

## Comparative Study of Health Properties and Nutritional Value of Durian, Mangosteen, and Snake Fruit: Experiments In vitro and In vivo

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In vitro and in vivo studies of the health and nutritional properties of durian (*Durio zibethinus* Murr.) were compared with snake fruit (*Salacca edulis* Reinw.) and mangosteen (*Garcinia mangostana*). Dietary fibers, minerals, and trace metals were comparable. Total polyphenols (mg of GAE/100 g of FW) and flavonoids (85.1 ± 6.1) were significantly higher ( $p < 0.05$ ) than in snake fruit (217.1 ± 13.2 (mg of CE/100 g of FW)), durian (309.7 ± 19.3 and 61.2 ± 4.9), and mangosteen (190.3 ± 12.1 and 54.1 ± 3.8). Antioxidant activity ( $\mu\text{M TE}/100\text{ g of FW}$ ) of durian measured by DPPH and ABTS assays (228.2 ± 13.4 and 2016.3 ± 81.1) was significantly higher ( $p < 0.05$ ) than in snake fruit (110.4 ± 7.9 and 1507.5 ± 70.1) and mangosteen (79.1 ± 5.9 and 1268.6 ± 62.3). HPLC/DAD analysis of durian ( $\mu\text{g}/100\text{ g of FW}$ ) showed that quercetin (1214.23 ± 116.7) was present at levels three times that of caffeic acid, and twice as high as *p*-coumaric and cinnamic acids. The correlation coefficients between the bioactive compounds of fruits and their antioxidant activities were high ( $R^2 = 0.99$ ). Male Wistar rats (25) were divided into five dietary groups: the control group was fed the basal diet (BD); in addition to BD, the cholesterol (Chol) group was supplemented with 1% of Chol; the diets of the Chol/Durian, Chol/Snake, and Chol/Mangosteen groups were supplemented with 5% of these fruits, respectively. It was found that diets supplemented with durian, and to a lesser degree with snake fruit and mangosteen, significantly hindered the rise in plasma lipids and the decrease in antioxidant activity. The nutritional values were comparably high. In conclusion, it could be suggested that inclusion of studied tropical fruits, especially durian, in known disease-preventing diets could be beneficial.

**KEYWORDS:** Exotic fruits; bioactive compounds; identification; antioxidant activity; rats; plasma lipid; and antioxidant levels

### INTRODUCTION

A significant decrease in morbidity and mortality from cardiovascular and other diseases among fruit and vegetable consumers was reported recently. The protective effects of these

natural products are related to their antioxidants: phenolic compounds and, to a lesser extent, dietary fibers. Consumption of a healthy diet rich in fruits and vegetables and reduced in saturated and total fat and cholesterol concomitantly reduced oxidative stress (1–4).

Many exotic tropical fruits are available to consumers of North America and Europe (5, 6). Among them is durian (*Durio*

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*zibethinus* Murr.), a native of Thailand, which is less investigated (7). It is natural that a new kind of fruit attracts consumers; however, the nutritional and health values of this fruit are not known to most of them. Therefore, we decided to identify the bioactive compounds and the antioxidant activities of durian and compare the results with those of other investigated fruits (8–12), such as snake fruit (*Salacca edulis* Reinw.) and mangosteen (*Garcinia mangostana* L.).

To receive reliable data on the investigated fruits, we used two complementary radical scavenging assays: [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) with Trolox equivalent antioxidant capacity (TEAC) and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Correlation was done with the polyphenols determined by the Folin–Ciocalteu (FC) method.

As far as we know, there are no published results of comparative investigations of durian, snake fruit, and mangosteen, including experiments on laboratory animals.

## MATERIALS AND METHODS

**Chemicals.** Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), BHA (butylated hydroxyanisole), ABTS<sup>+</sup> [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)], Folin–Ciocalteu reagent, cholesterol of analytical grade (USP), DPPH (1, 1-diphenyl-2-picrylhydrazyl), and all standards of phenolic acids and flavonoids were obtained from Sigma Chemical Co., St. Louis, MO.

**Samples.** Mature samples of durian, native of Thailand (*Durio zibethinus* Murr. cv *Mon Thong*), snake fruit, native from Southeast Asia (*Salacca edulis* Reinw.), and Mangosteen, native from Malaysia (*Garcinia mangostana* L.), harvested in 2006, were purchased at a local market in Bangkok. Harvesting and determination of maturity was carried out by very skilled Thai workers. They combined the following techniques: day count, character of fruit spines, tapping the fruit, and color and shape of all fruit. Only durian fruits were cut with peduncle intact and brought down carefully. The samples were left for 1 day and cut open to get mature durian flesh with firm texture and no smell. Some of the fruits were left for another 4 days to ripen until their flesh became soft and smelled normal.

The edible parts of the above-mentioned fruits were prepared manually without using steel knife. The fruits were cleaned with tap water and dried, using five replicates of five fruits each. 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.

**Dietary Fibers.** Dietary fibers in the selected samples were analyzed by the modified AOAC method (13). Samples were treated with heat-stable  $\alpha$ -amylase, protease, and amyloglucosidase, followed by centrifugation (15 min, 3000 g) to separate the soluble and insoluble fractions and then dialysis against water.

**Extraction of Polyphenols.** Defatted lyophilized fruit samples were extracted from a 50 mg aliquot with 5 mL of 50% methanol/water with heating at 90 °C for 3 h for free polyphenols (FP) and under the same conditions with 5 mL of 1.2 M HCl in 50% methanol/water for total polyphenols (TP). The samples were cooled, diluted to 10 mL with methanol, and centrifuged for 5 min at 4000 g with a benchtop centrifuge to remove solids (2).

