Antioxidant properties and bioactive constituents of some rare exotic Thai fruits and comparison with conventional fruits

In vitro and in vivo studies

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The aim of this research was to investigate the bioactivity of durian, snake fruit and mangosteen, rare exotic Thai fruits. These fruits were compared among them and with conventional fruits: durian with mango and avocado, and snake fruit with mangosteen and kiwifruit in order to find the preferable diet for human consumption. The contents of polyphenols, flavonoids, flavanols, tannins, anthocyanins, ascorbic acid and carotenoids, and the level of antioxidant potential by ABTS, DPPH, FRAP and CUPRAC in different extracts (methanol, water, acetone, and hexane) were determined. The presence of polyphenols (flavonoids and phenolic acids) in the investigated samples was characterized by Fourier transform infrared (FT-IR) spectroscopy and three-dimensional fluorimetry (3D-FL). The in vitro studies were carried out on 25 male Wistar rats, divided into 5 diet groups, each of 5. 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), mangosteen (Chol/Mangosteen). After the experiment diets supplemented with exotic fruits significantly hindered the rise in plasma lipids and hindered 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 among them depending on the extraction procedure. FT-IR and 3D-FL can be used as additional tools for identification and comparison of bioactive compounds. Supplementation of diets with exotic fruits positively affects plasma lipid profile and antioxidant activity in rats fed cholesterol-containing diets.

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1. Introduction

There is convincing evidence that fruits and vegetables are playing a beneficial role in the prevention and even treatment of different diseases (Aramwit, Bang, & Srichana, 2010; Borochov-Neori et al., 2008; Nakamura, Nagata, Oba, Takatsuka, & Shimizu, 2008; Takachi et al., 2008; Ou et al., 2002).

There has also been a great interest in the potential health benefits of exotic fruits due to their antioxidant content and bioactive compounds. Higher synergistic activity is shown when combinations of natural antioxidants were used (Hait-Darshan, Grossman, Bergman, Deutsch, & Zurigl, 2009). Among the exotic fruits durian (Durio zibethinus Murr.) is less investigated than the snake fruit (Salacca edulis Reinw) and mangosteen (Garcinia mangostana) (Arancibia-Avila et al., 2008; Gorinstein et al., 2009). Therefore, it was decided to compare durian with two other exotic fruits (snake fruit and mangosteen) and with the wide consumed mango (Mangifera indica L.), kiwifruit (Actinidia chinensis) and avocado (Persea americana). It has been shown that the above-mentioned fruits possessed high nutritional and bioactive properties (Aralas, Mohamed, & Bakar, 2009; Kho, Ismail, Mohd-Esa, & Idris, 2008; Masibo & He, 2008; Pedraza-Chaverri, Cárdenas-Rodríguez, Orozco-Ibarra, & Pérez-Rojas, 2008; Ribeiro, Barbosa,
Queiroz Knödler, & Schieber, 2008; Terasawa, Sakakibara, & Murata, 2006). However, these data were determined using different antioxidant methods (Leontowicz, Leontowicz, Drezewicki, et al., 2007; Leontowicz, Leontowicz, Jastrzebski, et al., 2007; Toledo et al., 2008; Park et al., 2009). In recent studies (Ikram et al., 2009; Isabelle et al., 2010; Leong & Shui, 2002) the characterization and comparison of the exotic fruits was based on the Singapore market, where the majority of the fruits were imported from Malaysia. Oppositely in this research the origin of exotic fruits was from Thailand. In order to obtain reliable results all samples of the studied fruits were investigated at the same stage of ripeness using unified methods for determination of the antioxidant potential. In our previous investigation the antioxidant potential (AP) was determined only by two assays: [2, 2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)] (ABTS) with Trolax equivalent antioxidant capacity (TEAC) and 1, 1-diphenyl-2-picylhydrayzidal radical (DPPH) (Haruenkit et al., 2007). In the present report two additional assays were used: Ferric-reducing/antioxidant power (FRAP) and Cupric reducing antioxidant capacity (CUPRAC) and also the previous two assays to analyze the exotic and conventional fruits of the new harvest. FT-IR and 3D fluorimetry were used in this study as additional analytical tools to characterize bioactive compounds of the different extracts.

Antihypercholesterolemic effects of different fruits were studied in vivo studies on animal models. Savithri, Appian, and Natesan Shamnugam (2009) investigated the reaction of Averrhoa bilimbii Linn. fruit and its extracts using Triton-induced hypercholesterolemia in rats as a model. Gallerah and Gallerah (2009) tested dried plums as a part of diet in lowering of lipids in rats. Valcheva-Kuzmanova et al. (2007) used as a part of a rat’s diet Aronia melanocarpa juice which is rich in anthocyanins. Beppu et al. (2009) have studied the hypolipidemic effects of ethanol extracts of Citrus depressa and Annona atemoya on KK ay mice fed with moderately high-fat diet. In spite of the reviewed data, it was interesting to compare the exotic fruits as supplementation of the diets in the present study. Therefore in vivo studies were carried out with the in vitro experiments.

As far as we know, no results of such comparative investigation of rare exotic fruits, mango, kiwifruit and avocado have been published.

