water (pH 3), because for polyphenols most extractions are carried out under acidic conditions. The polyphenols are more stable in low pH, and the acidic condition helps polyphenols to stay neutral [25,26]. The aqueous extracts are important for the further use in the interaction of polyphenols with HSA, because such reactions appeared in human metabolism under similar conditions [40]. The present results (Table 1) are in line with other reports [20], evaluating that among various ripe kiwifruit grown in South Korea, ‘Bidan’ had the highest total phenolics and antioxidant capacity, and the lowest total flavonoids; whereas, another cultivar ‘Chiak’ had the highest level of total flavonoids, but the lowest antioxidant capacity. It was reported that the raw ‘Hayward’ kiwifruit had a VC content of 0.55 mg/g fresh weight (FW) [11]. A. arguta [20,41] showed lower results of VC of about 1.5–2.0 mg AA/g FW and TPs of 656–1400 mg GAE/kg FW than the obtained values in Table 1, but the present data well agreed with similar characterizations of the same fruits by other investigators [42]. The evaluation of 62 consumed fruits [18] showed that persimmon with 112.09 mg GAE/100 g FW was the seventh most beneficial fruit in terms of antioxidant and phenolic properties, a result that agrees well with the present results.

The secondary metabolites were determined by MS and presented below. The ethanolic extracts for ADH [M-H]− (% in MS) showed the following results: catechol (m/z 110 (6)), caffeic acid (m/z 179 (8)), quinic acid (m/z 191 (85)), syringic acid (m/z 198 (20)), kaempferol (m/z 286 (9)), quercetin (m/z 301 (10)), rutin (m/z 609 (12)) and hesperidin (m/z 610 (8)). AEB ([M-H]− (% in MS) samples were characterized by the following peaks: catechol (m/z 110 (8)), protocatechuic acid (m/z 154 (12)), caffeic acid (m/z 179 (6)), quinic acid (m/z 191 (100)), syringic acid (m/z 198 (14)), kaempferol (m/z 286 (12)), quercetin (m/z 301 (18)), rutin (m/z 609 (12)) and hesperidin (m/z 610 (11)). The main peaks for AAC [M-H]− (% in MS) were identified as follows: catechol (m/z 110 (8)), caffeic acid (m/z 179 (7)), quinic acid (m/z 191 (87)), syringic acid (m/z 198 (19)), quercetin (m/z 301 (20)), and hesperidin (m/z 610 (10)). DKF spectra recorded six main peaks in the extracts: vanillic acid (m/z 168 (29)), gallic acid (m/z 169 (80)), caffeic acid (m/z 179 (18)), kaempferol (m/z 286 (7)), epicatechin (m/z 288(11)), and quercetin (m/z 301 (8)). As can be seen, ethanol extracts of kiwifruit cultivars characterized by chlorogenic acid of the [M-H]− deprotonated molecule and the ion corresponding to the deprotonated quinic acid (m/z 191), as the most abundant compound, in accordance with other reports [6,43]. Caffeic, quinic, and syringic acids, catechol, quercetin and hesperidin were identified in different amounts in all kiwifruit extracts with the highest
in AEB. Although there is a difference in the profile of compounds identified in the samples, but the majority of compounds such as caffec acid, quercetin and kaempferol were present in investigated extracts, except AAC. The secondary metabolites varied depending on the pedoclimatic, genetic and extractive conditions, in agreement with the cited reports [1,5,32].