**Polyphenol Determination.** The Folin–Ciocalteu method was used (14), and the measurements were performed at 765 nm with gallic acid as the standard. The results were expressed as milligrams of gallic acid equivalents (GAE)/100 g of FW.

**Flavonoids.** The absorbance of flavonoids (extracted with 5% NaNO<sub>2</sub>, 10% AlCl<sub>3</sub>·6H<sub>2</sub>O, and 1 M NaOH) was measured at 510 nm with the standards prepared similarly with known (+)-catechin concentrations. The results were expressed as milligrams of catechin equivalents CE/100 g of FW.

**Table 1.** Applied Composition Gradient of the Binary ACN + H<sub>2</sub>O Mobile Phase

time (min)	program	ACN (%)	H <sub>2</sub> O (%)
0–2	constant composition	5	95
0–22	composition change	rise from 5 to 25	drop from 95 to 75
22–32	composition change	rise from 25 to 55	drop from 75 to 45
32–50	constant composition	55	45

**Minerals (Na, K, Mg, Ca) and Trace Elements (Fe, Cu, Zn, and Mn).** Lyophilized samples (0.8 g) were mineralized in a microwave oven with concentrated HNO<sub>3</sub>. The concentrations of above-mentioned elements were estimated by a Perkin-Elmer 5100 ZL atomic absorption spectrometer (Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, England), using the flame method for Na, K, Mg, Ca, Fe, Cu, and Zn and a flameless method for Mn.

**Antioxidant Activities.** Determination of the antioxidant activity of the studied fruits was done as follow:

The ABTS<sup>+</sup> [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation was generated by the interaction of ABTS (250  $\mu$ M) and K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (40  $\mu$ M). The absorbance was monitored exactly 1 and 6 min at 734 nm after the addition of 990  $\mu$ L of ABTS<sup>+</sup> solution to 10  $\mu$ L of fruit extracts or Trolox standards in methanol or phosphate-buffered saline (pH 7.4). For the modified assay, ABTS was dissolved in 20 mM acetate buffer (pH 4.5) and prepared with potassium persulfate as described above (15).

In the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay the volume of fruit extracts was adjusted to 100  $\mu$ L by adding MeOH. A 0.1 mM methanolic solution of DPPH was added (5  $\mu$ L). Changes in the sample's absorbance were measured at 517 nm. BHA was used for comparison. Two antioxidant assays (DPPH and ABTS) were compared for the same periods of time (10, 30, 60, and 120 min) and the same concentration of the investigated fruit's methanolic extracts (10 mg/mL). Trolox equivalent antioxidant capacity (TEAC) was expressed as micromolar trolox equivalents (TE)/100 g of FW (15).

**High-Performance Liquid Chromatography with the Diode Array Detection (HPLC/DAD) Analysis.** The analysis was performed on the P580A LPG liquid chromatograph with the Gina 50 autosampler and the UVD340V DAD diode array detector (Gynkotek/Dionex, Germering, Germany); the column was a Tosoh Biosep cartridge filled with TSK gel ODS-80 TM, 5  $\mu$ m, 250 mm length, 4.6 mm i.d. The chromatographic column was thermostated at 40 °C. The 50  $\mu$ L volumes of durian polyphenol extracts were introduced to the HPLC/DAD system. Ethanol solutions (0.1 mg mL<sup>-1</sup>) of vanillic, caffeic, *p*-coumaric, and cinnamic acids, morin, quercetin, myricetin, apigenin, and campherol were used as standards. The analyses were carried out with the gradient of the mobile phase composition (Table 1).

**Rats and Diets.** The Animal Care Committee of the Warsaw Agricultural University had approved this study. The mean weight of the Wistar rats ( $n = 25$ ) at the beginning of the experiment was 115 g. Rats were divided into five groups of five and housed in the first part of the experiment (1–21 days) in plastic cages and then (22–26 days) in metabolic cages of the same Tecniplast, 21020, Italy. These groups were named control, cholesterol (Chol), Chol/Durian, Chol/Snake, and Chol/Mangosteen (10, 11). For the 26 days of the experiment, the rats of all groups were fed a basal diet (BD). The control group was given only the BD. The BD of the other groups was supplemented with 10 g/kg of nonoxidized Chol (NOC) for the Chol group, 10 g/kg of NOC and 50 g/kg of durian for the Chol/Durian group, 10 g/kg of NOC and 50 g/kg of snake fruit for the Chol/Snake group, and 10 g/kg of NOC and 50 g/kg of mangosteen for the Chol/Mangosteen group. To assess the nutritional value and the efficiency of the used diets, the digestibility of their dry matter (DM) and crude protein (CP), feed intake, body gains, and feed and protein efficiency ratios (FER and PER) were determined (7, 16–19). 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 total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and plasma antioxidant activity were determined. The ABTS<sup>+</sup> and DPPH tests were adopted for determi-