2. Materials and methods

2.1. Chemicals

Six-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picylhydrayzidal (DPPH), Folin–Ciocalteau reagent (FCR), lanthanum(III) chloride hexahydrate, FeCl₃ ×6H₂O, CuCl₂ ×2H₂O, 2,9-dimethyl-1,10-phenanthroline (neocuproine) were purchased from Sigma Chemical Co., St Louis, MO, USA. 2, 4, 6-Trimpyridyl-s-triazine (TPTZ) was purchased from Fluka Chemie, Buchs, Switzerland. All reagents were of analytical grade. Deionised and distilled water was used throughout.

2.2. Samples and preparation

Durian (D. zibethinus Murr. cv Mon Thong), snake fruit (S. edulis Reinw. cv Sumalee), mango (M. indica L. cv Nam Dok Mai No. 4) were harvested from a 25-year-old Mon durian orchard, in Chantaburi Province, eastern Thailand in 2009 and were at the same stage of ripeness. Kiwifruit (A. chinensis, cv ‘Hayward’) cultivar at its commercial maturity stage was harvested in the orchard (Heanam County, Jeonnam Province, Korea, 2009). Avocado (P. americana) was donated by Mehadrin Tnupor Export (MTEX) L.P, Be’erot Yitzhak, Israel. All fruits were cleaned with tap water and dried, using about 1 kg of fruits with five replicates. The edible parts of the above-mentioned fruits were prepared for this investigation 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). A weighed portion (50–100 g) was then lyophilized for 48 h (Virtis model 16-324), 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.

2.3. Determination of dietary fibers and microelements

Dietary fibers in the selected samples were analyzed by the modified AOAC method. The samples were treated with heat stable α-amylose, protease, and amyloglucosidase, followed by centrifugation (15 min, 3000 g) to separate the soluble and insoluble fractions and dialysis against water, which substituted ethanol precipitation of soluble dietary fiber (Prosksy, Asp, Schweizer, De Vries, & Furda, 1992; Mañas, Bravo, & Saura-Calixto, 1994).

Determination of minerals (Na, K, Mg, and Ca) and trace elements (Fe, Cu, Zn, and Mn) was done as follows. Lyophilized fruit samples (0.8 g) were mineralized in a microwave oven with concentrated HNO₃. The concentrations of elements were estimated by a Perkin-Elmer 5100 ZL atomic absorption spectrometer (Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, U.K.), using the flame method for Na, K, Mg, Ca, Fe, Cu, and Zn and the flameless method for Mn.

2.4. Determination of the contents of the main bioactive compounds, Fourier Transform Infrared (FT-IR) spectra of polyphenols and fluorimetry

The presence of polyphenols in the investigated fruit samples was studied by Fourier Transform Infrared (FT-IR) spectroscopy. A Bruker Optic GmbH Vector FT-IR spectrometer (Bruker Optic GMBH, Attingen, Germany) was used to record IR spectra. A potassium bromide microdisk was prepared from finely ground lyophilized powder of 2 mg of fruit samples with 100 mg of KBr (Sinelli, Spinardi, Di Egidio, Mignani, & Casiraghi, 2008).

Two-dimensional fluorescence measurements (2D-FL) were done using a model FP-6500, Jasco Spectrofluorometer, serial N261332, Japan. Fluorescence emission spectra for all fruit samples at a concentration of 0.25 mg/mL were taken at emission wavelength (nm) of 330, and recorded from wavelength of 265 to a wavelength of 310 nm, at emission wavelengths of 685 nm from 300 to 750 nm; and at excitation of 350 nm from 370 to 650 nm. Standard of 0.01 mM catechin or quercetin in methanol was used. The three-dimensional spectra (3D-FL) of water extracts of the investigated fruits were collected with subsequent scanning emission spectra from 270 to 750 nm at 1.0 nm increments by varying the excitation wavelength from 260 to 350 nm at 10 nm increments. The scanning speed was set at 1000 nm/min for all measurements. All measurements were performed with emission mode and with intensity up to 1000 (Wulf, Geyer, Nicolai, & Zude, 2005; Yin, Li, Ding, & Wang, 2009).

Phenols were extracted from lyophilized fruits with 100% methanol, acetone, hexane and water (concentration 25 mg/mL) at room temperature twice during 3 h. Lyophilized fruit samples were also extracted from a 50-mg aliquot with 5 mL of 60% methanol/water with heating at 90°C for 3 h for unconjugated polyphenols (UCP) and under the same conditions with 5 mL of 1.2 M HCl in 60% methanol/water for conjugated polyphenols (CP) with some modifications. The samples were cooled, diluted to 10 mL with methanol and centrifuged for 5 min at 4000×g to remove solids (Hertog, Hollman, & Venema, 1992; Gorinstein et al., 2008; Perez-Jimenez & Saura-Calixto, 2005; Vinson, Su, Zubic, & Rose, 2001).