### Table 1. Bioactive substances and binding properties of kiwifruit and persimmon water extracts.

| Extracts of Fruits | ADH | AEB | AAC | DKF |
|------------------|-----|-----|-----|-----|
| TPs, mg GAE      | 6.27 ± 0.43  c | 32.37 ± 1.34  a | 16.94 ± 0.81  b | 4.31 ± 0.21  c |
| TFAs, mg CE      | 1.89 ± 0.22 b | 2.18 ± 0.14 b | 4.46 ± 0.31 a | 1.11 ± 0.18 b |
| TFLs, mg CE      | 130 ± 6.17 b, c | 30 ± 2.21 c | 1790 ± 16.21 a | 83 ± 3.54 b |
| TNs, mg CE       | 2.21 ± 0.12 b | 0.57 ± 0.11 c | 4.18 ± 0.31 a | 3.94 ± 0.21 a |
| VC, mg AA        | 4.07 ± 0.25 b, c | 36.51 ± 1.65 a | 6.69 ± 0.43 b | 2.31 ± 0.23 c |
| ABTS, µM TE      | 20.42 ± 1.17 b, c | 86.14 ± 3.23 a | 49.42 ± 2.34 b | 16.48 ± 0.86 c |
| FRAP, µMTE       | 10.28 ± 0.84 c | 42.97 ± 2.45 a | 24.47 ± 1.67 b | 9.03 ± 0.34 c |
| CUPRAC, µMTE     | 25.98 ± 0.96 c | 103.81 ± 5.43 a | 65.21 ± 3.56 b | 19.61 ± 0.93 c |
| DPPH, µMTE       | 12.45 ± 1.12 c | 51.67 ± 2.34 a | 30.08 ± 1.98 b | 9.58 ± 0.76 c |
| Fl of peak a, A.U.| 465.5 ± 7.8 a | 279.2 ± 4.3 b | 320.1 ± 4.4 b | 490.2 ± 5.4 a |
| Fl of peak b, A.U.| 822.6 ± 10.6 a | 753.1 ± 7.4 a | 783.5 ± 8.7 a | 827.7 ± 6.5 a |
| BP, peak a%      | 18.2 ± 1.1 c | 50.9 ± 2.34 a | 43.7 ± 1.2 b | 13.8 ± 0.6 a |
| BP, peak b%      | 3.1 ± 0.2 a | 11.3 ± 1.1 a | 7.7 ± 0.6 b | 2.5 ± 0.1 c |

Values are means ± SD per gram dry weight (DW); n = 5 samples per cultivar, each subsampled and analyzed 5 times. Values in rows and columns with different superscript letters (a–d) are significantly different (p < 0.05). Abbreviations: ADH, Actinidia (A.) delicosa cv. Hayward; AEB, A. arguta cv. Cheongsan; DKF, Diospyros kaki Thunb. cv. Fuyu; TPs, total phenols; GAE, gallic acid equivalent; CE, catechin equivalent; TFAs, total flavonoids; TFLs, total flavanols; TNs, total tannins; VC, vitamin C; AA, ascorbic acid; ABTS, 2, 2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; FRAP, Ferric-reducing/antioxidant power; CUPRAC, Cupric reducing antioxidant capacity; DPPH, 1, 1-Diphenyl-2-picrylhydrazyl method; TE, trolox equivalent; FI, fluorescence intensity; A. U., arbitral units; HSA, human serum albumin; BP, binding properties of polyphenols with HSA, bold letters a and b showed fluorescence peaks of HSA; % FI, peak a, A.U. 465.5 ± 7.8; peak b is equal to 848.9 ± 10.5.