**Table 2.** Dietary Fibers and Antioxidants in Durian, Snake Fruit, and Mangosteen<sup>a</sup>

indices	Durian	Snake fruit	Mangosteen
TDF, g/100 g of FW	1.20 ± 0.10 a	1.10 ± 0.10 a	0.90 ± 0.09 b
IDF, g/100 g of FW	0.80 ± 0.08 a	0.75 ± 0.07 a	0.60 ± 0.06 b
SDF, g/100 g of FW	0.40 ± 0.05 a	0.35 ± 0.04 a	0.30 ± 0.03 b
TP, mg of GAE/100 g of FW	309.7 ± 19.3 a	217.1 ± 13.2 b	190.3 ± 12.1 b
FP, mg of GAE/100 g of FW	37.1 ± 1.9 a	33.2 ± 1.7 a	14.0 ± 0.9 b
TF, mg of CE/100 g of FW	85.1 ± 6.1 a	61.2 ± 4.9 b	54.1 ± 3.8 c
FF, mg of CE/100 g of FW	21.2 ± 1.4 a	14.1 ± 0.9 b	7.2 ± 0.5 c
TDPPH, mM TE/100 g of FW	228.2 ± 13.4 a	110.4 ± 7.9 b	79.1 ± 5.9 c
FDPH, mM TE/100 g of FW	35.3 ± 1.8 a	31.2 ± 1.6 a	19.4 ± 1.2 b
TABTS, mM TE/100 g of FW	2016.3 ± 81.1 a	1507.5 ± 70.1 <sup>b</sup>	1268.6 ± 62.3 c
FABTS, mM TE/100 g of FW	321.2 ± 21.1 a	318.1 ± 20.1 a	260.4 ± 14.2 b

<sup>a</sup> Values are means ± SD of 5 measurements. Means in rows without letters in common differ significantly ( $P < 0.05$ ). Abbreviations used: TDF, total dietary fibers; FW, fresh weight; IDF, insoluble dietary fibers; SDF, soluble dietary fibers; TP, total polyphenols; FP, free polyphenols; TF, total flavonoids; FF, free flavonoids; TDPPH, (1,1-diphenyl-2-picrylhydrazyl) determined in the fruit extracts of total polyphenols; FDPH, in the extracts of free polyphenols; TABTS, [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] in the fruit extracts of total polyphenols; FABTS, in the fruit extracts of free polyphenols.

nation of the plasma antioxidant activity. ABTS<sup>+</sup> was done exactly as described previously with 10  $\mu$ L of plasma (20). In the DPPH test, the reaction mixture (25  $\mu$ L of plasma, 75  $\mu$ L of methanol, and 0.8 mL of 75  $\mu$ M DPPH) was maintained in the dark at room temperature for 90 min, and the absorbance at 517 nm was then recorded (21). The results of antioxidant activity were expressed in millimolar trolox equivalents (TE)/L.

**Statistical Methods.** Values of the results of in vitro and in vivo experiments are given as means ± SD of five measurements. Where appropriate, data were tested by two-way ANOVA using GraphPad Prism, version 2.0 (GraphPad Software, San Diego, CA), following by Duncan's new multiple range test to assess differences between groups means. Differences of  $P < 0.05$  were considered significant.

## RESULTS

**In vitro. Dietary Fibers.** Total dietary fibers (TDF, g/100 g of FW) (classified as a polysaccharide that escapes hydrolysis by animals and human digestive tract enzymes) was between 0.9 and 1.2 for mangosteen and durian, respectively. The highest concentration of the dietary fibers was in durian (Table 2); however, it was only significant vs mangosteen ( $P < 0.05$ ). The proportions of insoluble fiber (IDF) fraction to soluble were, on average, in the ranges  $67.17 \pm 0.87$  and  $32.83 \pm 0.88\%$ , respectively (Table 2).

**Polyphenols and Flavonoids.** The amounts of total and free polyphenols (mg of GAE/100 g of FW) and total and free flavonoids (mg of CE/100 g of FW) for durian, snake fruit, and mangosteen were ranged from 309.7 to 190.3 and 37.1 to 14.0 and from 85.1 to 54.1 and 21.2 to 7.2, respectively (Table 2).

**Minerals and Trace Elements.** The contents of Na, K, and Ca (Table 3) were significantly higher in durian ( $P < 0.05$ ) than in other fruits. Mg was estimated as the highest in mangosteen, but without significance ( $P > 0.05$ ). In the three studied fruits, the trace elements were in the following order: Fe > Mn > Zn > Cu. Only Fe and Cu were significantly higher in durian and Mn in snake fruit ( $P < 0.05$ ).

**Antioxidant Activities.** A combination of DPPH, ABTS, and Folin assays were used. These assays take into account the wide variety and range of action of antioxidant compounds presented in actual fruits, but all these assays are based on electron transfer. The assays were prolonged for 120 min at the same temperature and same concentration of the extract (10 mg/mL) in order to see the changes in the maximum of antioxidant activity in comparable scale (panels A and B in Figure 1), because the time that is used in routine assays is not enough for the fruit extracts (15). From the kinetic curves, it can be concluded that

**Table 3.** Minerals (Na, K, Mg, and Ca, mg/100 g of FW) and Trace Elements (Fe, Mn, Zn, and Cu,  $\mu$ g/100 g of FW) in Durian, Snake Fruit, and Mangosteen<sup>a</sup>

indices	Durian	Snake fruit	Mangosteen
Na	2.1 ± 0.1 b	1.9 ± 0.1 b	1.1 ± 0.1 b
K	201.2 ± 13.7 b	191.2 ± 12.6 b	101.3 ± 9.6 b
Mg	11.23 ± 1.1 b	7.16 ± 0.5 a	13.2 ± 1.2 b
Ca	18.32 ± 0.3 c	6.11 ± 0.4 a	12.3 ± 1.1 b
Fe	991.1 ± 18.5 c	301.7 ± 11.2 a	512.6 ± 13.3 b
Mn	145.2 ± 9.9 a	249.9 ± 11.7 b	112.6 ± 8.4 a
Zn	32.3 ± 2.9 a	35.1 ± 2.9 a	31.6 ± 2.7 a
Cu	24.1 ± 1.8 b	8.4 ± 0.6 a	8.7 ± 0.6 a

