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ORIGINAL PAPER

Hanna Leontowicz · Maria Leontowicz · Jerzy Drzewiecki · Ratiporn Haruenkit · Sumitra Poovarodom · Yong-Seo Park · Soon-Teck Jung · Seong-Gook Kang · Simon Trakhtenberg · Shela Gorinstein

Bioactive properties of Snake fruit (*Salacca edulis Reinw*) and Mangosteen (*Garcinia mangostana*) and their influence on plasma lipid profile and antioxidant activity in rats fed cholesterol

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Abstract Two exotic fruits (Snake fruit and Mangosteen) were characterized by polyphenols, proteins and antioxidant potentials and by their influence on plasma lipids and antioxidant activity in rats fed cholesterol. The content of polyphenols (14.9 ± 1.5 and 9.2 ± 0.8 mg GAE g^{-1}) and antioxidant potential (46.7 ± 4.7 and 72.9 ± 7.4 μmol TE g^{-1}) in Snake fruit was significantly higher than in Mangosteen ($P<0.05$). Twenty male Wistar rats were divided into four dietary groups: Control, Chol, Chol/Snake and Chol/Mangosteen. After 4 weeks of the experiment diets supplemented with Snake fruit and to a lesser degree with Mangosteen significantly hindered the rise in plasma lipids and hindered a decrease of antioxidant activity. Changes were found in fibrinogen fraction, such as solubility and mobility by the number of protein bands detected in SDS-electrophoresis: Chol/Snake differed from Chol/Mangosteen. In conclusion, Snake fruit and Mangosteen contain high quantity of bioactive compounds, therefore positively affect plasma lipid profile and antioxidant activity in rats fed cholesterol-containing diets. Such positive influence is higher in rats fed diet with added Snake fruit.

Keywords Snake fruit · Mangosteen · Total polyphenols · Plasma lipids · Antioxidant activity · Rats

Introduction

There is a convincing evidence that fruits and vegetables are playing a beneficial role in prevention and even treatment of different diseases [1]. Therefore, many investigators proposed different diets rich in both fruits and vegetables [2–4]. Now at the fruit markets of North America and Europe different kinds of fruits, including tropical and subtropical, can be found [5–7]. Some of them such as mango, guava,

H. Leontowicz · M. Leontowicz
Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, 02-787 Warsaw, Poland

J. Drzewiecki
Plant Breeding and Acclimatization Institute, Radzikow, Poland

R. Haruenkit
Faculty of Agricultural Technology, King Mondkut Institute of Technology, Ladkrabang, Bangkok, Thailand

S. Poovarodom
Department of Soil Science, King Mondkut Institute of Technology, Ladkrabang, Bangkok, Thailand

Y.-S. Park
Department of Horticultural Science, Mokpo National University, Muan Jeonnam 534-729, Korea

S.-T. Jung
Department of Food Engineering, Mokpo National University, Muan Jeonnam 534-729, Korea

S.-G. Kang
Food Industrial Technology Research Center, Mokpo National University, Muan Jeonnam 534-729, Korea

S. Trakhtenberg
Kaplan University Medical Center, Rehovot, Israel

S. Gorinstein (✉)
Department of Medicinal Chemistry and Natural Products, School of Pharmacy, The Hebrew University - Hadassah Medical School, Jerusalem 91120, Israel
e-mail: gorin@cc.huji.ac.il
Tel.: +972-2-6758690
Fax: +972-2-6757076

papaya, star fruit and persimmon are well known to most of consumers [6, 7]. However, Snake fruit, Salacca (*Salacca edulis Reinw*) and Mangosteen (*Garcinia mangostana*) are less known and also less investigated [8–11].

It is very important to know if in addition to good taste, these fruits also possess high bioactivity and high total antioxidant potential, and positively influence plasma lipids and antioxidant activity. If these fruits will contain high amount of bioactive compounds, then their inclusion in disease-preventing diets will be justified. Therefore, it was decided to determine *in vitro* the contents of polyphenols, antioxidant potentials and protein profiles in Snake fruit and Mangosteen and then to assess *in vivo* their influence on plasma lipids and antioxidant activities in rats fed cholesterol supplemented diet.

As far as we know, this is the first investigation *in vivo* of Snake fruit and Mangosteen.