### 3.3. Antioxidant Capacities

Evaluation of the antioxidant capacities by 2, 2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), ferric reducing antioxidant power (FRAP), cupric ion reducing capability (CUPRAC) and 1, 1-Diphenyl-2-picrylhydrazyl scavenging radical (DPPH) assays showed the highest values by CUPRAC between 103.81 ± 5.43 and 19.61 ± 0.93 µMTE/g DW for all kiwifruit cultivars and persimmon (Table 1). The results calculated by ABTS, FRAP, CUPRAC and DPPH which connected with different kiwifruit cultivars are in agreement with several reports. So, the IC50 values of ABTS radical cation scavenging activities showed that A. arguta determined to be 1.26 mg/mL in comparison with A. delicosa cv. Hayward of 22.72 mg/mL, presenting the strongest antioxidant activities among all tested kiwifruit cultivars, such as A. chinensis, A. polygama and A. macroasperma [6,20]. The estimated results of AAC (Table 1) showed that the ABTS values were 2.4 times greater than ADH. The antioxidant capacities of kiwifruit flour ‘Hayward’ in vitro by DPPH and FRAP assays were 20 and 25 µmol TE/g DW, respectively, and the free phenolic content was 14.57 mg GAE/g DW [7]. These results are superior to those presented in Table 1, where the DPPH and FRAP values were 12.45 and 10.28 µmol TE/g DW, respectively, and TPs were 6.27 mg GAE/g DW. These values exhibit good correlation between the polyphenols and their antioxidant capacities, showing that the cited results [7] were 1.6, 2.43 and 2.32 times higher for DPPH, FRAP and TPs, respectively, than the presented ones in Table 1. The values of persimmon ‘Fuyu’ showed TPs of 4.31 mg GAE/g DW, antioxidant capacities (µmol TE/g DW) by ABTS, FRAP and DPPH methods—16.48, 9.03 and 9.58, respectively, and VC of 2.31 mg AA/g DW (Table 1). These results can be compared with cv. Vanilla, in which total polyphenols were estimated of 1 mg GAE/g FW, antioxidant capacities by ABTS—5 µmol TE/g FW, FRAP—6 µmol TE/g FW, and DPPH—0.3 mg GAE/g FW. VC content was in the range of 0.1–0.2 mg/g FW [44]. Two innovative products (kaki and kiwi) were characterized by their bioactivity [8]. The total phenolics have ranged from 2.1 mg GAE/g DW (kiwi) to 8.7 mg GAE/g DW (kaki), while dried fruit antioxidant
capacity was from 23.09 μmol Fe^{2+}/g DW to 137.5 μmol Fe^{2+}/g DW, as was shown in previous results [17]. The most important phytochemical class including apple, kiwi, and kaki dried fruits (from 74.6% to 93.3%), as it was demonstrated in other reports were phenolics [26]. The evaluation of bioactive substances of presently investigated fruits, including polyphenols, tannins, flavonols, flavonoids, vitamin C and antioxidant capacities (Table 1) well agreed with the data, discussed in recent investigations [39,45,46]. A positive and highly significant correlation between the contents of total phenols and radical scavenging capacities against all used radicals, and especially against ABTS and DPPH in persimmons, are in line with described data [21].

3.4. Binding Properties of Bioactive Compounds of Investigated Fruits with Serum Protein Measured by Fluorescence

The results of changing in the fluorescence intensity during interaction of polyphenols with HSA are shown in Tables 1 and 2 and Figure 2. For the standards were chosen gallic acid and epicatechin. The quenching of polyphenols extracted from persimmon ‘Fuyu’ and kiwifruit ‘Hayward’ were in the same range and were comparable with gallic acid and among other kiwifruit cultivars were the lowest. As it was described previously [1,2,5], the quenching of the protein depends on the amount of the fruit extract added to the reaction, then fluorescence intensity decreases, by the shifting in the emission value. The change of the fluorescence intensity after interaction with HSA was compared with HSA before interaction, and the peaks a and b were measured, and the binding properties were calculated. The binding properties measured by 2D-FL (Table 2) differ from the values estimated by 3D-FL (Table 1, Figure 2). In 3D-FL the main changes were found according to the decrease of fluorescence intensity mostly in peak a. The results showed that the binding properties of ‘Bidan’ were greater than other investigated fruits and estimated of about 51%, according to the changes in the fluorescence intensity of peak a (Table 1).

Table 2. Fluorometric measurements in two-dimensional fluorescence analysis (2D-FL) of kiwifruit and persimmon water extracts.

| Samples          | λ_{em.} nm | FI A.U. | BP (%) |
|------------------|------------|---------|--------|
| HSA              | 355        | 953.2 ± 6.9^a | -      |
| HSA + ADH        | 350        | 830.1 ± 4.8^a,b | 12.0 ± 1.4^c,d |
| HSA + GA         | 354        | 817.8 ± 3.2^b | 14.2 ± 1.5^c |
| HSA + Epica     | 335        | 729.4 ± 3.1^b,c | 23.5 ± 1.7^b |
| HSA + AAC        | 355        | 676.3 ± 2.8^c | 29.1 ± 1.3^a,b |
| HSA + AEB        | 357        | 626.6 ± 2.6^c | 34.3 ± 2.8^a |
| HSA + DKF        | 355        | 865.4 ± 4.4^a,b | 9.2 ± 0.9^d |

Values are means ± SD per gram dry weight (DW); n = 5 samples per cultivar, each subsampled and analyzed 5 times. Values in rows and columns with different superscript letters (a–d) are significantly different (p < 0.05). Abbreviations: HSA, human serum albumin; GA, gallic acid; Epica, Epicatechin; λ_{em} nm, maximum emission shift.