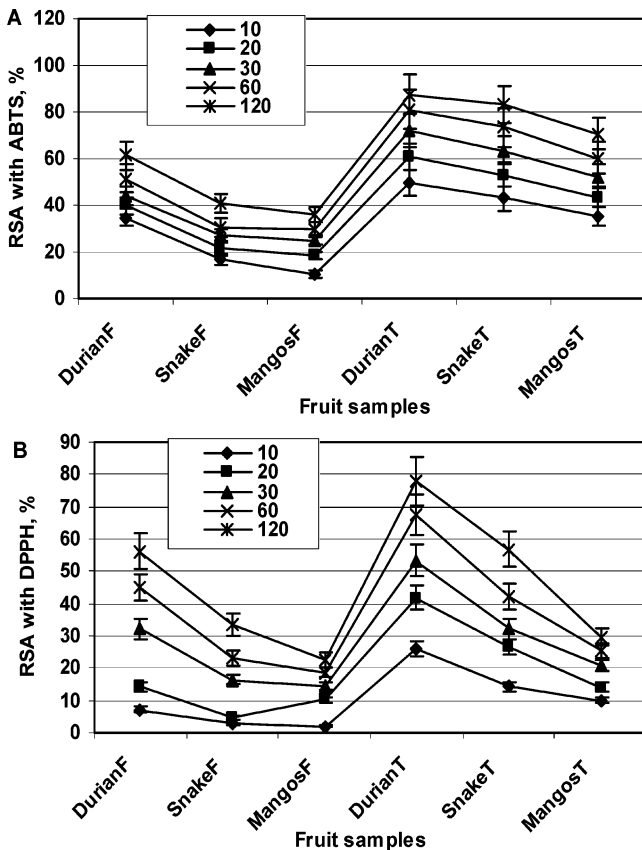
<sup>a</sup> Values are means ± SD of 5 measurements. Means in rows without letters in common differ significantly ( $P < 0.05$ ).

in two assays (ABTS, Figure 1A, and DPPH, Figure 1B) of free and total polyphenol extracts, durian has the highest activity, which increases with the prolonged time of the assay (the lowest at 10 min and the highest at 120 min). The maximum antioxidant activities were calculated after 120 min of the scavenging reaction (Table 2). The contents of total and free polyphenols and flavonoids and related antioxidant activities, as determined by DPPH and ABTS assays, were significantly higher in durian ( $P < 0.05$ ) than in both other fruits (Table 2, Figure 2A).

The calculated correlation between the antioxidant activity determined by DPPH and ABTS and their polyphenols was about 0.9998 and 0.9894 (Figure 2B) for extracts of total polyphenols and 0.9914 and 0.9873 for free polyphenol extracts. Flavonoids in total phenol extract showed nearly the same patterns as polyphenols: 0.9996 and 0.9905 for DPPH and ABTS, respectively. In free polyphenol extracts, the correlation was slightly lower and showed about 0.9232 and 0.7814. Therefore a very good correlation was observed between the antioxidant activity determined by ABTS and DPPH in free and total polyphenol extracts, and a moderate one was obtained with flavonoids in free extracts only.

**Identification of Bioactive Compounds.** Compounds identified by means of HPLC/DAD (Figure 3) in the extract from the durian sample had the following retention times ( $t_R$ , min) and amounts ( $\mu$ g mg<sup>-1</sup> of lyophilized sample): vanillic acid (2.80, 0.011); caffeic acid (3.50, 0.017); *p*-coumaric acid (6.58, 0.023); cinnamic (9.43, 0.029); morin (23.13, 0.024); quercetin (31.65, 0.053); myricetin (32.04, 0.014); apigenin (33.92, 0.027) and campherol (34.26, 0.095).

These results show that the most important phenolic acids (caffeic, *p*-coumaric, sinamic, and vanillic) and flavonoids

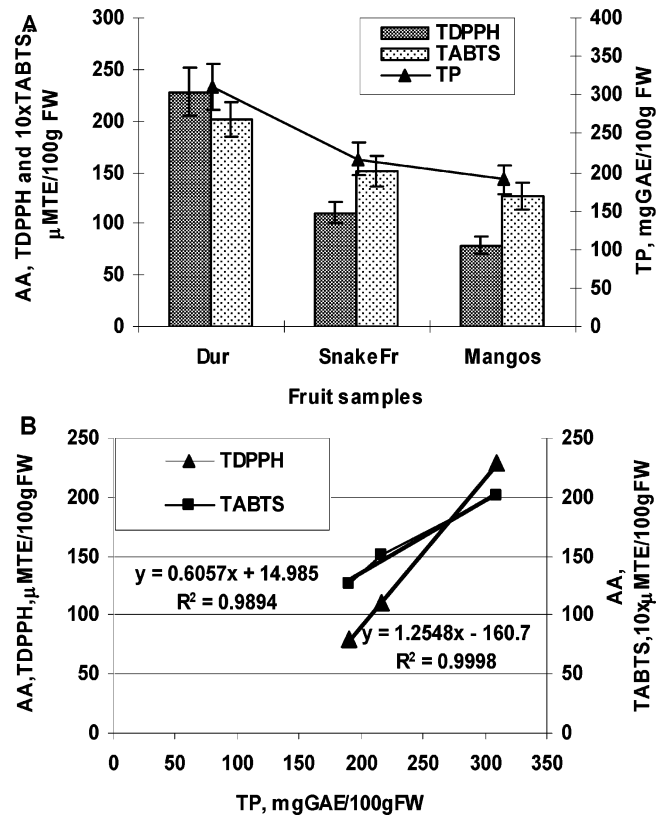


**Figure 1.** Radical scavenging activity (RSA, %) of total (T) and free (F) polyphenol extracts from exotic fruits determined by two assays: **A**, with ABTS, **B**, DPPH during different periods of time 0, 10, 20, 30, 60, and 120 min. Standard errors represent SD of five measurements. Abbreviations: DurianF, durian free polyphenols; SnakeF, snake fruit free polyphenols; MangosF, mangosteen free polyphenols; DurianT, durian total polyphenols; SnakeT, snake fruit total polyphenols; MangosT, mangosteen total polyphenols; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; ABTS<sup>+</sup>, [2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical cation.