Materials and methods

Reagents

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, Folin-Ciocalteau reagent, β-carotene, gallic acid, butylated hydroxyanisole (BHA) and sodium dodecyl sulphate (SDS) were obtained from Sigma Chemical Co., St. Louis, MO, USA. All reagents were of analytical grade. Deionized and distilled water were used throughout the experiment.

Sample preparation

Snake fruit (*Salacca edulis Reinw*) and Mangosteen (*Garcinia mangostana*) were purchased from the fruit market in Bangkok, Thailand, by one of the investigators and lyophilized.

Fruit lyophilized samples were treated with solvents of different lipophilicity (methanol, ethanol and acetone in water) to obtain fractions for testing the antioxidant activity [12]. To 10–100 mg of fruit powder, either 1 mL of water, methanol/water (70:30), or ethanol was added. After 24 h of incubation under agitation at 4 °C in the dark, all suspensions were centrifuged at 2,570 × g for 10 min and the supernatants were collected. The pellets were washed with 0.5 mL solvent, left for 2 h at 4 °C in the dark and centrifuged. Supernatants from the same solvent were combined. The extracts were designated as fraction A (water), fraction B (methanol/water (70:30), and fraction C (ethanol).

A highly lipophilic fraction (fraction D) was prepared from 1 g of fruit powder in 50 mL acetone/water (75:25 v/v) for 2 h in the dark and vacuum-filtered through a Büchner funnel. The filtrates were transferred to a decanting funnel; 150 mL of diethyl ether was added. The upper phase, which contained the lipophilic compounds, was washed several times with water. The ether solution was filtered through a solid bed of Na_2SO_4 and dried. The residue was dissolved in acetone. The clear supernatants and the solutions of the pure compounds were stored at − 80 °C until use.

Determination of total polyphenols

Total polyphenols were determined by Folin-Ciocalteu method and measured at 765 nm. The results are given in milligrams of gallic acid [13] equivalent per gram dry weight (DW).

Determination of total antioxidant potential by 2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt ($ABTS^{\bullet+}$) and $K_2S_2O_8$.

$ABTS^{\bullet+}$ radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). After addition of 990 μL of $ABTS^{\bullet+}$ solution to 10 μL of Trolox standards (final concentration 0–20 μM) in phosphate buffered saline (PBS), the absorbance was monitored exactly 1, 6 and 9 min after the initial mixing. The percentage decrease of the absorbance at 734 nm was calculated and plotted as a function of the concentration of the samples and of Trolox for the standard reference data [14]. When the samples were dissolved in ethanol, hexane or dichloromethane, the $ABTS^{\bullet+}$ solution was diluted with ethanol. For samples dissolved in water, acetone, or methanol/water (70:30), the dilution was done with water. Standards such as β-carotene, BHA and glutathione were dissolved in dichloromethane, and Trolox in both water and ethanol. This method was applied for the determination of antioxidant potential of fruit samples and plasma antioxidant activity.

Determination and separation of proteins

Fruit lyophilized samples of 50 mg each were dissolved in sample buffer: 2% SDS; 10% glycerol, 2% mercaptoethanol, 0.002% bromophenol blue and 0.62 M Tris HCl, pH 6.8. Electrophoresis was performed with the Hoeffer SE 600 vertical unit (Hoeffer Pharmacia Biotech Inc., San Francisco, CA 94107, USA) according to Laemmli method [15], using polyacrylamide gels (resolving gel $T = 13.7\%$, $C = 1.7\%$, stacking gel $T = 3.8\%$, $C = 1.8\%$) with gel size of 180 mm × 160 mm × 1.5 mm. Sample size was 5 μL. The run was carried out at 25 mA per gel until the end of electrophoresis. Gels were stained with 0.25% Coomassie Brillant Blue R in methanol/water/glacial acetic acid (5:5:1 v/v), destained in water and scanned in transmission light with an Agfa SNAPSCAN 1236 (Agfa-Gevaert N.V Belgium, Agfa SnapScan 1236 s Color image scanner).