Binding properties of ‘Hayward’ and ‘Fuyu’ were comparable and showed 18% and 14%, respectively (Table 1, Figure 2). The high binding properties of the investigated fruits can be explained by their antioxidant capacities. The antioxidant capacities of ‘Bidan’ by DPPH and ABTS assays were 4.2 times greater than for ‘Hayward’ and the binding properties of ‘Bidan’ were also greater than for ‘Hayward’ by 2.8 times (Table 1). These results are in full agreement with the correlation analysis which demonstrated that the phenolic and flavonoid contents are responsible for increasing the scavenging activities of DPPH and ABTS, where protocatechuic and chlorogenic acids were the predominant phenolic acids in kiwifruit pulp, as well as gallic acid, epicatechin, and catechin [47]. The fluorescence results indicate that there was a static quenching mechanism in the interactions of gallic acid (GA) with BSA [48] and support the data obtained in Table 2, where the binding properties of gallic acid were slightly higher that for kiwifruit ‘Hayward’. Epicatechin showed as well relatively high binding properties which were closed to the ability of kiwifruit A. Arguta (Table 2). These results are consistent with others [49], where the interaction of copper complexed with (-)-epigallocatechin-3-gallate (EGCG)
and bovine serum albumin (BSA) was investigated using fluorescence. The fluorescence quenching efficiency of BSA by EGCG was enhanced after the formation of the complex of EGCG with copper. The EGCG-Cu complex exhibited a higher apparent binding affinity to BSA compared with EGCG alone. The binding affinities to HSA were ranked in the order that EGCG showed the highest value between the investigated compounds [40]. The synergetic effect of pure standards in comparison with the mixture of different antioxidants in the investigated fruits showed higher reactivity than the pure substances themselves [13,14,39]. Molecular docking supported the obtained results.

3.5. Docking Studies

Fluorescence studies revealed the binding properties of kiwifruit and persimmon water extracts with HSA. To delineate the mechanisms at a molecular level, 15 compounds identified from kiwifruit and persimmon extracts were analyzed through docking studies. Ligands exhibiting high binding affinity towards HSA are shown in Figure 3. Caffeic acid, quinic acid, catechol, syringic acid, hesperidin and quercetin were identified to be the major ingredients [1,5,39] that are common among the three varieties of kiwifruit (‘Hayward’, ‘Bidan’ and ‘Cheongsan’) used in the current study (Table 3). From the interaction analysis, hesperidin and quercetin were identified as the top scorers among the compounds screened from the kiwifruit. Hesperidin was found to have a docking and binding energy score of −168.71 and −140.3 kcal/mol, respectively. Whereas, quercetin exhibited the highest dock score of −163 and binding energy score of −116.8 kcal/mol. Hesperidin (Figure 4) formed five conventional hydrogen bond interactions with three residues (Leu115, Arg117 and Leu185).

Table 3. The ligands from kiwifruit showing interaction with HSA represented with binding energy (BE), dock score (DS), van der Waals (VDW) and H-bond (HB) energy.

