Nutritional and Pharmaceutical Applications of Bioactive Compounds in Tropical Fruits

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Abstract

During the past two decades our international scientific group investigated in vitro the physicochemical and nutritional characteristics of some cultivars of durian at various stages of ripening, mangosteen and new cultivars of snake fruit in comparison with widely used avocado, kiwifruit and mango, and in vivo their influence on laboratory animals. The main objective of the present study was to screen and compare the properties of water and ethanol extracts of selected exotic fruits and the influence of their physiologically active compounds on human health. The bioactive compounds were extracted with water and ethanol using similar procedures as are used in pharmaceutical applications and daily fruit consumption. Various analytical methods were used to detect biologically active metabolites derived from exotic fruits (polyphenols, flavonoids, flavanols, tannins, anthocyanins and ascorbic acid), antioxidant radical scavenging assays (DPPH, FRAP, CUPRAC, and ABTS), Fourier transform infrared (FT-IR) and ultraviolet (UV) spectroscopy, two (2D-FL) and three-dimensional (3D-FL) fluorimetry. The correlation between the polyphenols and other bioactive compounds, and their antioxidant activities, is reported for the studied fruit extracts. The properties of the soil, where the investigated fruits were grown, were studied as well. Supplementation of diets with exotic fruits positively affected the plasma lipid profile and antioxidant activity in rats fed cholesterol-containing diets. The interaction between drugs and serum albumin plays an important role in the distribution and metabolism of drugs. The properties of polyphenol extracts of exotic fruits showed the ability to quench serum albumin by forming complexes similar with the ones formed between the proteins and pure flavonoids such as catechin and quercetin. In conclusion, new application of fluorimetry and FTIR spectroscopy for rapid estimation of the quality of exotic fruits in particular and for any fruits and

vegetables in general is presented. It is necessary to promote a consumption of exotic fruits (a rich source of natural antioxidants) as a supplement to everyday human diet and for pharmaceutical applications.

INTRODUCTION

At this time of globalization, many tropical fruits can be found at the markets of Europe and North America. Most customers are not familiar with the nutritional values of these fruits in spite of the fact that the consumption of new exotic fruits, with their high nutritional and sensory values, has significantly increased in the past few years (Park et al., 2009; Poovarodom et al., 2010; Haruenkit et al., 2010; Dembitsky et al., 2011; Gorinstein et al., 2011; Leontowicz et al., 2011).

The aim of this research was to investigate the bioactivity of exotic Thai fruits such as durian, snake fruit and mangosteen, in water and ethanol extracts by the application of fluorimetry and FTIR spectroscopy. As far as we know, no results of such investigation have been published before.

MATERIALS AND METHODS

All chemicals were purchased from Sigma Chemical Co., St Louis, Missouri, USA. Samples of 'Mon Thong' durian (*Durio zibethinus* Murr. 'Mon Thong'), a new cultivar of snake fruit (*Salacca edulis* 'Sumalee'), and mangosteen (*Garcinia mangostana*) were harvested and collected in their ripe stage in 2011 from a 25-year-old commercial orchard in Chanthaburi province of eastern Thailand. All fruits were cleaned with tap water and dried, using five replicates of five fruits each. The edible parts of the fruits were prepared manually without using steel knives. The peeled fruits were weighed, chopped and homogenized under liquid nitrogen in a high-speed blender (Hamilton Beach Silex professional model) for 1 min. A weighed portion (50-100 g) was then lyophilized for 48 h (Virtis model 10–324), and the dry weight was determined. The samples were ground to pass through a 0.5-mm sieve and stored at -20° C until the bioactive substances were analyzed.

In Vitro Studies

Polyphenols, extracted from lyophilized fruits with water or with 50% ethanol (at a concentration of 25 mg mL^{-1}) for 48 h at room temperature, were determined by Folin-Ciocalteu method. Flavonoids, flavanols, tannins, anthocyanins, quercetin and ascorbic acid were determined spectroscopically as previously described. The antioxidant potentials were estimated by four complementary assays: ferric reducing antioxidant power (FRAP); 2,2-azino-bis (3-ethyl-benzothiazoline- 6-sulfonic acid) diamonium salt ($ABTS^{\cdot+}$); 1-diphenyl-2-picrylhydrazyl method (DPPH); and cupric reducing antioxidant capacity (CUPRAC) (Park et al., 2009; Haruenkit et al., 2010; Poovarodom et al., 2010; Gorinstein et al., 2011; Leontowicz et al., 2011). The presence of polyphenols in the fruit extracts and the interaction between polyphenols and bovine serum albumin (BSA) were studied by Fourier Transform Infrared (FT-IR) spectroscopy (Nicolet iS 10 FT-IR Spectrometer) and by 3-D fluorescence (3D-FL).