Rats and diets

The Animal Care Committee of the University had approved this study protocol, which was successfully used in our previous investigation [16]. Twenty Wistar male rats with mean weight 115 g at the beginning of this study were

used in this experiment. The rats were housed in individual plastic cages in an air-conditioned room (temperature 21–22 °C and relative humidity 55–65%). These rats were divided into four diet groups (Control, Chol, Chol/Snake and Chol/Mangosteen), each of five. During 4 weeks of the experiment the rats of all four 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 a BD only. To the BD of the Chol group, 1% of cholesterol was added. The BD of the two other groups (Snake and Mangosteen) was supplemented with 1% of cholesterol and 5% of the exotic fruits as freeze-dried powder, respectively. In the diets nonoxidized cholesterol of analytical grade was used, which was obtained from Sigma Chemical, St Louis, MO. The dietary cholesterol was checked according to the HPLC method [17] and no presence of cholesterol oxides was found. The cholesterol batches were mixed carefully with the BD (1:99) just before the diets were offered to the rats. Several prior experiments on laboratory animals and human studies have shown that cellulose has no significant hypocholesterolemic effects [18]. Therefore, cellulose was used as a control fibre. The diets contained as percentage of energy 65% of carbohydrates, 26% of protein and 9% of fat. Their calculated energy was from 395.4 to 400.4 kcal 100 g^{-1}, and the differences were not significant.

As in our previous experiment [16], all rats were fed once a day at 10.00 h ad libitum. They had unrestricted access to drinking water. The food intake was monitored daily and body gains every week.

Before and at the end of the experiment the blood samples were taken from the left atrium of the heart. Plasma was prepared and used for laboratory tests, which included determination of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), and TC in liver and plasma antioxidant activity [19]. The $ABTS^{\bullet+}$ test was adopted for determination of the plasma antioxidant activity [20]. The results showed trolox equivalent antioxidant capacity (TEAC) and were expressed as millimole per litre TE (Trolox Equivalent). The antioxidant activity was determined in the full plasma, as well as in two fractions: ethanol-soluble (ethanol/plasma) and acetone-soluble (acetone/plasma).

Serum fibrinogen was precipitated with methanol, then purified by sequential DEAE anion-exchange chromatography, dialyzed against water for 72 h, and lyophilized [21]. As it was mentioned the mobility and protein profile of plasma was determined by Laemmli method [15].

Statistics

The results of this investigation *in vitro* are means ± SD of three measurements. 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.

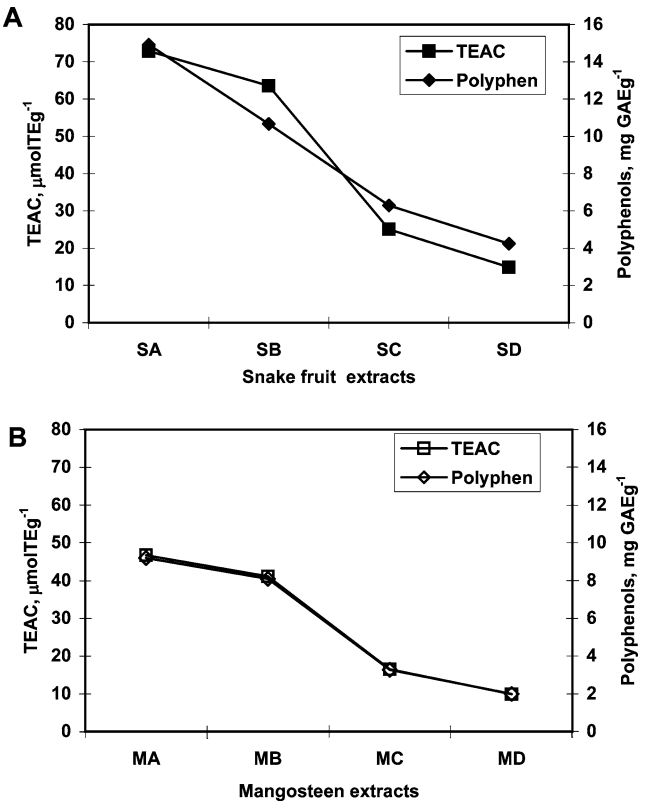

Fig. 1 Antioxidant activities and polyphenols in subfractions from Snake fruit (**A**) and Mangosteen extracts (**B**). Snake fruit (S): SA water, SB methanol/water, SC ethanol, SD acetone/water. Mangosteen (M): MA water, MB methanol/water, MC ethanol, MD acetone/water