| S. No | PubChem ID | Compounds        | HSA + Kiwifruit |
|------|------------|------------------|-----------------|
|      |            |                  | BE   | DS   | VDW  | HB   |
| 1    | 5280863    | Kaempferol       | −106.3| −138.14| −102.8| −3.4 |
| 2    | 5280805    | Rutin            | −78.9 | −82.84| −61.9 | −16.9 |
| 3    | 689043     | Caffeic acid     | −75.0 | −99.36| −71.5 | −3.5 |
| 4    | 6508       | Quinic acid      | −69.4 | −95.18| −63.0 | −6.3 |
| 5    | 289        | Catechol         | −50.8 | −61.67| −47.3 | −3.4 |
| 6    | 10742      | Syringic acid    | −0.5  | −107.18| −77.2 | −3.2 |
| 7    | 10621      | Hesperidin       | −140.3| −168.71| −126.1| −14.1 |
| 8    | 5280343    | Quercetin        | −116.8| −163   | −106.2| −10.5 |
| 9    | 72         | Protecatechuic acid | −69.0 | −90.03| −66.2 | −2.7 |
Hesperidin and quercetin were identified to be the major ingredients \cite{1,5,39} that are common among the three varieties of kiwifruit (‘Hayward’, ‘Bidan’ and ‘Cheongsan’) used in the current study (Table 3). From the interaction analysis, hesperidin and quercetin were identified as the top scorers among the compounds screened from the kiwifruit. Hesperidin was found to have a docking and binding energy score of $-168.71$ and $-140.3$ kcal/mol, respectively. Whereas, quercetin exhibited the highest dock score of $-163$ and binding energy score of $-116.8$ kcal/mol. Hesperidin (Figure 4) formed five conventional hydrogen bond interactions with three residues (Leu115, Arg117 and Leu185).

Table 3. The ligands from kiwifruit showing interaction with HSA represented with binding energy (BE), dock score (DS), van der Waals (VDW) and H-bond (HB) energy.

| S. No | PubChem ID | Compound      | HSA + Kiwifruit BE  | DS        | VDW       | HB       |
|-------|------------|---------------|---------------------|-----------|-----------|----------|
| 1     | 528086     | Kaempferol    | $-106.3$            | $-138.14$ | $-102.8$  | $-3.4$   |
| 2     | 528080     | Rutin         | $-78.9$             | $-82.84$  | $-61.9$   | $-16.9$  |
| 3     | 689043     | Caffeic acid  | $-75.0$             | $-99.36$  | $-71.5$   | $-3.5$   |
| 4     | 6508       | Quinic acid   | $-69.4$             | $-95.18$  | $-63.0$   | $-6.3$   |
| 5     | 289        | Catechol      | $-50.8$             | $-61.67$  | $-47.3$   | $-3.4$   |
| 6     | 10742      | Syringic acid | $0.5$               | $-107.18$ | $-77.2$   | $-3.2$   |
| 7     | 10621      | Hesperidin    | $-140.3$            | $-168.71$ | $-126.1$  | $-14.1$  |
| 8     | 528034     | Quercetin     | $-116.8$            | $-163$    | $-106.2$  | $-10.5$  |
| 9     | 72         | Protocatechuic acid | $-69.0$            | $-90.03$  | $-66.2$   | $-2.7$   |

Figure 3. Top scoring ligands (stick model) showing interactions with the receptor binding site of human serum albumin (space filling model).

Figure 4. The interactions of the highest docking score ligands from different cultivars of kiwifruit and persimmon with HSA receptor protein. (A) hesperidin; (B) quercetin; (C) kaempferol; (D) protocatechuic acid (3,4-dihydroxybenzoic acid). 2D view of ligands and their bonding type are represented in (E–H).
In addition, a carbon–hydrogen bond interaction with Pro113 and a pi-donor hydrogen bond interaction with Tyr161 and Gly189 were exhibited. Other key interactions include pi-sigma (Ile142), pi-alkyl and alky interactions (Tyr138, Pro118 and Leu115). Quercetin formed three hydrogen bond interactions with Leu135 and Arg117 and one carbon–hydrogen bond and pi-donor hydrogen bond interaction with Tyr138. It formed van der Waals interaction with Leu135 and other stabilizing interactions such as pi-pi stacked (Tyr161), amide-pi stacked (Tyr161) and pi-alkyl interactions (Ala158, Ile142 and Leu182). Kaempferol and rutin are the major compounds reported only in ‘Hayward’ and ‘Bidan’ and not in Actinidia arguta. Kaempferol and rutin have a dock score of $-138.14$ and $-82.84$ and binding energy of $-106.3$ and $-78.9$, respectively. Among the ligands, rutin has the highest hydrogen bond energy of $-16.9$ kcal/mol on interactions with the receptor protein HSA. The highest interactions are evident from six hydrogen bonds from Tyr161, Lys190, Pro113 and Glu141 and other carbon–hydrogen and pi-donor hydrogen bond interactions from Arg186, Ser193 and Leu115. Besides, it forms pi-alkyl (Arg186 and Leu115) and pi-sigma interactions with HSA (Leu115). Protocatechuic acid (3, 4-dihydroxybenzoic acid) was identified only in ‘Bidan’ cultivar and has shown $-90.03$ as dock score and $-69.0$ kcal/mol as binding energy. It formed one conventional hydrogen bond interaction with Leu154, pi-donor hydrogen bond with Tyr161 and van der Waals interaction with Leu139. The other scaffold stabilizing interactions such as pi-pi stacked (Tyr138), amide-pi stacked (Tyr138) and pi-alkyl (Ala158) were also observed.