In Vivo Studies

The Animal Care Committee of the Warsaw University of Life Sciences (SGGW), Warsaw, Poland, approved this study. The in vivo studies were carried out on 25 male Wistar rats, divided into 5 diet groups, each of 5 animals. During 30 days of the experiment the rats of all 5 groups were fed basal diet (BD), which included wheat starch, casein, soybean oil, vitamin and mineral mixtures. The rats of the Control group were fed only the BD. The BD of the other 4 groups was supplemented with 1% of nonoxidized cholesterol (NOC) (Chol group), 1% of NOC in each group and 5% of lyophilized fruits: durian (Chol/Durian), snake fruit (Chol/Snake), and mangosteen (Chol/Mangosteen). At the end of the experiment, the rats were anaesthetized, using diethyl ether, and the blood samples were taken from the left atrium of the heart. Total cholesterol (TC), low-density

lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and plasma antioxidant activity were determined.

RESULTS AND DISCUSSION

The soil where the investigated fruits were grown was a sandy loam with the following properties: pH 5.4, EC 296 μS cm^{-1}, organic matter 3.7%, available P (Bray II) 1400 mg kg^{-1}, exchangeable (NH_4OAc) K, Ca and Mg 105, 1773 and 75.2 mg kg^{-1}, respectively; extractable (DTPA) Fe, Mn, Cu and Zn 104, 14.9, 15.8 and 7.2 mg kg^{-1}, respectively. These results did not differ from a previous study (Poovarodom et al., 2010).

The contents of polyphenols, flavanols, and anthocyanins in water extracts of mangosteen were significantly higher than in other two fruits (Table 1A). Flavonoids, tannins, and ascorbic acid in mangosteen were higher than in durian and equal to snake fruit. Similar results were obtained for the ethanol extracts: polyphenols, flavonoids, and flavanols were significantly higher in mangosteen than in other fruits (Table 1B). According to ABTS and FRAP, the highest antioxidant activity in water extracts was in mangosteen (Table 2A), and CUPRAC and DPPH values in mangosteen were similar to snake fruit, but not always significant (Table 2A). All values of antioxidant potentials in the ethanol extracts were higher in mangosteen that in other fruits (Table 2B). The total phenolics reported by others (Ashraf et al., 2011) were in the range of 690.62-998.29 mg L^{-1} (2.32-3.34 mg g^{-1} DW), which are similar to our data. The total flavonoids and vitamin C contents were found in the range of 211.36-220.34 (0.71-0.74 mg CE g^{-1} DW) and 18.87-25.1 mg L^{-1} (0.063-0.084 mg g^{-1} DW), respectively. These values were similar for flavonoids and higher in the content of vitamin C compared to Leontowicz et al. (2011). Such variations in the content of bioactive compounds can be explained by the year of growing, the stage of ripening, and mostly by the different extracts used in various studies. Other reports showed a big variation in the reported data (Kongkachuichai et al., 2010; Fu et al., 2011). The latter authors showed that mangosteen had the lowest FRAP (0.11 ± 0.01 μmol Fe (II) g^{-1} FW) in comparison with many other fruits, including durian (7.41 ± 0.58 μmol Fe (II) g^{-1}). TEAC values (μmol Trolox g^{-1} FW) showed a slight difference: 4.98 ± 0.17 for durian and 3.34 ± 0.01 for mangosteen. The phenolic contents (mg GAE 100 g^{-1} FW) for durian was 79.15 ± 3.05 and for mangosteen 43.68 ± 1.34. Opposite results were reported by Jayakumar and Kanthimathi (2011) based on antioxidant and proliferative properties of durian and mangosteen. Free radical scavenging activities of mangosteen extract prepared by different extraction methods (Pothitirat et al., 2010) were in accordance with our results. Maximum amounts of total phenolic compounds (26.96 g GAE 100 g^{-1} extract) and total tannins (46.83 g tannic acid equivalents 100 g^{-1} extract), and also DPPH-scavenging activity (EC_{50} 12.84 μg mL^{-1}) was achieved in 50% ethanol. This solvent is appropriate for extracting free radical-scavenging components, phenolic compounds, and tannins. The highest amount of quercetin was in durian 76.9±3.3 mg g^{-1}, lower in mangosteen 68.7±4.2, and lowest in snake fruit 61.3±2.2. These results differ slightly from our previous study in which the highest content of quercetin was detected in ripe durian (68.9±3.3 mg g^{-1}) (Haruenkit et al., 2010). These results were predictable – the tested samples were grown under slightly different climatic conditions and in another year.