The polyphenols were determined by Folin–Ciocalteau method with measurement at 750 nm with spectrophotometer (Hewlett-Packard, model 8452A, Rockville, USA). The results were expressed as mg of gallic acid equivalents (GAE) per g DW (Singleton, Orthofer, &
2.5. Determination of antioxidant potential

The AP was determined by four complementary assays:

1. 2, 2-Azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS\textsuperscript{+}) method for the screening of antioxidant activity is reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants, including flavonoids, hydroxycinnamates, carotenoids, and plasma antioxidants. The pre-formed radical monocation ABTS is generated by oxidation of ABTS with potassium persulfate and is reduced in the presence of such hydrogen-donating antioxidants. The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption are taken into account when determining the antioxidant activity. ABTS\textsuperscript{+} radical cation was generated by the interaction of ABTS (7 mM/L) and \( K_2 S_2 O_8 \) (2.45 mM/L). This solution was diluted with methanol until the absorbance in the samples reached 0.7 at 734 nm (Re et al., 1999).

2. Ferric-reducing/antioxidant power (FRAP) assay measures the ability of the antioxidants in the investigated samples to reduce ferric-tripiridyltriazine (Fe\textsuperscript{3+} \textbullet TPTZ) to a ferrous form (Fe\textsuperscript{2+}), which absorbs light at 593 nm (Benzie & Strain, 1996).

3. Cupric reducing antioxidant capacity (CUPRAC): This assay is based on utilizing the copper(I)–neocuproine \textbullet Cu(I)–Nc reagent as the chromogenic oxidizing agent. The absorbance at 450 nm was recorded against a reagent blank (Apak, Guclu, Ozyurek, & Karademir, 2004).

4. Scavenging free radical potentials were tested in a methanolic solution of 1, 1-Diphenyl-2-picrylhydrazyl method (DPPH). The degree of decoloration of the solution indicates the scavenging efficiency of the added substance. In its radical form, DPPH has an absorption band at 515 nm which disappears upon reduction by antiradical compounds. DPPH solution (3.9 mL, 25 mg/L) in methanol was mixed with the sample extracts (0.1 mL), then the reaction progress was monitored at 515 nm until the absorbance was stable (Brand-Williams, Cuvelier, & Berset, 1995).

2.6. Animal study

The Animal Care Committee of the Warsaw University of Life Sciences (SGGW), Warsaw, Poland had approved this study. The experiments were done on young growing 3–4 weeks old Wistar male rats (n = 25) with the mean weight of 111 g at the beginning of the trial. During the experiment the feed intake was about 4.4 g per day \textbullet 30 days of the period of the experiment = 111 g + 132 g = 243 g. At the end of the experiment the rats were about 7–8 weeks old. The rats were divided into 5 groups of 5 in each group and were named Control, Chol, Chol/Snake, Chol/Mangosteen, Chol/Durian. However the diet groups containing mango, kiwi and avocado were not investigated in in vivo studies, because of the similarity to the exotic fruits.

2.7. Diets

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) of analytical grade (Chol group), 1% of NOC and 5% of lyophilized following fruits: snake fruit (Chol/Snake), mangosteen (Chol/mangosteen), durian (Chol/durian). In most of the experiments in vivo cellulose was used as control fiber (Anderson, Jones, & Riddell-Mason, 1994). In Control groups animals were supplemented with a percentage of cellulose similar to the percentage of supplementation with fruit in the other groups. 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 and plasma was prepared according to test regulations and used for laboratory tests, which included the determination of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) and plasma antioxidant activity (PAA). Also liver and aorta histopathology was examined.

ABTS, DPPH and FRAP were adopted for the determination of plasma antioxidant activity.

2.8. Statistical analysis

The results of this investigation in vivo are means \textpm SD, n = 5. Differences between groups were tested by two-way ANOVA. In the assessment of the antioxidant potential, Spearman correlation coefficient (R) was used. Linear regressions were also calculated. The P values of <0.05 were considered significant.

