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RESEARCH ARTICLE

Hypolipidemic Potential of Roselle Calyces *(Hibiscus sabdariffa)* Extracts and Passion Fruit *(Passiflora edulis)* Juice with Pulp Concentrate Formulations in High Fat Diet-Fed Rats

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Abstract

Obesity contributes to cardiometabolic risk factors, including type 2 diabetes mellitus, hypertension, and dyslipidemia. In this study, we aimed to investigate the hypolipidemic potential of various formulations of Roselle Calyces (*Hibiscus sabdariffa*) extracts and Passion Fruit (*Passiflora edulis*) juice with pulp concentrate on high-fat diet (HFD) induced obese rats. Male Sprague Dawley rats were divided into seven groups: (1) control group, (2) HFD group, (3) HFD treated with passion fruit juice with pulp concentrate group, (4) HFD treated with roselle calyces extract group, (5) HFD treated with mix roselle calyces extract plus passion fruit juice with pulp concentrate in powdered form group, (6) HFD treated with a jelly drink containing polyphenol-rich roselle calyces extract and passion fruit juice with pulp concentrate group, and (7) HFD treated with simvastatin group. The results revealed that HFD-fed rats exhibited an increase in body weight, atherogenic and adiposity indices, serum triglyceride (TG), total cholesterol (TC), low-density lipoproteins (LDL), and decreased high-density lipoproteins (HDL) levels compared to control rats. Interestingly, all of these formulas namely the roselle calyces extract, the passion fruit juice with pulp concentrate, the combination of roselle calyces extract plus passion fruit juice with pulp concentrate (RP powder), and the jelly drink containing polyphenol-rich roselle calyces extract and passion fruit juice with pulp concentrate could normalize lipid profiles levels including TG, TC, LDL and increased HDL in HFD-fed rats. Our findings provide significant information for the commercial development of functional ingredients or foods with hypolipidemic properties.

Keywords: Hypolipidemia, Roselle calyces extract, Passion fruit, Obesity

Introduction

Obesity has reached a global epidemic level since 1997, as declared by the World Health Organization (WHO). The primary causes of obesity are excessive intake of calorie-dense food, particularly a high-fat diet (HFD), and reduced physical activity. Obesity contributes to cardiometabolic risk factors, including

type 2 diabetes mellitus, hypertension, sleep disorders, and dyslipidemia [1]. While significant progress has been made in understanding the molecular mechanisms underlying these metabolic disorders, successful treatment options remain limited. Similarly, current pharmacological treatments for obesity are often ineffective and frequently entail significant side effects with prolonged use [2-4].

Dyslipidemia, characterized by hypercholesterolemia and hypertriglyceridemia, is a major risk factor for cardiovascular disease, stroke, and type 2 diabetes mellitus [5]. It can be modified through lifestyle changes and medication [6]. According to the National Health and Nutrition Examination Survey 2003-2006, lipid abnormalities were present in 53% of US adults [7]. Recently, the low-density lipoprotein receptor (LDLR) and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase have emerged as therapeutic targets for the treatment of dyslipidemia and cardiovascular disease due to their cholesterol-lowering effects and additional cholesterol-independent or pleiotropic effects. These include improving endothelial function, attenuating vascular remodeling, and inhibiting vascular inflammatory response [8,9]. However, adverse effects of these cholesterol-lowering agents have been reported [10].

Hibiscus sabdariffa (*H. sabdariffa*), commonly known as roselle, is a tropical plant often used as a raw material for a local soft drink. It has a traditional use against hypertension, inflammation, and liver disorders [11]. The therapeutic effects of *H. sabdariffa* are attributed to its bioactive and functional components, such as phenolic acids, flavonoids, anthocyanins, organic acids, and dietary fiber [12]. Previous studies have demonstrated that *H. sabdariffa* extract reduced serum triglycerides, cholesterol, and LDL cholesterol levels both in animal models of obesity [13,14] and in obese individuals [15]. However, the effects of *H. sabdariffa* extract on body weight, blood pressure, and lipid profile in HFD-fed rats have not yet been investigated, particularly in combination with other plant extracts.