The FTIR-spectra of water and ethanol extracts of the investigated fruits are shown in Figures 1A and 1B. The matching (%) of the peaks in the region from 4000 to 650 cm^{-1} and from 1806-806 cm^{-1} (Fig. 1A, Table 3) showed the highest matching (99%) between snake fruit and mangosteen in two regions of polyphenols extracted with ethanol. The peaks of polyphenols in water and ethanol extracts (Figs. 1A and 1B; Table 4) were compared with different standards. The matching of tannic acid and mangosteen in water and ethanol extracts was the highest between the phenolic acids (Fig. 1A, insert of tannic acid). The highest matching was estimated in the region of 3404-3206 cm^{-1} between quercetin and the investigated fruits in water and ethanol extracts: quercetin:snake fruit between of 50-52%; and 3000-2911 cm^{-1} of 81-84%; quercetin:durian between 3404-3206 cm^{-1} of 51-52%; and 3000-2911 cm^{-1} of 82-80%; and quercetin:mangosteen between

3404-3206 cm^{-1} of 0.51-0.50 and 3000-2911 cm^{-1} of 81-84% (Fig. 1B, insert of quercetin). These matching results show for the first time that FTIR spectra can be used for a rapid estimation of extracted bioactive compounds. The water extracts were relatively lower in the matching values than the ethanol ones, indicating that the yield of extracted bioactive compounds was lower in water than in ethanol extracts. Quercetin showed the highest matching in the investigated fruit extracts in comparison with flavonoids hesperidin and fisetin; caffeic, *p*-coumaric, gallic and tannic acids.

In our previous study, the FTIR-spectra data showed that the main bands in the durian samples were from 1700 to 800 cm^{-1} (1637, 1415, 1137, 1103, 1056, 995 and 923 cm^{-1}). A shift in the difference between the standards and the investigated samples can be explained by the method of extraction of the main polyphenols (Haruenkit et al., 2010; Leontowicz et al., 2011).

The results of the fluorescence of the investigated fruits are shown on Figure 2. One main and one minor peak appear at the approximate locations for durian (Fig. 2A, λem/ex 340/280 nm with FIU 737.6 and λem/ex 650/280 nm with FIU 105); mangosteen (Fig. 2B, λem/ex 330/280 nm with FIU 480 and λem/ex 620/280 nm with FIU 85.3); snake fruit (Fig. 2C, λem/ex 340/280 nm with FIU 979.3 and λem/ex 660/280 nm with FIU 120.9). The polyphenol spectra in water extracts are similar among these fruits. The results of binding affinity of BSA and extracted polyphenols of durian, snake fruit and mangosteen showed that the main quenching effect was with mangosteen (Fig. 2D, with the lowest line of FI=358). The main peak is at λem/ex=335-345/280 nm.

Our results showed that the fluorescence is significantly quenched because the conformation of the BSA changes in the presence of pure flavonoids and fruit extracts (Leontowicz et al., 2011). This interaction between quercetin and BSA was investigated using tryptophan fluorescence quenching. Our result agrees with others that quercetin, as an aglycon, is more hydrophobic and demonstrates strong affinity toward BSA. The data obtained by 2D-FL and 3D-FL fluorescence of quercetin binding in our studies correspond with Papadopoulou et al. (2005). Other results differ from ours, probably because of the variety of antioxidant abilities of pure flavonoids and different ranges of fluorimetry scanning ranges used in a similar study (Shi et al., 2010).

There are no publications on applications of 3D fluorescence spectra, therefore our present conclusions that 3-D fluorescence can be used as an additional tool for the characterization of the polyphenol extracts correspond with the previous data (Gorinstein et al., 2011). The biological relevance of quercetin interaction in the human organism is important from the point that this molecule of polyphenolic type extensively binds to human serum albumin (HSA), the most abundant carrier protein in blood. Our in vitro results of interaction of BSA and quercetin can be compared with other reports in vivo, showing the protective effects of quercetin on hepatic injury induced by different chemical reactions.