3. Results and discussion

3.1. Dietary fibers and microelements

The contents of total, insoluble and soluble dietary fibers in durian, snake fruit, mangosteen, mango and kiwifruit differ minimally (Table 1, P >0.05), and in avocado was significantly higher than in...
the other five fruits (P < 0.05). Other authors showed similar results in the amount of dietary fibers (Mahattanatawee et al., 2006), where fourteen tropical fruits from south Florida including green and ripe mangos were studied. It was found that total dietary fiber changed from 0.9 to 7.2 g/100 g FW, which is higher than the data shown in Table 1. The content of Na in all 6 studied fruits was different (Table 2), but not significantly (P > 0.05). The significantly highest content of K, Mg and Ca was registered in kiwifruit (P < 0.05). The content of Fe and Mn in mango was significantly higher than that in the other five fruits (P < 0.05). Also the content of Zn in mangosteen was higher than that in the other five fruits but not significantly (P > 0.05). The content of Cu in mango and durian was significantly higher than that in the other four fruits (P < 0.05). From multidimensional pattern recognition techniques (Poovarodom et al., 2010), the factor analysis (FA) was involved in the mineral composition of fruits. The results of FA of durian, avocado and mango fruits, as well as snake fruit, mangosteen and kiwifruit, based on the content of macro- and micro-elements K, Ca, Mg, Na, Fe, Mn, Cu, Zn, are depicted on a score plot of factors. Avocado and mango are more similar on the element profile than durian when compared with avocado or mango (Poovarodom et al., 2010). The potassium/magnesium ratio for snake fruit was 21.10 and for kiwifruit: 20.16; and the magnesium/calcium ratio for snake fruit was 2.76 and for kiwifruit 0.50. The obtained present results on minerals and microelements in the new harvest of the investigated fruits (Table 2) are in agreement with our previous data on the same fruits which were collected two years ago (Gorinstein et al., 2010; Haruenkit et al., 2007; Haruenkit et al., 2010; Poovarodom et al., 2010). As was found in this investigation, the content of minerals in the studied fruits vary significantly (P < 0.05). K has the significantly highest content among the studied minerals (P < 0.05). Among the fruits the significantly highest content of minerals (P < 0.05) was in kiwifruit and mangosteen: of K, Mg and Ca and Fe and Mn, respectively. 3.2. Bioactive compounds Fluorimetric measurements showed (Table 3) the following peaks (nm) with the fluorescence intensity units (FIU): durian, mangosteen, snake fruit, mango, avocado and kiwifruit with the range of the spectra λ ex (290–400 nm) with emission of 685 and a peak of 343–344 nm and the lowest of 240.14 FIU for snake fruit and the highest of 276.58 for mango (Fig. 1A, B). Catechin of 0.001 mM as a standard for flavonoids was measured at the same conditions and showed the same peak as the fruit extracts at 343.5 nm with a higher FIU of 666.83 (Fig. 1A, B). At excitation wavelength of 350 nm with the range of the spectra λ ex (370–650 nm) the following peaks appeared: the first peak appeared at 390.5–391.5 nm with FIU in the range from 43.8 to 77.3. A shift appeared in the second peak for the fruits: from 423.5 nm to 448 nm and FIU from 11.1 for durian to 70.0 for avocado. Catechin showed one peak at 390.5 with FIU of 35.1. At emission of 330 nm the recording was for all fruits with the shift of the main peak, detected between 280.5 and 282.5 nm with 202.4 and 484.0 for avocado and mangosteen, respectively. The second peak was measured exactly at the same wavelength of λ ex 300 nm with 90.1 for avocado and the highest was for snake fruit of 149.3, respectively. Catechin showed one peak at 282 with FIU of 700.4 (Fig. 1A, B). At emission of 450 nm for mangosteen, snake fruit, mango and avocado one peak in the range between 303 and 359 appeared with 33.07 and 37 FIU for avocado and

### Table 2

Contents of minerals and trace elements in the studied fruits (mg kg⁻¹ DW).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Durian</th>
<th>Snake fruit</th>
<th>Mangosteen</th>
<th>Mango</th>
<th>Kiwifruit</th>
<th>Avocado</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>220.2 ± 11.4</td>
<td>254.3 ± 12.2</td>
<td>242.5 ± 12.1</td>
<td>289.1 ± 14.1</td>
<td>250.2 ± 12.1</td>
<td>231.2 ± 11.6</td>
</tr>
<tr>
<td>K</td>
<td>15.94 ± 42.6</td>
<td>12.473 ± 32.4</td>
<td>13.159 ± 30.8</td>
<td>14.181 ± 41.8</td>
<td>18.686 ± 49.0</td>
<td>12.592 ± 42.2</td>
</tr>
<tr>
<td>Mg</td>
<td>691.2 ± 29.7</td>
<td>628 ± 29.2</td>
<td>626 ± 27.9</td>
<td>545 ± 23.2</td>
<td>834 ± 31.9</td>
<td>684 ± 29.5</td>
</tr>
<tr>
<td>Ca</td>
<td>199.8 ± 10.1</td>
<td>242.8 ± 12.1</td>
<td>316.7 ± 14.9</td>
<td>273.1 ± 15.8</td>
<td>1637 ± 42.1</td>
<td>343 ± 12.1</td>
</tr>
<tr>
<td>Fe</td>
<td>6.71 ± 0.3</td>
<td>14.6 ± 0.3</td>
<td>14.19 ± 0.7</td>
<td>19.1 ± 0.9</td>
<td>23.1 ± 0.9</td>
<td>17.3 ± 0.8</td>
</tr>
<tr>
<td>Mn</td>
<td>8.26 ± 0.4</td>
<td>11.44 ± 0.5</td>
<td>30.58 ± 1.5</td>
<td>7.8 ± 0.5</td>
<td>21.7 ± 1.3</td>
<td>7.4 ± 0.4</td>
</tr>
<tr>
<td>Zn</td>
<td>4.92 ± 0.3</td>
<td>11.41 ± 0.6</td>
<td>12.54 ± 0.7</td>
<td>9.02 ± 0.3</td>
<td>11.44 ± 0.6</td>
<td>8.91 ± 0.4</td>
</tr>
<tr>
<td>Cu</td>
<td>4.92 ± 0.3</td>
<td>3.71 ± 0.3</td>
<td>1.63 ± 0.3</td>
<td>5.12 ± 0.3</td>
<td>1.09 ± 0.3</td>
<td>3.96 ± 0.3</td>
</tr>
</tbody>
</table>

Values are means ± SD of 5 measurements. Means in rows without superscript letters in common differ significantly (P < 0.05).
mango, respectively. The second peak was only in snake fruit, avocado and catechin at 286 nm with 9.72 FIU. At emission of 740 nm the following data were shown: the main peak was detected at 371, 371.5 and 372 for all fruits except snake fruit, with FIU from 239.5 for durian and 364.4 for mango (Table 3) and the second peak was at 739.5 nm and 739.2 with 43.14 for kiwifruit and 125.8 for mango. Catechin showed two peaks at 371.5 and 739.5 with 590.86 and 311.90.