(quercetin, morin, myricitin, apigenin, and campherol) having high antioxidant activity were found in durian. The absolute data based on the dry weight of the lyophilized sample were recalculated in order to allow a comparison; therefore, phenolic acids ( $\mu\text{g}/100\text{ g}$  of FW) caffeic ( $389.5 \pm 34.2$ ), *p*-coumaric ( $526.9 \pm 48.8$ ), cinnamic ( $664.4 \pm 61.1$ ) and vanillic ( $252.0 \pm 24.9$ ) were in relatively high amounts. From flavonoids, the highest was campherol ( $2176.5 \pm 216.8$ ), followed by quercetin ( $1214.23 \pm 116.7$ ), apigenin ( $618.6 \pm 60.8$ ), morin ( $549.8 \pm 53.3$ ), and myricitin ( $320.7 \pm 31.9$ ).

**In vivo.** The supplementation of 1% Chol and/or 5% of exotic fruits has no effect on protein content in the diets (% DM), which ranged from 10.8 (Chol/Mangosteen) to 11.3 (Chol) (Table 4). Dry matter digestibility was high in all groups of rats fed BD, cholesterol, and/or exotic fruits (94.5–95.2%). The differences between the groups were not significant, and all other indices (feed intake, body weight gains, feed and protein efficiency ratios) were not affected ( $P > 0.05$ ).

After the completion of the trial, the increase in the TC in the Chol diet group vs that in the control was significant ( $P < 0.05$ ). In the other three groups (Chol/Durian, Chol/Snake, and Chol/Mangosteen), the increase in TC was hindered (Table 5). However, in only Chol/Durian group was the hindrance significant ( $P < 0.05$ ). The same patterns in the changes of

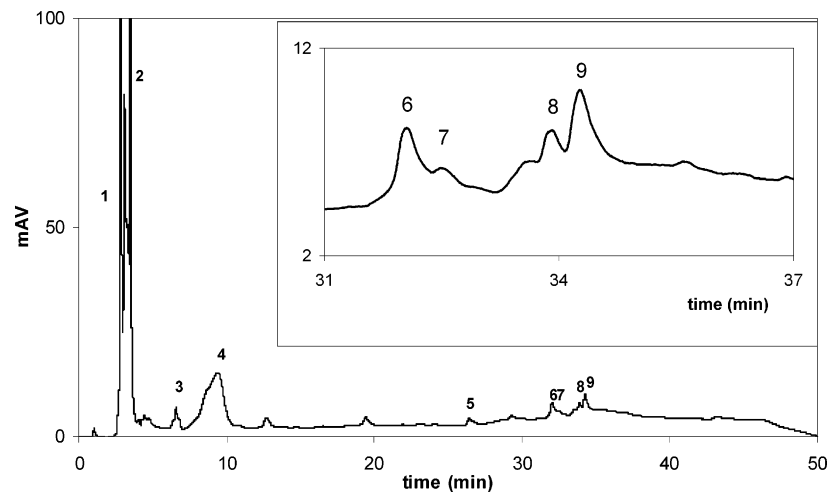


**Figure 2.** **A**, Total phenols and the antioxidant activities by DPPH and ABTS in total polyphenol extracts from Durian (Dur), Snake fruit (Snake Fr) and Mangosteen (Mangos). **B**, correlation coefficients ( $R^2$ ) calculated by linear regression analysis for fruit extracts between:  $\blacktriangle$ , TP in 50%Met/HCl (mg of GAE/100 g of FW, X) to DPPH ( $\mu\text{M TE}/100\text{ g}$  of FW,  $Y_1$ ); and  $\blacksquare$ , TP in 50%Met/HCl (mg of GAE/100 g of FW, X) to ABTS ( $\mu\text{M TE}/100\text{ g}$  of FW,  $Y_2$ ). Standard errors represent SD of five measurements. Abbreviations: AA, antioxidant activity; TDPDH, (1,1-diphenyl-2-picrylhydrazyl radical) determined in the fruit extracts of total polyphenols; TABTS, [2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] determined in the fruit extracts of total polyphenols; TE, trolox equivalent; TP, total polyphenols; GAE, gallic acid equivalents.

LDL-Chol levels were registered. The changes in the HDL-Chol and TG levels were not significant ( $P > 0.05$ ).

A decrease in the plasma antioxidant activity (AA) was found in all groups of rats (Table 5) fed cholesterol-containing diets (Chol, Chol/Durian, Chol/Snake, and Chol/Mangosteen), but in the Chol/Durian, Chol/Snake, and Chol/Mangosteen groups, the decrease in the AA was hindered. Only in the rats of the Chol/Durian group was the hindrance significant ( $P < 0.05$ ). This indicates that the bioactivity of durian was significantly higher than of the two other fruits. The following data show the correlation between the changes in plasma lipid levels and AA. So, durian, snake fruit, and mangosteen in the Chol/Durian, Chol/Snake, and Chol/Mangosteen diet groups significantly hindered the rise of plasma lipids versus Chol diet group: TC = 14.9, 10.1, and 9.0%; LDL-C = 21.6, 15.2, and 11.2%; TG = 17.7, 12.7, and 11.4%, respectively (Table 5, Figure 4). The decrease in the antioxidant activity determined by ABTS and DPPH was about 24.0, 17.6, and 11.7% and 28.1, 21.1, and 10.5%, respectively.