Results and discussion

in vitro experiment

The ability of the Mangosteen extract (ME) to scavenge ABTS was lower by about 36% than that of Snake fruit extract (SE). The highest trolox equivalent antioxidant capacity (TEAC, µmol TE g^{-1}) was estimated in fraction A in Snake fruit extract (SE) corresponding to 72.9 ± 7.5, following by fraction B: 63.6 ± 6.5, then fraction C: 25.1 ± 2.6 and then fraction D: 14.9 ± 1.6. Corresponding fractions from Mangosteen were about 35% lower than the Snake fruit (Fig. 1). For comparison, the radical scavenging effect of β-carotene, Trolox, BHA and glutathione was measured and compared with that of fruit extracts. β-carotene showed an antioxidant activity superior to Trolox in the TEAC assay. Total phenols (mg GAE g^{-1} DW) were determined in all fractions: (A) 14.93 ± 1.5; (B) 10.66 ± 1.0; (C) 6.29 ± 0.64; and (D) 4.24 ± 0.5. The amount of polyphenols in Mangosteen samples was about 30% less than in the Snake fruit samples. The contribution of total polyphenols to the antioxidant potential of Snake fruit and Mangosteen was high, and the correlation coefficient was about 0.94–0.96 (Fig. 2).

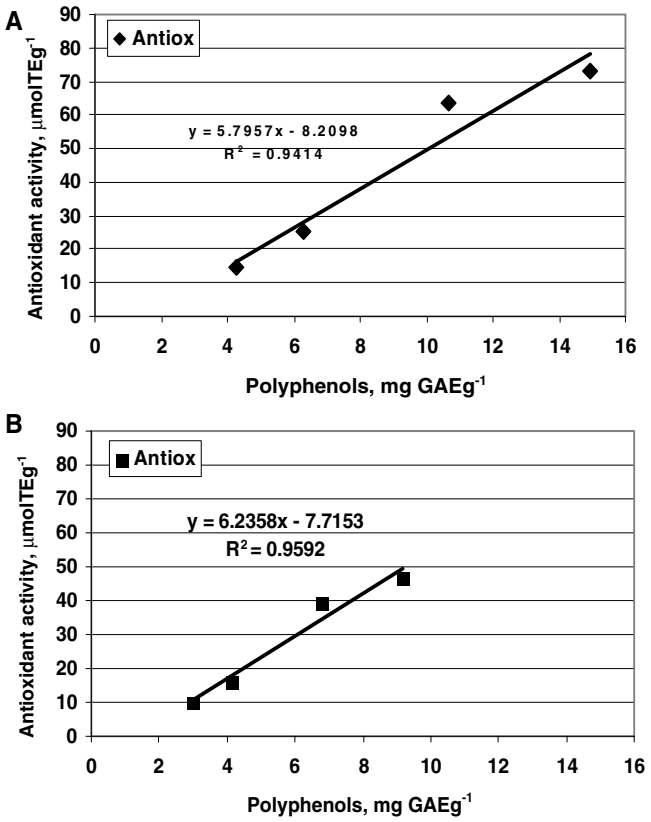

Fig. 2 Correlation between antioxidant activity and polyphenols in Snake fruit (**A**) and Mangosteen (**B**)

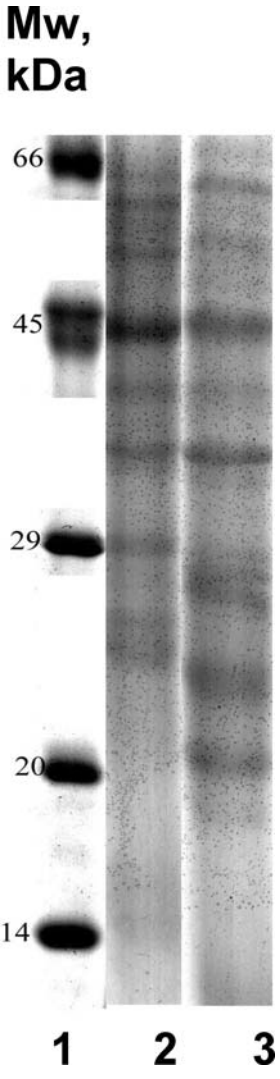

Fig. 3 Comparison of the band intensity of fruit proteins extracted with sample buffer containing SDS and 2-ME and separated by SDS-PAGE. Lane 1, molecular markers (kDa): 66-albumin; 45-ovalbumin; 36-glyceralaldehyde-3-phosphate dehydrogenase; 29-carbonic anhydrase; 24-trypsinogen, PMSF treated; 20-trypsin inhibitor, 14-α-lactalbumin; lane 2-Mangosteen, lane 3-Snake fruit