In three-dimensional fluorescence spectra the excitation and the emission (Fig. 1E, F, G) wavelengths and the fluorescence intensity were used as the axes in order to investigate the information of the fruit samples, and the contour spectra provided more information. The x-axis represents the emission spectra from 270 to 750 nm, while the y-axis is the excitation spectra from 260 to 350 nm for: E1, E2, and E3 (durian, snake fruit, and mangosteen); F1, F2, and F3 (durian, avocado, and mango); G1, G2, and G3, (snake fruit, mangosteen, and kiwifruit), respectively. The result shows that the three-dimensional fluorescence cross maps of fruits in comparison with standards (catechin and gallic acid) are obviously different (Gorinstein et al., 2010; Poovarodom et al., 2010). One main peak and one minor can easily be observed at the approximate location for durian (Fig. 2 E1, F1, λem/λex 340/275 nm with FIU 737.6 and λem/λex 650/275 nm with FIU 105); snake fruit (Fig. 2 E2, G1, λem/λex 340/275 nm with FIU 979.3 and λem/λex 660/275 nm with FIU 1203); mango (Fig. 1 E3, G2, λem/λex 330/275 nm with FIU 480 and λem/λex 620/275 nm with FIU 85.3); avocado (Fig. 1 F2, λem/λex 320/275 nm with FIU 999 and λem/λex 610/275 nm with FIU 67.3); mango (Fig. 1 F3, λem/λex 340/275 nm with FIU 539.8 and λem/λex 640/275 nm with FIU 65.4); kiwifruit (Fig. 3 G1, λem/λex 340/275 nm with FIU 705.2 and λem/λex 660/275 nm with FIU 94.5). As can be seen the polypehnon spectra in water extracts are similar among these fruits, but differ from the methanol extracts.

3.2.1. FT-IR spectra

The comparison of three exotic fruits durian, snake fruit and mangosteen showed more intensive bands in durian (Fig. 2A, a) than the other samples (Fig. 2A, b and c). The main bands in durian sample were from 1700 to 800 cm⁻¹ (1637, 1415, 1137, 1103, 1056, 995 and 923 cm⁻¹). The other two samples were similar and overlaid in the same area. Fig. 2B presents three spectra of durian (a), avocado (b) and mango (c). The bands were in the same region, and some additional bands were detected in the overlaying, such as 1595, 1435 and 1262 cm⁻¹. The same bands appeared in these three samples. Fig. 2C shows the comparison between snake fruit (a), mangosteen (b), and kiwifruit (c). One additional band was detected at 1725 cm⁻¹. The wavelength numbers of FT-IR spectra for catechin at 831, 1040, 1112, 1144, 1285, 1478, 1512 and 1611 cm⁻¹ were assigned to –C=H alkenes, –C–O alcohols, –C=O alcohols, –OH aromatic, C–O alcohols, C–H alkenes, C=C aromatic ring and C=C alkenes, respectively. Gallic acid showed the following wavelength numbers (cm⁻¹): 866, 1026, 1227, 1451, 1542 and 1619. A shift in the difference between the standards and the investigated samples can be explained by the method of extraction of the main polyphenols. FT-IR and 3 D fluorometry were used as rapid methods for comparison of methanol extracts from the studied fruits as an additional indicator of similarity or difference between the studied fruits, based on the bands and peaks in the polyphenol region. These analytical techniques can be recommended for any plant extracts.

The contents of total polyphenols (mg GAE g⁻¹ DW) were in range from 2.58 to 8.46 in water extracts for durian, avocado, mango, for kiwifruit, mangosteen and snake fruit (P<0.05, Figs. 3, 4, Table 4). These data are similar to the cited results on bioactive compounds (Arancibia-Avila et al., 2008; Gorinstein et al., 2009; Gorinstein et al., 2010; Leontowicz, Leontowicz, Drzewiecki, et al., 2007; Leontowicz, Leontowicz, Jastrzebski, et al., 2007) and other authors (Corral-Aguayo, Yahia, Carrillo-Lopez, & Gonzalez-Aguilar, 2008; Rocha Ribeiro, Queiroz, Lopes Ribeiro de Queiroz, Milagres Campos, & Pinheiro Sant’Ana, 2007; Wolfe et al., 2008). The contents of total flavonoids (mg CE g⁻¹ DW, Table 4) in water extracts were in the range from 1.523 to 0.163 for durian, snake fruit, mangosteen, avocado, kiwifruit and mango. These results on flavonoids are corresponding with the cited literature (Park et al., 2008, 2009; Robles-Sanchez et al., 2009; Toledo et al., 2008), Luximmon-Ramma, Bahouran, and Crozier (2003) found that the content of flavonoids in eleven exotic fruits commonly consumed in Mauritius was from 21 to 712 μg g⁻¹ FW. The contents of flavonals (μg CE/g DW, Table 4) for water extracts were in the range from 67.05 to 2.11 for durian, avocado, mango, snake fruit, mangosteen and kiwifruit, respectively (P<0.05). These results are in agreement with our previous published data (Arancibia-Avila et al., 2008; Harunenkit et al., 2007; Toledo et al., 2008) and other cited reports (Seeram, 2008), where some tropical fruits and among them mangosteen were investigated for phenolic phytochemicals such as, anthocyanins, flavonals, flavanols, proanthocyanidins, ellagitanins, gallotannins, xanthones, and coumarins. The contents of anthocyanins (mg CGE/g DW, Table 4) for water extracts were in range from 17.12 to 2.51 for all 6 investigated fruits, respectively (P<0.05). The obtained anthocyanin contents are in agreement with our previous published data (Arancibia-Avila et al., 2008; Toledo et al., 2008) and data of other authors (Luximmon-Ramma et al., 2003). These authors found that the content of proanthocyanidins in eleven commonly consumed exotic fruits from Mauritius was from 7 to 2561 μg g⁻¹ FW.