There are two regions (1700-1600 and 1550-1500 cm^{-1}) in the spectrum unique to the protein secondary structure (amides I and II). The shift in amide I and amide II peaks of BSA indicate the interaction between BSA and quercetin. The agreement of the peaks in the interaction between BSA and quercetin and BSA and the durian ripe extract (BSA+Quercetin):(BSA+DRipe) was about 99.9%, showing that the polyphenol extract of durian behaves similarly to pure quercetin. The best match was achieved with investigated fruits in comparison with pure quercetin. Our results are consistent with the cited in vivo results on application of pure quercetin, where it was shown that the pure quercetin behaves as do polyphenol extracts from durian fruit.

The in vivo studies showed an increase in plasma lipids level in all groups fed cholesterol-containing diets (Table 5). However, the diets supplemented with durian, snake fruit and mangosteen decreased TC by 4.5-5.3% and LDL-C by 6.2% (both not significant), while in the case of TG this decrease was significant and amounted to 26.3%. A significant decrease of plasma antioxidant activity was registered in Chol group vs. Control group, but only for the data obtained from the ABTS assay. A significant increase in plasma antioxidant activity was noted in the rat group supplemented with ripe durian

vs. the Chol Group – by 26.3% and 36.4%, according to ABTS and FRAP, respectively. A correlation between the increase of the plasma lipids and decrease in the plasma antioxidant activity: was observed – in the group of rats with the highest increase of plasma lipids (Chol), the decrease of the antioxidant activity was significantly higher than in other diet groups.

The fruits are a rich source of dietary antioxidants. Proanthocyanidins play one of the major roles, therefore, in this report in vitro studies were carried out on the interaction of quercetin with a protein molecule such as BSA. It is important to underline that not in every case it is necessary to use the fruits instead other treatments. This is in accordance with our results that cancer patients should use caution before consuming mangosteen products as they can potentially interact with cancer treatments and also affect blood sugar levels (Yeung, 2006).

FTIR, 2D-FL, and 3D-FL methods were applied to determine the effects of flavonoid complexation on the secondary structure of the protein by the quenching of the albumin fluorescence and the enhancement of the flavonoid fluorescence.

The contents of polyphenols, flavonoids, quercetin, flavanols, ascorbic acid and tannins in fruits and the antioxidant potentials as determined by four complementary assays (CUPRAC, DPPH, ABTS and FRAP) varied. Blood samples from rats fed cholesterol-containing diet, when supplemented with fruits, showed properties associated with reduced likelihood of heart and liver damage. The results of this investigation support our hypothesis and support the use of these fruits in human nutrition.

CONCLUSIONS

Exotic fruits at different stages of ripening, especially fully ripe, constitute an excellent source of effective natural compounds. The fruits possess antioxidant and health-protective properties for livers and aortas of rats fed cholesterol-enriched diet. The diets supplemented with exotic fruits significantly hindered the rise in plasma lipids and reduced the decrease in the plasma antioxidant activity. In conclusion, the contents of bioactive compounds and the antioxidant potential are relatively high in the studied fruits and varied depending on the extraction procedure. These fruits can be recommended for everyday consumption in fresh and dry forms as well as for pharmaceutical applications in water and ethanol extracts.

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Tables

Table 1. Bioactive compounds in water (A) and 50% ethanol (B) extracts[1, 2, 3] of the studied fruit samples.

A

Samples	Polyphenols (mg GAE)	Flavonoids (mg CE)	Flavanols (µg CE)	Tannins (mg CE)	Asc acid (mg)	Anthocyan (µg GC)
Durian	1.39±0.2[a]	0.400±0.01[a]	14.44±1.4[a]	6.96±0.7[a]	8.85±0.9[a]	26.68±2.8[a]
Snakefru	2.86±0.3[b]	0.189±0.01[b]	30.15±3.1[b]	5.31±0.4[b]	19.21±1.9[b]	26.66±2.4[a]
Mangost	5.70±0.7[c]	0.263±0.01[b]	86.62±8.9[c]	6.72±0.7[a]	22.18±2.1[b]	36.71±3.7[b]

B

Samples	Polyphenols (mg GA)	Flavonoids (mg CE)	Flavanols (µg CE)	Tannins (mg CE)
Durian	1.89±0.8[a]	0.21±0.01[a]	8.49±0.9[a]	8.00±0.7[a]
Snakefru	5.19±0.7[b]	0.47±0.03[b]	20.38±2.1[b]	6.29±0.6[b]
Mangost	13.81±1.4[c]	2.36±0.21[c]	1272.61±128.9[c]	8.48±0.9[a]

[1] Values are means ± SD of 5 measurements.