Passiflora edulis (*P. edulis*), commonly known as passion fruit, is extensively found in tropical and subtropical regions and has gained popularity due to its nutritional and health benefits. To date, more than 110 phytochemical constituents, with flavonoids and triterpenoids being the most abundant, have been identified in different parts of *P. edulis* [16]. Various extracts, fruit juice, and isolated compounds from *P. edulis* have been reported to exhibit a wide range of health impacts and biological activities, including antioxidant, anti-hypertensive, anti-tumor, anti-diabetic, and hypolipidemic activities [17-20]. However, research exploring the influence of *P. edulis* fruit extract in various forms on body weight, blood pressure, and lipid profiles in HFD-fed rats is still limited.

Functional foods are nutrient-rich foods that contain active compounds such as phytochemicals, antioxidants, and other substances, offering potential additional benefits beyond basic nutrition. They have gained popularity in recent years within health and wellness communities. Functional foods encompass a wide range of food types, including processed and whole foods and fortified, enriched, or enhanced products. To accommodate busy lifestyles, numerous ready-to-eat functional foods have been introduced to the market. Among these products, ready-to-drink jelly stands out as a popular beverage due to its convenient packaging in carry sachets. Based on our previous study which demonstrated significant hypolipidemic effects of *H. sabdariffa* and *P. edulis* extracts in experimental animals, a jelly drink containing these extracts was developed for the implementation in a clinical trial. The jelly drink, named the RP product, contains polyphenol-rich roselle calyces extract and passion fruit juice with pulp concentrate. This product was designed to promote health improvements and can be classified as an enriched product due to the inclusion of additional ingredients. However, it is possible that some of these additional ingredients may have been destroyed during the product preparation process. Therefore, it is important to consider strategies in the food science process to preserve the effectiveness of these additional ingredients. The remaining active ingredients of the RP product were identified based on our previously published results [21].

This study aimed to evaluate the effects of a jelly drink containing polyphenol-rich roselle calyces extract and passion fruit juice with pulp concentrate, compared to roselle calyces extract, passion fruit juice with pulp concentrate, and a combination of roselle calyces extract plus passion fruit juice with pulp concentrate (RP powder), on body weight, systolic blood pressure, serum

triglyceride, total cholesterol, low-density lipoproteins and high-density lipoproteins levels in HFD-fed rats. The observed results will provide valuable insights for the commercial development of functional ingredients or foods with anti-dyslipidemic properties, utilizing *H. sabdariffa* and *P. edulis* as sources of functional bioactive compounds.

Materials and methods

Animals

Male Sprague Dawley rats weighing 150 - 200 g and approximately 7 - 8 weeks old were obtained from Nomura Siam International Co., Ltd. (Bangkok, Thailand). The rats were raised at the Center for Animal Research, Naresuan University, Phitsanulok, Thailand, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. All animal procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals and were approved by the Animal Ethics Committee of Naresuan University, Thailand (approval number: NU-AE621023). The experiment involving acute toxicity was performed at the animal center of the Expert Center of Innovative Herbal Products and was approved by the Animal Ethics Committee of Thailand Institute of Scientific and Technological Research (TISTR) (approval number: T-62023).