The correlation between the most important fractions of lipid spectrum such as low-density cholesterol and the antioxidant activity in plasma was as follows: LDL-Chol vs AAABTS,  $R^2 = 0.9882$ ; and LDL-Chol vs AADPPH,  $R^2 = 0.9388$ .



**Figure 3.** Chromatogram obtained from the durian extract at the wavelength ( $\lambda$ ) of 253 nm. The identified compounds: (1) vanillic acid, (2) caffeic acid, (3) *p*-coumaric acid, (4) cinnamic acid, (5) morin, (6) quercetin, (7) myricetin, (8) apigenin, and (9) campherol.

**Table 4.** Dry Matter of Diets, Crude Protein, and Their Digestibility (%), Feed Intake and Body Gains (g/day) after Feeding with Different Diets<sup>a</sup>

indices	control	Chol	Chol/Durian	Chol/Snake	Chol/Mang
DM of diets	92.81 ± 0.79 a	92.88 ± 0.29 a	92.68 ± 0.38 a	92.35 ± 0.48 a	92.76 ± 0.83 a
DM of CP	11.32 ± 0.41 a	11.25 ± 0.38 a	11.00 ± 0.25 a	10.82 ± 0.33 a	10.81 ± 0.19 a
Dig of DM	94.50 ± 0.48 a	94.48 ± 0.47 a	94.83 ± 0.13 a	94.93 ± 0.60 a	95.23 ± 0.55 a
Dig of CP	91.05 ± 1.31 a	90.05 ± 1.48 a	90.07 ± 1.73 a	91.04 ± 0.49 a	91.30 ± 1.23 a
feed intake	13.99 ± 0.68 a	14.09 ± 0.19 a	13.94 ± 0.11 a	13.99 ± 0.69 a	13.13 ± 0.44 a
body gains	3.50 ± 0.20 a	3.70 ± 0.40 a	3.80 ± 0.40 a	3.50 ± 0.20 a	3.40 ± 0.30 a
FER	0.25 ± 0.01 a	0.26 ± 0.02 a	0.27 ± 0.03 a	0.25 ± 0.01 a	0.26 ± 0.03 a
PER	0.44 ± 0.01 a	0.43 ± 0.04 a	0.43 ± 0.05 a	0.44 ± 0.01 a	0.44 ± 0.06 a

<sup>a</sup> Values are means ± SD, *n* = 5. Means in rows without letters in common differ significantly (*P* < 0.05). Abbreviations used: Chol, cholesterol; Snake, snake fruit; DM, dry matter; CP, crude protein; Dig, digestibility; FER, feed efficiency ratio, PER, protein efficiency ratio.

**Table 5.** Plasma Lipids (mM/L) and Antioxidant Activity (mM TE/L) after Feeding with Different Diets<sup>a</sup>

indices	control	Chol	Chol/Durian	Chol/Snake	Chol/Mang
TC	2.21 ± 0.11 a	2.69 ± 0.12 b	2.29 ± 0.11 a	2.42 ± 0.11 b	2.45 ± 0.12 b
LDL-Chol	0.87 ± 0.04 a	1.25 ± 0.06 b	0.98 ± 0.05 a	1.06 ± 0.05 b	1.10 ± 0.06 b
HDL-Chol	1.34 ± 0.07 a	1.44 ± 0.07 a	1.31 ± 0.06 a	1.36 ± 0.06 a	1.35 ± 0.06 a
TG	0.69 ± 0.04 a	0.76 ± 0.04 a	0.73 ± 0.04 a	0.74 ± 0.04 a	0.76 ± 0.05 a
AA (ABTS)	1.49 ± 0.07 a	0.98 ± 0.05 b	1.29 ± 0.06 a	1.19 ± 0.06 b	1.11 ± 0.06 b
AA (DPPH)	0.83 ± 0.04 a	0.57 ± 0.03 b	0.73 ± 0.04 a	0.69 ± 0.04 b	0.63 ± 0.04 b

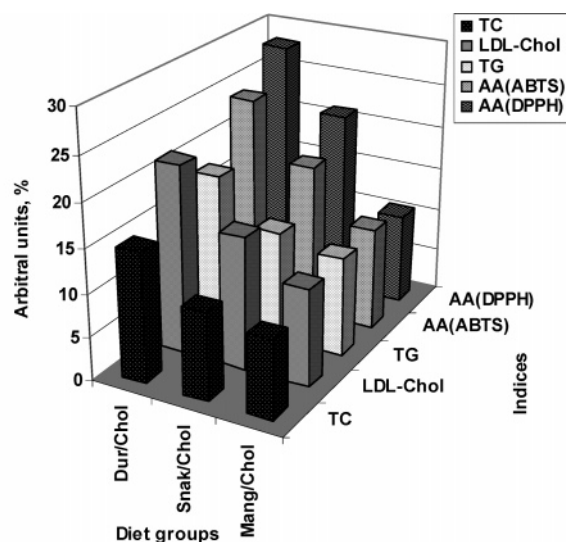
<sup>a</sup> Values are means ± SD, *n* = 5. Means in rows without letters in common differ significantly (*P* < 0.05). Abbreviations used: TC, total cholesterol; LDL-Chol, low-density lipoprotein cholesterol; HDL-Chol, high-density lipoprotein cholesterol; TG, triglycerides; AA, antioxidant activity; ABTS, [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)]; DPPH, 1,1-diphenyl-2-picrylhydrazyl; Chol, cholesterol; Snake, snake fruit; Mang, mangosteen.