The contents of ascorbic acid (mg g⁻¹ DW, Table 4) for water extracts were in the range from 2.52 to 15.25 for investigated samples, respectively (P<0.05). The contents of ascorbic acid in investigated samples were higher than determined by others: for kiwifruit 1.88–3.00 (Tavarini, Degl’Innocenti, Remorini, Massai, & Guidi, 2008), 2.06 (Amadio, Colelli, Hasey, & Kader, 2007), and 1.69 mg g⁻¹ FW (Jung, Lee, Bae, & Choi, 2007). It was found that the content of vitamin C was in the range from 8 to 1426 μg g⁻¹ FW in analyzed eleven commonly consumed exotic fruits from Mauritius (Luximmon-Ramma et al., 2003).

The contents of tannins (mg CEG⁻¹ DW, Table 4) for water extracts were in the range from 0.27 to 6.48 for the investigated samples. Total carotenoids and β-carotene (μg/g) were from 1.47 to 15.18 and 0.38 to 13.62, respectively. The obtained results on tannins and carotenoids for the investigated fruits are in agreement with our previous published data (Gorinstein et al., 2010; Park et al., 2009; Poovarodom et al., 2010). As can be seen, the contents of the bioactive compounds in water extracts in the studied fruits differ significantly. The higher content of polyphenols and tannins was in snake fruit, flavonoids, flavanols and anthocyanins — in durian, vitamin C — in kiwifruit, total carotenoids and β-carotenoids — in mango. Most of the bioactive compounds in exotic and conventional fruits were comparable (P<0.05). Durian contains the significantly highest contents of flavonoids, flavanols and anthocyanins followed by snake fruit — the significantly highest content of polyphenols and tannins (P<0.05 in both cases).
3.3. Fruits antioxidant activity

It was found that the 1) ABTS data were in range of 10.32±1.6 to 19.78±2.1 μMTE/g for durian, avocado and mango (P<0.05, Fig. 3); 2) FRAP data were in the range of 18.43±1.8 to 22.65±2.3 μMTE/g for avocado, durian and mango (P<0.05, Fig. 3); 3) CUPRAC data were in range of 15.62±1.6 to 20.13±2.1 μMTE/g for durian, avocado and mango (P<0.05, Fig. 3); 4) The obtained results of antioxidant values of the fruits are in agreement with our previous published data (Gorinstein et al., 2010; Haruenkit et al., 2010; Poovarodom et al.,

Fig. 2. FTIR spectra of lyophilized water extracts: A durian (a), snake fruit (b) and mangosteen (c); B: durian (a) avocado (b) and mango (c); C, snake fruit (a), mangosteen (b) and kiwifruit (c).
antioxidant activity was in mangosteen, followed by kiwifruit and mango. Our data were slightly lower (Figs. 3, 4, Table 4).