Determination and separation of proteins in fruits and in plasma

Buffer-soluble (Fig. 3) fruit proteins were separated into numerous components. The molecular weight range of detected components was from 10 to 66 kDa. The patterns obtained for proteins extracted from both fruits were similar. The majority of the protein bands fall into the MW ranges 20–66 kDa. The bands with MW less than 12 kDa were diffused. Intensive major components were concentrated between protein bands of 24 and 45 kDa. Snake fruit showed more intensive bands between 20 and 24 kDa than Mangosteen.

in vivo experiment

Weight gains, food consumption and food efficiency ratio in all four diet groups after the conclusion of the trial are varying (Table 1), however not enough to be significant ($P>0.05$). Diet supplemented with Snake fruit and to a lesser degree with Mangosteen (Table 2) significantly hindered the rise in plasma lipids, which was connected with cholesterol feeding ($P<0.05$).

As can be expected, liver total cholesterol (TC) concentration after the experiment was significantly higher in the rats of the Chol, Chol/Snake and Chol/Mangosteen groups than in Control. However, the increase in the liver TC concentration in Chol/Snake and Chol/Mangosteen groups was significantly less than in Chol group. The TC concentration in rats of the Chol group (Fig. 4) was 4.15 times higher than in the Control group. In Chol/Snake and Chol/Mangosteen groups the concentrations were only 3.05 and 3.19 times higher than in Control, respectively ($P<0.005$ in both cases). The above mentioned data indicate that supplementation of the diets with Snake fruit and Mangosteen significantly hindered the increase in liver TC concentration.

Table 1 Weight gains, food consumption and food efficiency in all the four groups of rats

Diet groups	Weight gains (g/day)	Food consumption (g/day)	Food efficiency ratio
Control	3.8 ± 0.4^a	13.94 ± 0.11^a	0.273 ± 0.03^a
Chol	3.7 ± 0.0^a	14.09 ± 0.19^a	0.263 ± 0.02^a
Snake	3.5 ± 0.2^a	13.99 ± 0.68^a	0.250 ± 0.01^a
Mangosteen	3.4 ± 0.3^a	13.13 ± 0.44^a	0.259 ± 0.03^a

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

Table 2 Plasma lipids (mmol/L) of rats fed diets with 1% Cholesterol (Chol) and with 5% of Snake fruit or Mangosteen

Diets	Control	Chol	Chol/Snake fruit	Chol/Mangosteen	2-way ANOVA (*P*-value)		
					Chol	Chol/Snake fruit	Chol/Mangosteen
TC	2.31 ± 0.11^{b}	2.69 ± 0.12^{a}	2.42 ± 0.11^{b}	2.45 ± 0.12^{b}	<0.001	<0.050	<0.050
LDL-C	0.92 ± 0.05^{c}	1.25 ± 0.06^{a}	1.06 ± 0.05^{b}	1.11 ± 0.05^{b}	<0.001	<0.050	<0.050
HDL-C	1.39 ± 0.07^{a}	1.44 ± 0.07^{a}	1.36 ± 0.07^{a}	1.34 ± 0.07^{a}	NS	NS	NS
TG	0.69 ± 0.04^{b}	0.79 ± 0.03^{a}	0.69 ± 0.03^{b}	0.70 ± 0.03^{b}	<0.001	<0.050	<0.050

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

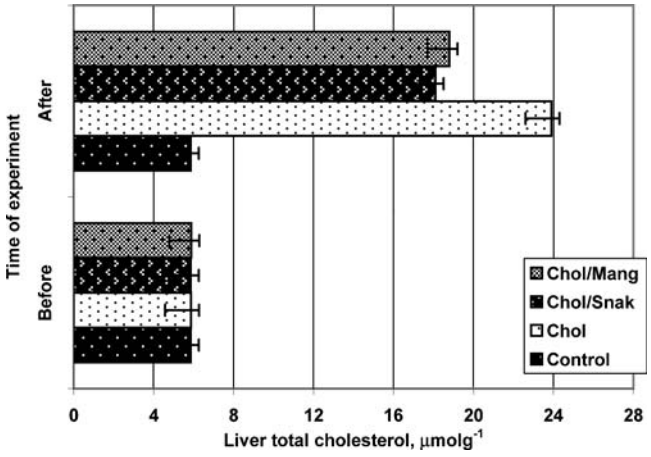

Fig. 4 Changes in the liver total cholesterol in Control, Chol, Chol/Mang and Chol/Snak groups after completion of the experiment ($n = 5$). Chol, cholesterol; Mang, Mangosteen; Snak, Snake fruit