[2] Values in columns for every bioactive compound with the same solvent bearing different superscript letters are significantly different ($P < 0.05$).

[3] per g dry weight. Abbreviations: snakefru, snakefruit; mangost, mangosteen; GAE, gallic acid equivalent; CE, catechin equivalent; CGE, cyanidin-3-glucoside equivalent; Asc, ascorbic.

Table 2. The antioxidant activity (μMTE g^{-1}) in water (A) and 50% ethanol (B) extracts [1, 2, 3] of the studied fruit samples.

A

Samples	ABTS	FRAP	CUPRAC	DPPH
Durian	4.10±0.5[a]	1.78±0.2[a]	3.53±0.3[a]	2.02 ±0.2[a]
Snakefruit	5.32±0.5[a]	2.87±0.4[a]	16.33±1.7[b]	4.11±0.4[b]
Mangosteen	19.74±1.9[b]	5.36±0.6[b]	19.75±1.9[b]	6.00±0.6[b]

B

Samples	ABTS	FRAP	CUPRAC	DPPH
Durian	8.66±0.9[a]	2.34±0.3[a]	13.93±1.4[a]	3.19±0.3[a]
Snakefruit	20.80±2.1[b]	6.82±0.7[b]	22.49±2.3[b]	7.47±0.7[b]
Mangosteen	56.20±5.7[c]	20.77±2.2[c]	51.17±5.2[c]	21.94±2.2[c]

[1] Values are means ± SD of 5 measurements.

[2] Values in columns for every value of antioxidant activity bearing different superscript letters are significantly different ($P<0.05$).

[3] per g dry weight. Abbreviations: ABTS, 2, 2-Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; CUPRAC, Cupric reducing antioxidant capacity; FRAP, Ferric-reducing/antioxidant power; DPPH, 1-Diphenyl-2-picrylhydrazyl method.

Table 3. Matching of the peaks (%) in the FTIR spectrum of extracted polyphenols in water (W) and ethanol (Et) between investigated fruits.

Extract samples	Matching of peaks (%) in 4000-650 cm^{-1}	Matching of peaks (%) in 1806-806 cm^{-1}
Snake/DurianW	63	60
Snake/MangW	56	56
Durian/MangW	75	78
Snake/DurianEt	96	97
Snake/MangEt	99	99
Durian/MangEt	98	98

Abbreviations: Snake, snake fruit; Mang, mangosteen.

Table 4. Matching of the peaks (%) in the FTIR spectrum of extracted polyphenols in water (W) and ethanol (Et) and standards.

Extract samples	Max matching of peaks (%) in 4000-650 cm^{-1}
Hesperidin/MangW/Et	62/56
Ferulic ac/MangW/Et	31/24
Tannic ac/MangW/Et	73/72
Fisitin//MangW/Et	47/42
Caffeic ac/SnakeW/Et	66/63
p-Coum ac/SnakeW/Et	58/61
Gallic ac/SnakeW/Et	67/69
Gallic ac/DurianW/Et	65/57
Gallic ac/MangW/Et	60/68
Quercetin/SnakeW/Et	81/84
Quercetin/DurianW/Et	82/80
Quercetin/MangW/Et	81/84

Abbreviations: Snake, snake fruit; Mang, mangosteen.

Table 5. Plasma lipids (mM L^{-1}) and antioxidant activity (mMTE L^{-1}) after feeding with different diets.