Our data can be compared with another report (Barreto, Benassi, & Mercadante, 2009), where in 18 pulps from tropical fruits ascorbic acid, total phenolics, flavonoids, carotenoids and free radical scavenger activity evaluated by the ABTS assay were determined. Fruits showed a relatively high amount of bioactive compounds. Free radical scavenger showed a high correlation with total polyphenolic compounds ($r = 0.99$) and flavonoids ($r = 0.86$). However, the correlation was found to be very poor with ascorbic acid ($r = 0.02$) and with total carotenoid levels ($r = 0.08$). The same correlation coefficients were evaluated in our research only with slightly higher value for flavonoids ($r = 0.92$), ascorbic acid ($r = 0.56$), and carotenoids ($r = 0.42$). Our results are in agreement with Vijaya Kumar Reddy, Sreeramulu, and Raghanath (2010), where fourteen commonly consumed fresh and ten dry fruits were studied. The antioxidant activity and polyphenols of both fresh and dry fruits showed marked variation, as it was shown in our data. Correlation analyzed between the polyphenols and antioxidant activity as assessed by the two methods showed that phenolics may contribute maximally to the ABTS ($r = 0.84$) and to lesser extent to DPPH ($r = 0.77$) in fresh fruits. Our data were slightly higher than the cited ones in the estimation of the correlation coefficients. The data in the literature is poor on the investigated exotic fruits, therefore our results were compared with a number of Brazilian exotic fruits, which were characterized in relation to their bioactive compounds and antioxidant capacity (Genovese, Da Silva Pinto, De Souza Schmidt Goncalves, & Lajolo, 2008). Camu—camu (Myrciaria dubia) presented the highest vitamin C and total phenolics contents (397 and 1797 mg/100 g FW, respectively) and the highest DPPH scavenging capacity. Coquinho (Butia capitata) also showed a significant vitamin C content (43 mg/100 g FW). A good correlation between total phenols and DPPH scavenging activity was found for fruits ($r = 0.987$). Quercetin and kaempferol derivatives were the main flavonoids present in all samples. According to our results, camu—camu and araca can be compared with the snake fruit and might be sources of bioactive compounds. Our results were in agreement with the results of others (Ikram et al., 2009), where antioxidant activity and total phenolic acids were determined by two similar antioxidants assays (DPPH and FRAP). Their findings showed that the fruits from Sallacca and Garcinia had higher antioxidant capacity compared to other studied genera. Durio [total phenolics (TP), mg GAE/g DW, and antioxidant capacity (AC) based on β-carotene bleaching assay, %] showed different ranges of TP and AC from 1.56 to 7.52 and AC from 73.87 to 60.36, depending on the cultivar and genera as for Durio zibenthinus (Durian Tutong, TP = 1.56 and AC = 73.87) for D. zibenthinus (Durian Isu Oren, TP = 4.32 and AC = 64.86) and for Durio kutejensis (Durian Isu Kuning, TP = 4.70 and AC = 54.05). Garcinia showed as well differences in the polyphenols [total phenolics (TP), mg GAE/g DW, and antioxidant capacity (AC) based on β-carotene bleaching assay, %] from the lowest for Garcinia atroviridis (Assam Gelugo) – TP = 3.49; middle of TP = 4.89 for Garcinia parvifolia (Kundung Sarawak) and the highest – TP = 85.10 for Garcinia pra tiniana (Cerapu) (Ikram et al., 2009). In Paththamakanporkorn, Puwastien, Nitithamyong, and Sirichakwal (2008) the data for mango—steer were similar: polyphenols (TP, mg GAE/g DW) TP = 3.49; middle of TP = 4.89 and AC from 73.87 to 60.36, depending on the cultivar and genera as for Durio zibenthinus (Durian Tutong, TP = 1.56 and AC = 73.87) for D. zibenthinus (Durian Isu Oren, TP = 4.32 and AC = 64.86) and for Durio kutejensis (Durian Isu Kuning, TP = 4.70 and AC = 54.05). Garcinia showed as well differences in the polyphenols [total phenolics (TP), mg GAE/g DW, and antioxidant capacity (AC) based on β-carotene bleaching assay, %] from the lowest for Garcinia atroviridis (Assam Gelugo) – TP = 3.49; middle of TP = 4.89 for Garcinia parvifolia (Kundung Sarawak) and the highest – TP = 85.10 for Garcinia prat iniana (Cerapu) (Ikram et al., 2009). In Paththamakanporkorn, Puwastien, Nitithamyong, and Sirichakwal (2008) the data for mango—steer were similar: polyphenols (TP, mg GAE/g DW) TP = 4.15, ORAC and FRAP (μM TE/g DW) – 30.56 and 25.43, respectively. In Leong and Shui (2002) l-ascorbic acid equivalent antioxidant capacity (AEC) calculated on dry weight (mg/g DW) was for snake fruit 17.16, followed by mangosteen of 7.65, kiwifruit – 7.60, mango – 6.48, and avocado of 4.16. The ranking orders of total antioxidant capacity were as follows: snake fruit > mangosteen > kiwifruit > mango > avocado (Leong & Shui, 2002).

Mango Nam Dok Mai (M. indica L.) was reported to have 5.2 mg GAE/g DW, ORAC and FRAP values (μM TE/g DW) of 96.6 and 36.8 (Paththamakanporkorn et al., 2008). Mango Kiew-sa-웨어 had lower data: total polyphenols about 2.94, ORAC and FRAP of 30.56 and 26.43. In

Fig. 3. Polyphenols and antioxidant activities of durian, mango and avocado extracts: A) Methanol (60%)-HCl extracts of conjugated polyphenols, and methanol (60%) extracts of unconjugated polyphenols; B) methanol polyphenols extracts, and water polyphenol extracts; C) acetone polyphenol extracts and hexane polyphenol extracts. Dur, Durango; Man, mango; Avo, avocado; CP, conjugated polyphenols; UCP, unconjugated polyphenols; POL, polyphenols; CUPRAC, cupric reducing antioxidant power; GAE, gallic acid equivalent; and Antiox, antioxidant. All data are calculated per gram dry weight.
mangosteen values of polyphenols were 4.15, ORAC and FRAP — 122.74 and 31.78. The rank of ORAC and FRAP antioxidant activity values in fruits was different: phenols: mango (Nam Dok Mai) > mangosteen > mango (Kiew-sa-weya); FRAP: mango (Nam Dok Mai) > mangosteen > mango (Kiew-sa-weya); ORAC: mangosteen > mango (Kiew-sa-weya) > ORAC. Therefore, such extraction was suggested in this study for the extraction of total phenols. These results are exactly in line with Hertog et al. (1992), reporting that the extraction efficiency could thus depend on the water/methanol ratio (Hertog et al., 1992; Perez-Jimenez & Saura-Calixto, 2005; Gorinstein et al., 2008). The order of the investigated exotic fruits is not exactly the same as in the cited literature. Such difference depends on the year of collection, the cultivar, the post harvest conditions, the extraction of bioactive compounds and the applied assays for the antioxidant determination.