From persimmon ‘Fuyu’ extract six compounds were identified such as gallic, vanillic and caffeic acids, epicatechin, kaempferol, and quercetin (Table 4). Among them, caffeic acid, kaempferol, and quercetin are reported for kiwifruit varieties. Quercetin and kaempferol have achieved high binding affinity with HSA with the dock score of $-163$ and $-138.14$, respectively [4,5]. The ligand epicatechin has a dock score of $-138.14$, binding energy of $-106.3$ kcal/mol, van der Waals energy of $-102.8$ and the highest H-bond energy of $-3.4$, which might have favored the interaction with HSA (Table 4).

| S. No | PubChem ID | Compounds          | HSA + Persimmon ‘Fuyu’ |
|-------|------------|---------------------|-------------------------|
|       |            |                     | BE         | DS         | VDW        | HB         |
| 1     | 370        | Gallic acid         | $-72$       | $-95.4$    | $-68.7$    | $-3.2$     |
| 2     | 72276      | Epicatechin         | $-103.2$    | $-137.4$   | $-92.6$    | $-10.4$    |
| 3     | 5280863    | Kaempferol          | $-106.3$    | $-138.14$  | $-102.8$   | $-3.4$     |
| 4     | 8468       | Vanillic acid       | $-71.6$     | $-95.25$   | $-68.1$    | $-3.4$     |
| 5     | 689043     | Caffeic acid        | $-75.0$     | $-99.36$   | $-71.5$    | $-3.5$     |
| 6     | 5280343    | Quercetin           | $-116.8$    | $-163$     | $-106.2$   | $-10.5$    |

Comparing the docking results, it was observed that the ‘Bidan’ cultivar among the kiwifruit has the maximum binding affinity with HSA and it is in good agreement with the fluorescence experiments where it showed the highest percentage of binding (51%) with HSA. All the kiwifruit cultivars (used in the current study) have hesperidin and quercetin ligands, which were predicted with high docking and binding energy values among all the ligands. Although, ‘Bidan’ shares similar ligands with ‘Cheongsan’, it possesses the distinct ligand named protocatechuic acid, which might have played a significant role in enhancing the binding affinity of the ligands to HSA receptor. Hence, ‘Bidan’ cultivar might have shown the highest binding in the fluorescence experiments. Interestingly, kaempferol and rutin are absent in ‘Cheongsan’. However, ‘Hayward’ and ‘Fuyu’ have shown the least interaction with HSA in fluorescence results. HSA, a globular protein of 585 amino acids, comprises three homologous domains: I (5–197 residues), II (198–382 residues) and III (495–585 residues). Each domain has been further subdivided in to subdomain A and B (IA, IB, IIA, IIB, IIIA and IIIB). HSA has been reported to bind to a wide range of ligands at multiple sites with different binding affinities.
From our docking analysis, it is apparent that all the ligands identified from kiwifruit and persimmon extracts have binding pockets in domain I of HSA.