Diet Groups	TC	LDL-C	TC/HDL	TG	ABTS	FRAP
Control	1.6 ± 0.3^{a}	1.1 ± 0.2^{a}	2.9 ± 0.2^{a}	0.9 ± 0.4^{a}	1.33 ± 0.1^{a}	0.13 ± 0.01^{a}
Control/Chol	2.1 ± 0.4^{b}	1.6 ± 0.4^{b}	4.3 ± 0.3^{b}	1.9 ± 0.5^{b}	1.18 ± 0.2^{b}	0.11 ± 0.01^{b}
Chol/Durian	2.0 ± 0.2^{b}	1.5 ± 0.3^{b}	4.0 ± 0.4^{b}	1.4 ± 0.6^{ab}	1.49 ± 0.2^{ab}	0.15 ± 0.01^{a}
Chol/Snake	2.0 ± 0.4^{b}	1.6 ± 0.4^{b}	4.2 ± 0.5^{b}	1.5 ± 0.4^{ab}	1.23 ± 0.1^{a}	0.13 ± 0.01^{a}
Chol/Mang	1.9 ± 0.3^{ab}	1.45 ± 0.5^{b}	3.8 ± 0.6^{ab}	1.6 ± 0.6^{ab}	1.25 ± 0.6^{a}	0.14 ± 0.06^{a}

Values are means ± SD, n = 5. Means in rows without superscript letters in common differ significantly ($P < 0.05$).

Abbreviations: TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL, high density lipoprotein cholesterol; TG, triglycerides; AA, antioxidant activity; ABTS, [2, 2'-azinobis (3-ethylbenzo-thiazoline-6-sulfonic acid)]; FRAP, Ferric-reducing/antioxidant power; Chol, cholesterol; Snake, snake fruit; Mang, mangosteen.