3.4. Investigation in vivo

One of the main risk factors of atherosclerosis is hypercholesterolemia. Decrease of LDL reduces the risk of this disease. Oxidized LDL cholesterol is the main contributor of the development of atherosclerotic lesions, because it stimulates macrophage cholesterol accumulation. Serum high-density lipoprotein (HDL) levels are inversely related to the risk of atherosclerosis. Fruits extracts of high antioxidative capacity and unique polyphenolic composition are reported to be beneficial in atherosclerosis prevention (Borochov-Neori et al., 2008), therefore the results with the exotic fruits in vivo are important for a better understanding of the bioactivity of the investigated fruits.

After the experiment in vivo diets supplemented with exotic and other fruits significantly hindered the rise in plasma lipids and hindered a decrease in the plasma antioxidant activity in rats fed cholesterol-containing diet. It was recorded that the level of plasma lipids in diet groups with investigated fruits vs. Chol group was significantly less (P < 0.05): TC — 12.1–10.2%, LDL-C — 13.3–11.4%, HDL-C — 12.2–10.8% and TG — 14.1–13.2% (Fig. 5). A significant decrease was registered in the plasma antioxidant activity in all cholesterol fed vs. Control group (P < 0.05). However, the decrease in the antioxidant activity in Durian/Chol vs. Chol Group was significantly less: by 16.9–14.7%, 21.9–20.8% and 11.8–10.5%, according to ABTS, DPPH and FRAP, respectively. The decrease in the plasma antioxidant activity was predictable. As was shown by Mahfouz and Kummerow (2000), cholesterol-rich diets have different effects on lipid peroxidation, cholesterol oxides, and antioxidant enzymes in rats and rabbits.

The above cited results of the present investigation which was conducted with the fruits collected from the new harvest are in agreement with our published data on the change in the plasma cholesterol spectrum and antioxidant activity with the exotic fruits from the previous harvest (Haruenkit et al., 2007; Leontowicz, Leontowicz, Drzewiecki, et al., 2007; Leontowicz, Leontowicz, Jastrzebski, et al., 2007). Our findings agree with those of Savithri et al. (2009), where A. polynophenolic flavonoids, Flava, flavanoids, Flava, flavanols, Antho, anthocyanins, Vit C, vitamin C, Totalcar, total carotenoids, β-Car, β-Carotenoids, CE, catechin equivalent; GAE, gallic acid equivalent, and CGE, cyanidin-3-glucoside equivalent.

Values are means ± SD. Means in rows without superscript letters in common differ significantly (P < 0.05).
In the liver (Beppu et al., 2009). Our results corresponded with this. The results were in agreement with Valcheva-Kuzmanova et al. (2007), where the apoE-deficient mouse, which develops atherosclerotic lesions rapidly when fed cholesterol, was used to determine the ability of dried plums to reduce atherosclerosis. Similarly, repeated oral administration of ethanol extracts of C. depressa and A. atemoya potently lowered the plasma triglyceride (TG) concentrations of KK&y mice fed a moderately high-fat diet for 4 weeks. Thus, reduced fatty acid mobilization from the adipose tissue by the A. atemoya extract may result in the reduction in TG synthesis in the liver (Beppu et al., 2009). Our results corresponded with this report on the hypolipidemic effects of C. depressa and A. atemoya. Our results were in agreement with Valcheva-Kuzmanova et al. (2007), where A. melanocarpa fruit juice (AMFJ) was applied orally for 30 days at doses of 5, 10 and 20 mL/kg. In rats fed the cholesterol-containing diets, AMFJ significantly hindered an increase in plasma lipids (total cholesterol, low-density lipoprotein cholesterol and triglycerides) because of cholesterol feeding. Body weight gains, liver weights and liver and aorta histopathology were not influenced either by high-

4. Conclusions

The contents of bioactive compounds and the antioxidant potentials are relatively high in all studied exotic fruits, such as durian, snake fruit, and mangosteen and traditional ones such as avocado, mango and kiwifruit. The contents of total polyphenols (mg GAE g−1 DW) were in the range from 2.58 to 8.46 in water extracts for durian, mango, and kiwifruit, respectively. Singlet oxygen scavenger activity was in the range from 32.9 to 64.5 μMTE/g for durian, mango and melon, respectively, and from 63.5 to 9.43 for kiwifruit, mangosteen and snake fruit, respectively. The supplementation of diets with these fruits positively affects plasma lipid profile and antioxidant activity in rats fed cholesterol-containing diets. The combination of in vitro with in vivo studies may allow to have a better information on the association between the content of bioactive compounds in the studied fruits and their effects on health.

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Fig. 5. Effects of supplementation to rats with exotic fruits in diets on: A) LDL-C, ABTS, TC; B) HDL-C, DPPH, TG. Cont, control diet; Cont/Chol, control diet with cholesterol; Chol/Dur, diet with cholesterol supplemented with durian; Chol/Mango, diet with cholesterol supplemented with mango; ABTS, 2,2′-azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt; and DPPH, 1-diphenyl-2-picrylhydrazyl method.
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