## DISCUSSION

In connection with the use of fruits in disease-preventing diets, the results of the studied exotic fruits are interesting and important (1–6). For these fruits to be included in disease-preventing diets, the contents of their bioactive compounds and their antioxidant activities have to be relatively high. Until now, some of these fruits were practically not investigated; durian was among them (7). Therefore, the aim of this investigation was to assess the health and nutritional properties of durian in *in vitro* and *in vivo* experiments and to compare the properties with those of better known fruits such as snake fruit and mangosteen.

The knowledge of minerals and trace elements in natural products is highly important. Fruits and vegetables are the main external source of these nutrients for animals and humans alike. The contents of minerals and trace elements in durian, snake fruit, and mangosteen obtained in this study differed from those published by other authors (22, 23). We did not expect that the

contents of the studied minerals and trace elements would be identical: the geographical regions, climatic conditions, cultivars, and level of maturity were different. It was impossible to find data for comparison of the same fruits that were investigated in this study: the major minerals (mg/100 g of FW) ranged from 2.1 to 201.2, whereas trace metals ranged from 0.024 to 0.991. The data reported in (22) were slightly higher for minerals (mg/100 g of FW), ranging from 7.7 to 433.3, and for trace elements, ranging from 0.116 to 1.91; the tropical fruits were relatively high in K (101–201) and low in Na (1–2) and Fe (0.3–1.0) levels. The range of K was from 36 to 1.782; that of Na was 48; and that of Fe from 0.7 to 8.4 (23). These results differ from those presented in this report; however, the tendencies were similar: the studied fruits were generally high in potassium and low in sodium levels. The contents of the studied trace elements were in the ranges published by other authors (23), and can be demonstrated as follow: K > Ca ≥ Mg > Na and Fe > Mn > Zn > Cu.



**Figure 4.** Hindering of the rise of plasma lipid (TC, total cholesterol; LDL-Chol, low-density lipid cholesterol; TG, triglycerides) and the decrease in plasma antioxidant activity by ABTS and DPPH vs Chol (Cholesterol) in Dur/Chol (Durian/Chol), Snak/Chol (Snake fruit/Chol) and Mang/Chol (Mangosteen/Chol) diet groups. Abbreviations: AA, antioxidant activity; DPPH, (1,1-diphenyl-2-picrylhydrazyl radical) determined in the fruit extracts of total polyphenols; ABTS, [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] determined in the fruit extracts of total polyphenols.

It is known that there is little information on the antioxidant activity on exotic fruits and their bioactive compounds. The presented results on polyphenols are in accordance with the data of others (2, 6, 8, 9, 12): it was expected that the free methanolic extracts contain significantly lower amount of polyphenols than the hydrolyzed ones. The patterns in changes of flavonoids are similar to the polyphenols with slightly lower correlation of antioxidant activity than in polyphenols. The comparison of the obtained results depends on the extraction procedure: all polyphenol extracts from mangosteen showed antioxidative activity, but the water and 50% ethanol extracts exhibited neuroprotective activity and high free-radical scavenging activity by DPPH (9). These phenolics, especially their condensed tannin fractions, displayed strong antioxidant activities and were similar to our results. In relation to the findings reported in (12), where the phenolics were extracted with 95% ethanol and 70% acetone, the results of polyphenols and their antioxidant activities were similar to the present data in vitro and in vivo on animal model. Our results obtained from the extracts, which were done at optimal extraction conditions with two variables, high temperature and prolonged time, were slightly lower than the data of others (24).

Our results of total soluble phenolics ( $\mu\text{gGAE/g}$  FW) were 1900, 2170, and 3097 and corresponded with tropical fruits from south Florida such as red guava, banana, and kiwi fruit (6); the antioxidant activities ( $\mu\text{M TE/g}$ ) were 3.17, 3.77, and 5.05, corresponded to banana (6), and were influenced by cultivar and ripening stage.

The present data of the three investigated fruits can be compared with commonly consumed exotic fruits from Mauritius (24), where the antioxidant activities by ABTS ( $\mu\text{M TE/g}$  of FW) ranged from 1 to 47 (Table 2, from 3 to 20), the total phenolics ( $\mu\text{g/g}$  of FW) ranged from 118 to 5638 (Table 2, from 140 to 3100), and the flavonoids ( $\mu\text{g/g}$  FW) ranged from 21 to 712 (Table 2, from 70 to 850). There were strong correlations between antioxidant activity (assessed by both ABTS and FRAP) and total phenolics (24), which exactly

correspond with our results. Flavonoids seemed to contribute less to the antioxidant potential of the fruits (24), but in our case the correlation was relatively high in the total and lower in the free polyphenol extracts ( $R^2 = 0.8272$ ). The highest antioxidant capacities were observed in red and yellow Chinese guava, starfruit, jamblon, and white guava (24). Our data could be placed in the middle of the reported list of Mauritian exotic fruits. The exotic fruits from different geographical regions were also characterized by high levels of total phenolics, and thus a significant source of phenolic antioxidants, which may have potential beneficial effects on health (3, 9, 24). Our results can be compared as well with the properties of natural cultivated and wild tropical fruits grown in southern Brazil. The global antioxidant activity presented as TEAC (Trolox equivalent antioxidant activity,  $\mu\text{M TE/g}$  of FW) in natural fruit pulps (3) were 13.3–111.2 (Table 2, from 3 to 20). The descending order according to antioxidant capacity is acerola > mango > strawberry > grapes > acai > guava > mulberry > graviola > passion fruit > cupuacu > pineapple (3). Most probably the place of durian is between guava and passion fruit (3).