These results were in accordance with other investigations. Molecular docking showed that (-)-epigallocatechin-3-gallate (EGCG) mainly bound to subdomain IIA and IIIA Results of competitive binding experiments confirmed that the location of EGCG binding in BSA was site I [40,49]. Molecular docking analysis highlighted that GA binds at Sudlow site I of BSA and binding of GA at site I of BSA is stable [48]. Remarkably, domain I has been reported to bind hemin and fatty acids, and thus the site has been represented as a major drug binding pocket. The major residues involved in binding are Tyr138, Tyr161, Arg114, His146, Lys190, Ile142, Arg117 and Leu182. Among these Tyr138 and Tyr161 are the crucial residues involved in drug recognition [50,51]. Our results are consistent with these earlier reports wherein the identified ligands were found to have interactions with these major residues. Thus, from our docking studies it is envisaged that the presence of more potential ingredients in the ‘Bidan’ cultivar might have led to multiple binding pockets in HSA and thereby it might have enhanced the binding affinity towards HSA. Kiwifruit has also been reported for preventing platelet aggregation and has shown an effect on Angiotensin converting enzyme (ACE) [52]. Quercetin identified as one among the top hits has been reported with effective antiviral activity against herpes viruses and Dengue virus [53]. Similarly, kaempferol, quercetin and hesperidin were reported as promising drug candidates to prevent enterovirus 71 replication [54]. Thus, bioactive flavonoids identified and investigated in the present study with potential binding ability with HSA have several health benefits to the human system both as an immune booster as well as in terms of antibacterial and antiviral activities [55].

4. Conclusions

The exotic fruits such as kiwifruit and persimmon have been investigated previously and suggested to be used as food supplementation, especially for patients with cardiovascular risk factors. Spectrophotometric and fluorometric methods and molecular docking were applied for characterization of several kiwifruit cultivars and persimmon. Antioxidant capacities directly correlated with the amount of polyphenols and the quenching properties of extracted fruit polyphenols and human protein albumin. In the present study several bioactive ligands with antioxidant properties were identified from kiwifruit and persimmon by molecular docking. Quercetin, kaempferol, quercetin, epicatechin and hesperidin as bioactive flavonoids, possess antibacterial and antiviral activities. These bioactive ligands are also present in several fruits and vegetables. Based on the obtained results of secondary metabolites, we have selected only the major hit compounds with high abundance. Docking was performed for the selected (highly abundant) compounds and reported with the binding affinity. Docking studies are in good agreement with the experimental results. Since the main objective of the study was to report that the investigated fruits can serve as a promising functional food with medicinal properties, we validated this hypothesis with robust in silico docking approaches. Therefore, consuming such fruits and vegetables as such or as a complex mixture of phytochemicals can provide beneficial effects to human health by protecting against several infections. In addition to nutritional and antioxidant properties, kiwifruit and persimmon have potential role in pharmacological applications.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/12/4218/s1, Table S1: List of ligands identified from different varieties of kiwifruit pulp (‘Hayward’, ‘Bidan’ and Actinidia arguta) and persimmon ‘Fuyu’ pulp.

Author Contributions: Conceptualization, R.M.B.S. and S.G.; methodology, Y.M.K. and S.G.K.; software, S.A.L.; validation, K.S.H. and Y.S.P.; formal analysis, K.S.H. and Y.K.P.; investigation, S.A.L. and Y.K.P.; resources, S.G.K. and Y.M.K.; supervision, S.G.; writing—original draft preparation, R.M.B.S. and Y.S.P.; writing—review and editing, R.M.B.S. and S.G. All authors have read and agreed to the published version of the manuscript.

Funding: R.M.B.S. is thankful to Rashtriya Uchchatar Shiksha Abhiyan (RUSA 2.0) [F.24-51/2014-U, Policy (TN Multi-Gen), Dept. of Edn, GoI].
Acknowledgments: Thanks from all authors of the manuscript to Elena Katrich, Dinorah Barasch and Alina Nemirovski from Institute for Drug Research, Hebrew University of Jerusalem, for their assistance in the measuring of some indices in fruits.

Conflicts of Interest: No conflict of interest between the authors.

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