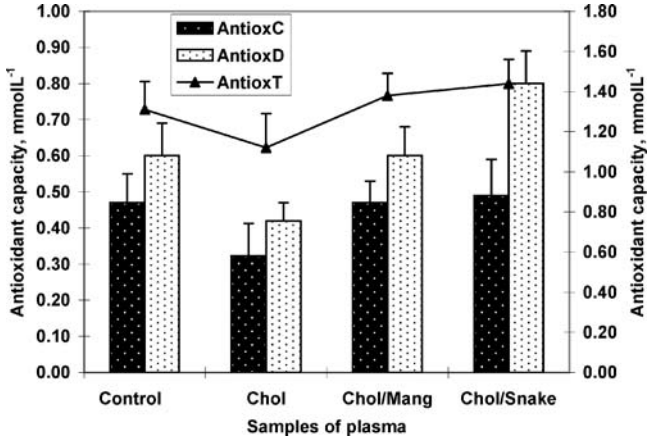

Fig. 5 Changes in antioxidant activities of the total plasma and its fractions (C and D) after completion of the experiment ($n = 5$). AntioxT, total plasma; AntioxC, plasma ethanol extract; AntioxD, plasma acetone extract

After completion of the trial, a significant decrease in the plasma antioxidant activity values in the rats fed with added cholesterol was found (Fig. 5). The decrease in the antioxidant activity in the groups of rats fed diets supplemented with fruits (Snake fruit and Mangosteen) was significantly less ($P < 0.05$) than in group of rats fed diet without fruits (Chol). The same relationship was in the extracted plasma fractions. The lipophilic fraction was higher than the ethanol in all the cases.

The protein profile of the serum samples showed that in fibrinogen fraction of Chol/Snake less protein bands and lower intensity was detected than in Chol/Mangosteen.

The main patterns were located in the range of 36–45 kDa (Fig. 6).

Now-a-days at the fruit markets of North America and Europe, there are different kinds of tropical and subtropical fruits [5–7]. The interest of consumers in these fruits is understandable: everyone likes something new, especially exotic fruits. In our previous investigations *in vitro* and *in vivo* on laboratory animals and humans, we studied different kind of fruits: traditional, citrus and some tropical fruits. All fruits have positive effect on the plasma lipid levels in experiments of laboratory animals and in investigations of humans, suffering from hyperlipidemia [6, 21, 22]. According to our data, the best result was registered when the diet was supplemented with red grapefruit [16]. Therefore, we recommended inclusion of these fruits in disease preventing diets. The answer of the question if the good taste of Snake fruit and Mangosteen justifies their supplementation to such diets was one of the important conclusions. As it was already underlined, some of exotic fruits are less known to consumers and also less investigated by scientists [8–11]. Among these exotic fruits are Snake fruit and Mangosteen, which were investigated only *in vitro*. Therefore, in this study these fruits were also used in experiment on animal model. The first step of the report involved the extraction and analysis of exotic fruits for total phenolic contents, determination of their antioxidant potential and their protein profile. The fruits were analyzed by electrophoresis to determine the content and profile of protein compounds, and the profiles were nearly similar.

Our results *in vitro* are corresponding to data of others who showed that Snake fruit is an excellent source of provitamin A carotenoids [1, 7, 23]. The extracted lipophilic fraction was low in these fruits. Most of the antioxidants were found in hydrophilic fraction, which also corresponds with other reports [24].

The total antioxidant potential in Snake fruit is significantly higher than in Mangosteen ($P < 0.05$). Also other reports discovered that antioxidant potential of Snake fruit is significantly higher than of Mangosteen (AEAC, L-ascorbic acid equivalent antioxidant capacity: 260 ± 32.5 versus 150 ± 23.3 mg 100 g^{-1} WW, edible parts) [7]. The