Figures

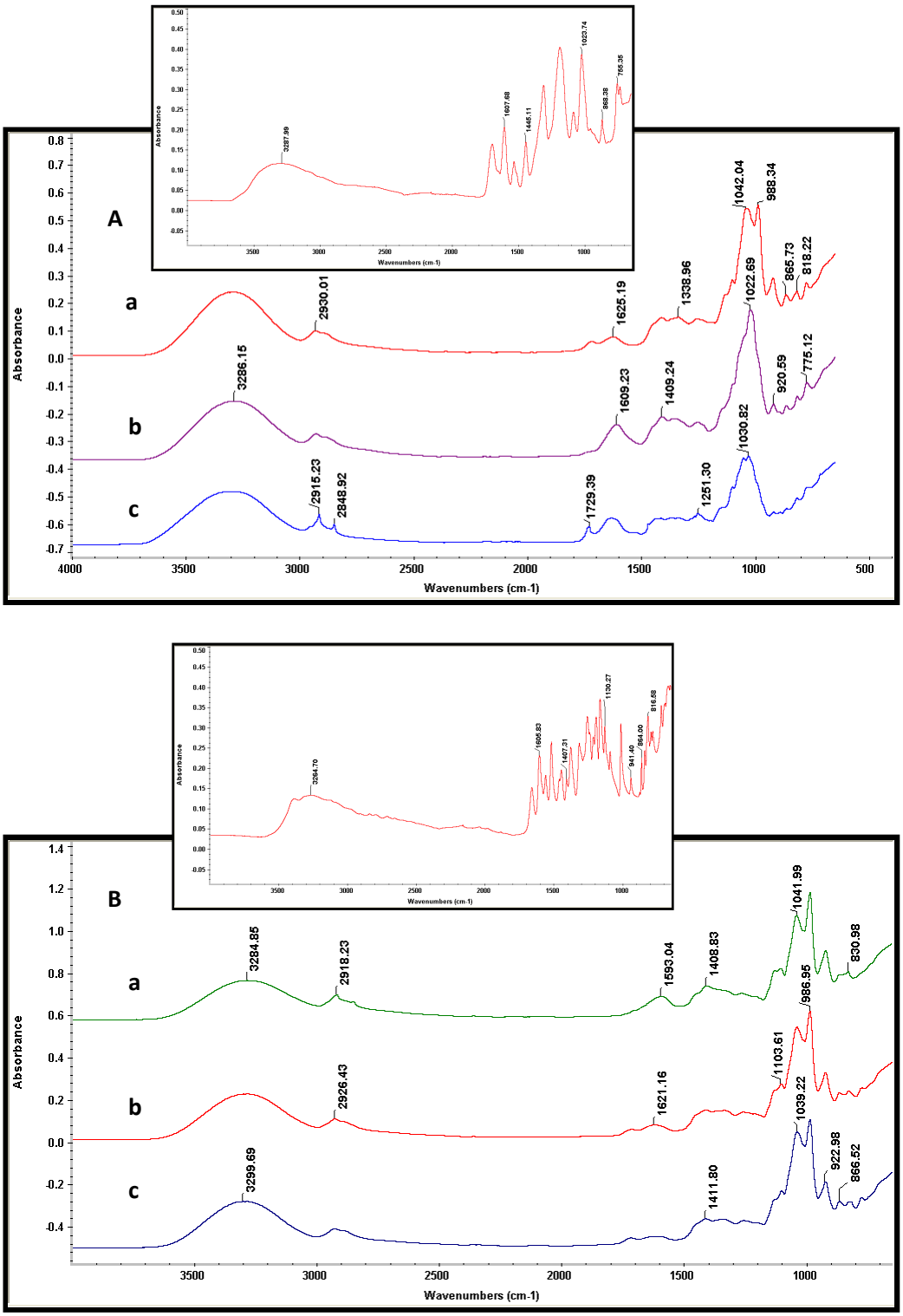

Fig. 1. FTIR spectra of A, water extracts of snake fruit (a), durian (b), mangosteen (c). Insert: tannic acid. B, 50% ethanol extract of durian (a), mangosteen (b), snake fruit (c). Insert: quercetin.

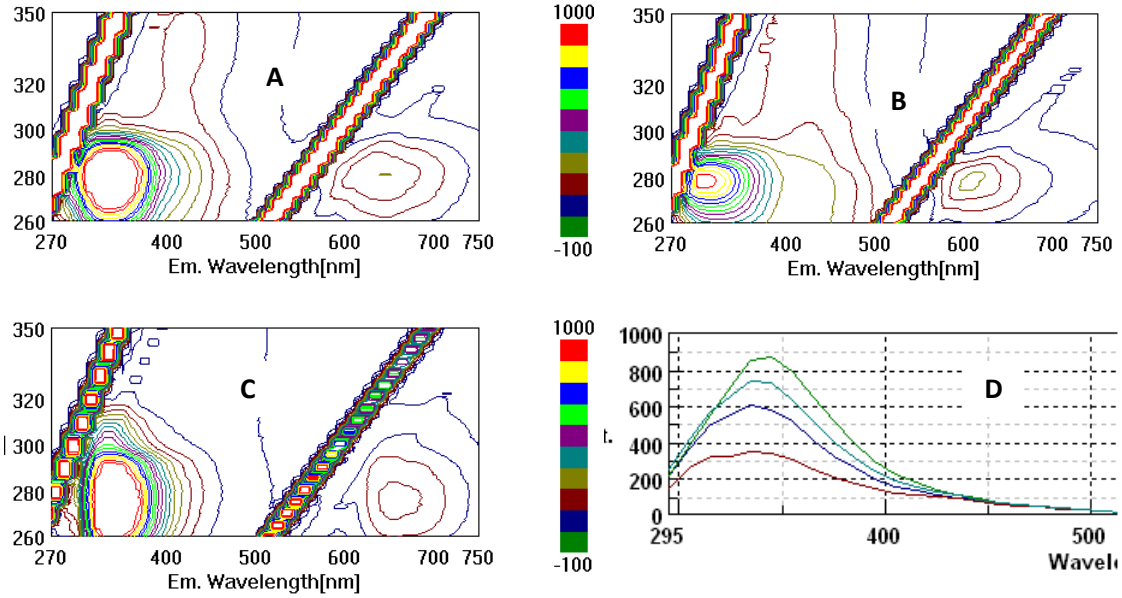

Fig. 2. Contour maps in three-dimensional fluorescence (3D-FL) of water extracts (2.5 mg mL^{-1}) of (A) durian, (B) mangosteen, and (C) snake fruit. The 3D-FL were run emission mode and fluorescence intensity up to 1000, emission wavelengths from 270 to 750 nm and excitation wavelengths from 260 to 350 nm; scanning speed was 1000 nm min^{-1}, emission wavelength on x-axis and excitation wavelength on y-axis. 2D-FL) spectra illustrate the interaction between BSA and water extracts of studied fruits. D, change in the fluorescence intensity as a result of binding affinity between BSA and water extracts of investigated fruits: 0.0132 µM BSA [upper line with fluorescence intensity (FI) of 876.8]; 0.0132 µM BSA + 50 µg ml^{-1} of water extract of durian 1 hour at 37°C (second line from the top with FI=742.9). 0.0132 µM BSA + 50 µg ml^{-1} of water extract of snake fruit 1 hour at 37°C (third line from the top with FI =605.3); 0.0132 µM BSA + 50 µg ml^{-1} of water extract of mangosteen (lower line with FI=358). Fluorescence intensities are on y-axis and emission wavelengths on x-axis.