The antioxidant capacity of quercetin was about three times higher than that of caffeic acid, twice as high as that of *p*-coumaric and cinnamic acids, and four times higher than that of vanillic acid. There are no data in the literature concerning the identification of the bioactive compounds in durian. Therefore, the present results of HPLC/DAD analysis showing the main phenolic acids and flavonoids in durian could not be compared, but these compounds are characteristic and typical for many fruits. Our previous data on the composition of phenolic acids in different citrus fruits are in accordance with the presented ones, concerning the distribution of caffeic and *p*-coumaric acids. Hydroxycinnamic acids were highly correlated with each other (25).

It was shown that a cholesterol-supplemented diet leads to a decrease in plasma antioxidant activity (10, 11, 25). However, in the present study, the plasma antioxidant activity decrease in groups of rats fed a fruit-supplemented diet, especially with durian, was significantly lower than that in the Chol group and corresponded with others, showing that the alcohol extracts from fruits are powerful antioxidants via the inhibition of LDL atherogenic modifications and lipid peroxides formation in hypercholesterolemic rats (4). The present results connected with plasma antioxidant activity can be compared with the reported ones determined by FRAP and DPPH free-radical assays (21) after supplementation of rat diets with tea epicatechins. The antioxidant activity by ABTS varied between 1.49 and 0.98 mM TE/L and could be compared with FRAP (21) values (from 0.05 to 1.8). DPPH scavenging activity has changed as well from 4 to 67% and could be compared (21) with the 18 and 85% values determined in this study. Similarly, the antioxidant capacity measured using both FRAP and DPPH free-radical assays was consistent with the change pattern of epicatechins and their epimers after an oral dose of 4000 mg gallicocatechin (21). In the supplementation with exotic fruits, the results were lower than with the gallicocatechin. This can be explained by different amounts of fruits being added to the diets and their antioxidant activities (DPPH scavenging activity: for durian, 77.90%; snake fruit, 56.57%; and mangosteen, 29.71% in comparison with gallicocatechin (21), about 80%, and epicatechin, 85%).

The nutritional values of exotic fruits, especially durian, and their influence on dry matter and protein digestibility are almost not known. Higher fiber amounts in human diets (6, 26) have been recommended in dietoprophylaxis and also for prevention of some health problems (coronary heart disease, cancer,

obesity). Dietary fiber fraction (apart from protein content in the diet) can influence the dry matter and protein apparent digestibility. Total dietary fiber in the investigated fruits can be compared with the results reported by others, and our data correspond to longan, dragon, and carambola (6). An increase in soluble dietary fiber in the diet can significantly decrease the digestibility and its performance (18). Our results on total dietary fiber were comparable with the data of others (26) where dragon fruit, durian, guava, longan, mango, and pineapple showed from 0.19% for longan and about 2.7% for guava. Therefore, the dietary fiber of durian was in the range of reviewed results (26).

In the present report, there was no effect of exotic fruits on performance expressed by FER and PER, calculated on the feed and protein intakes and body gains. No differences between growth parameters of the cholesterol group of rats fed diets with exotic fruits were requested. It can be concluded that these three fruits have no influence on performance, because their utilization in the body (evaluated on diet digestibility) was similar.

In our previous study, different results were obtained in rats that were also fed a semipurified diet (0.3% Chol) with pectin supplementation (10%) from highly methoxylated citrus pectin, apple pomace, potato fiber, and sugar beet pulp. Dry matter digestibility (16) was significantly lower (89.09–93.8%) than in the control (95.0%). There was no effect of apple peels and pulp (5%) in rats fed a semipurified diet (without Chol) on dry matter digestibility, which was, on average, 92.5% (17). Apple peel and pulp decreased this digestibility in rats fed semisynthetic (17) or atherogenic standard diets. The supplementation of cholesterol diets with pectins from different sources lowers the feed consumption and body weight gain, which is an important index in obesity. This reaction was not observed in rats fed diets with durian, snake fruit, and mangosteen. Polysaccharide gel (PG) extracted from fruit hulls of durian is a source of soluble fiber (mainly pectins), which is used in pharmaceutical preparations and food products (7). Protein content in durian was slightly higher than in the others exotic fruits. Also, a long-term consumption of PG from durian did not affect the relative weight gain in mice (7), but during the first two weeks, excreted feces were very soft, which showed the laxitative property of this polysaccharide fiber. There was no effect of high doses of PG from durian on toxicity for mice and rats. A decrease in protein digestibility in rats with pectin from different sources in the semisynthetic diets was noted (16). Protein digestibility in control rats was higher (94.3%), because rats were younger than in the present report, but the reaction after feeding with pectin was strong (digestibility was 4% lower). Protein digestibility varied from 90.1 to 91.3% in Chol and Chol/Mangosteen, respectively, and can be compared only with data from other fruits, because no data were found on the exotic fruits investigated in this report. For fresh fruits, the protein score by protein digestibility was 75.6/64.3%, for dried fruits 65.6/48.1%, for legumes 89.2/69.6%, and vegetables 88.5/73.4% (19). In vitro availabilities of major minerals (% of total) varied from 11.1 to 86.2%, whereas for minor minerals, it ranged from 13 to 72.5% (22). All nutritional tests indicate a high nutritional value of the studied fruits. In conclusion, (i) all studied exotic fruits, especially durian, contain high quantities of bioactive compounds and possess high antioxidant activity; (ii) diets supplemented with durian and to a lesser degree with snake fruit and mangosteen hindered the rise in plasma lipid levels and in the decrease of plasma antioxidant activity in rats fed cholesterol; and (iii) it is suggested that inclusion of all studied

exotic fruits, especially durian, in known disease-preventing diets could be beneficial.

## ABBREVIATIONS

Trolox, (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid); ABTS<sup>+</sup>, [2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)]; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; TEAC, Trolox equivalent antioxidant capacity; FER, feed efficiency ratio; PER, protein efficiency ratio.

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