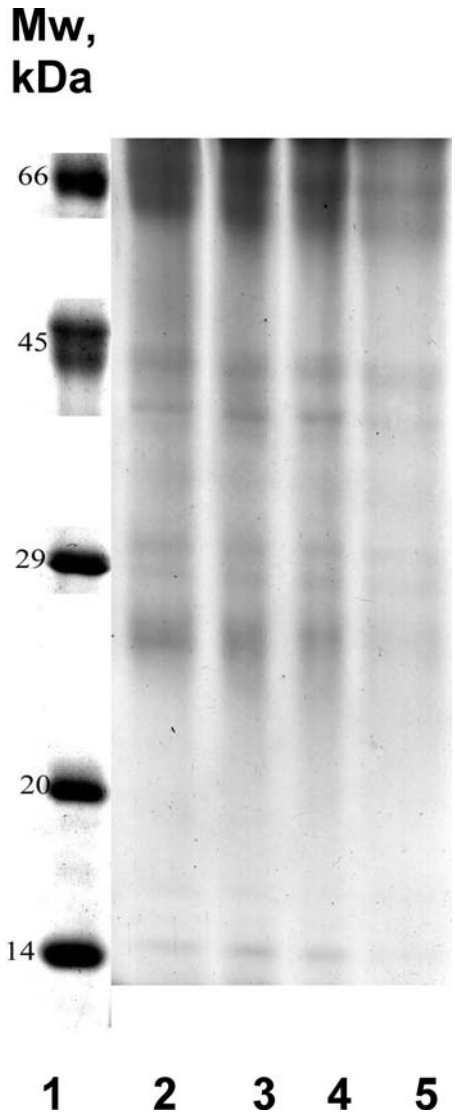

Fig. 6 Comparison of the band intensity of plasma proteins extracted with sample buffer containing SDS and 2-ME and separated by SDS-PAGE. Lane 1, molecular markers (kDa): 66-albumin; 45-ovalbumin; 36-glyceralaldehyde-3-phosphate dehydrogenase; 29-carbonic anhydrase; 24-trypsinogen, PMSF treated; 20-trypsin inhibitor, 14-α-lactalbumin; Lane 2, Control; Lane 3, Cholesterol; Lane 4, Chol/Mangosteen, Lane 5-Chol/Snake

comparison with other well-known exotic fruits put Snake fruit in the order of guava and star fruit, and Mangosteen was between mango, papaya and kiwi fruit [7]. Only few authors claim that there is no correlation between the total polyphenol content and the total antioxidant potential [25]. However, in most reports was shown that high total polyphenol content leads to high total antioxidant potential and there is a linear correlation between these two indices [26–28]. We found, as it was shown in our previous investigations, the same relationships between these two indices [6, 16, 21, 29]: The total phenolic levels of fruits were highly correlated with their antioxidant potentials ($R^2 = 0.96$). Lotito and Frei [30] have shown that high antioxidant potential of individual antioxidant compounds is not necessary correlated with high total antioxidant potential of whole fruits: The total antioxidant potential of whole fruits reflected all antioxidant compounds including inter alia ascorbic acid, dietary fibers and other compounds.

The investigation *in vivo* has shown that after 4 weeks of different feeding, the diet supplemented with Snake fruit and to a lesser degree with Mangosteen hindered a rise in plasma lipids and in decrease of plasma antioxidant activity in rats fed cholesterol-containing diet. This decrease in the plasma antioxidant activity was predictable: Also other investigators observed that cholesterol supplemented diet leads to a decrease in plasma antioxidant activity [31, 32]. Such changes appeared not only in the total antioxidant activity, but also in the extracted fractions (ethanol- and acetone-soluble).

No significant changes in the plasma lipid levels and antioxidant activity were observed in the Control group.

The fibrinogen protein fraction after Chol/Snake diet has shown less protein bands and with lower intensity than for Chol/Mangosteen. Fibrinogen is one of the plasma proteins. Evidence links fibrinogen with coronary atherosclerosis and blood coagulation. Our findings indicate that one of the positive benefits of fruit consumption was to diminish the production of fibrinogen and its stability, which reduces the potential risk exerted by this protein, therefore from the health point these results are positive.

Positive results of this investigation could justify the inclusion of Snake fruit and Mangosteen in atherosclerosis preventing diets.

However, it is well known that the results of experiments on laboratory animals can not be automatically applied to humans. Therefore, it is a need for further investigations of human volunteers, suffering from hyperlipidemia.

Conclusion

Snake fruit and Mangosteen apart of a good taste have high concentration of bioactive compounds, high antioxidant potential and positively affect plasma lipid profile and plasma antioxidant activity in rats fed cholesterol-containing diets. The degree of this positive influence is higher in rats fed diet supplemented with Snake fruit. It is suggested that Snake fruit supplemented diet could be useful for patient suffering from hypercholesterolemia.

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