Rats were grouped and housed together, with 2-3 rats per cage, in an accredited animal room maintained at constant humidity (40% - 60%), temperature (22.0 ± 1.0 °C), and a 12/12-h light-dark cycle. They were given a one-week acclimatization period. Following acclimatization, the rats were divided into seven groups as follows: (1) control group (C; n = 7) fed a standard rat diet, (2) HFD group (n = 7), (3) high-fat diet treated with passion fruit juice with pulp concentrate group (Passion fruit; n = 5), where the rats were fed a high-fat diet and orally administered passion fruit juice with pulp concentrate at a dose of 500 mg/kg body weight (BW), (4) high-fat diet treated with roselle calyces extract group (Roselle; n = 5), where the rats were fed a high-fat diet and orally administered roselle calyces extract at a dose of 500 mg/kg BW, (5) high-fat diet treated with a mixture of roselle calyces extract and passion fruit juice with pulp concentrate in powder form group (RP powder; n = 5), where the rats were fed a high-fat diet and orally administered 250 mg/kg BW of roselle calyces extract powder and 250 mg/kg BW of passion fruit juice with pulp concentrate powder, (6) high-fat diet treated with a jelly drink containing polyphenol-rich roselle calyces extract (250 mg/kg BW) and passion fruit juice with pulp concentrate (250 mg/kg BW) group (RP product; n = 6) administered via oral gavage, and (7) high-fat diet treated with simvastatin group (Simvastatin; n = 5) administered via oral gavage at a dose of 40 mg/kg BW. The standard diet provided 10% of energy from fat containing 4.5 g of fat/100 g of pellets, while the HFD provided 36% of the total energy intake containing 20 g of fat/100 g of pellets. The standard diet and HFD are given in ad libitum. The rats' body weights were measured weekly.

After 8 weeks, rats underwent a 24-h fast and were euthanized by intraperitoneal injection of pentobarbital (50 mg/kg). Blood samples were collected via cardiac puncture, centrifuged at 1,500×g for 15 min at 4 °C to obtain serum, and stored at −20 °C for further analysis of lipid profiles measurement. The experimental design is depicted in **Figure 1**: Animal procedure of the study.

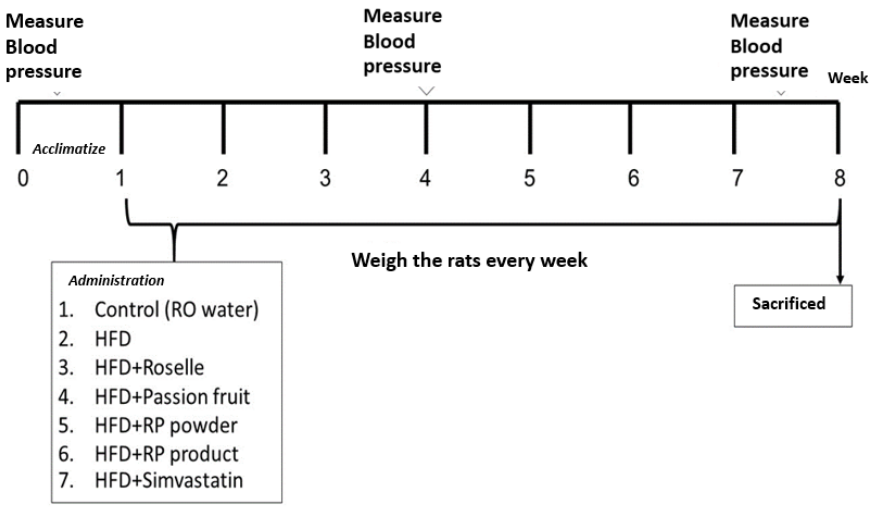

Figure 1 Schematic diagram showing all experimental procedures.

Preparation mix roselle calyces extract plus passion fruit juice with pulp concentrate powder and a jelly drink containing polyphenol-rich roselle calyces extract and passion fruit juice with pulp concentrate (RP product)

Passiflora edulis f. *flavicarpa* Deg. L. fruits were sourced from Chiang Mai, Thailand, while *Hibiscus sabdariffa* (Roselle) flowers were obtained from Sila Loi, Prachuap Khirikhan, Thailand. Both samples were authenticated by a taxonomist at the Plant Varieties Protection Office and deposited at the Forest Herbarium, Royal Forest Department, Ministry of Agriculture and Cooperatives in Bangkok, Thailand. The plants are identified by BK numbers 071159 and 082283, respectively. For the present study, the raw materials were prepared differently. The purple passion fruit juice with pulp was blended and filtered three times using a cotton cloth, followed by freeze-drying. The freeze-drying process yielded 12.12% of the weight of the dried mixture of juice with pulp compared to its liquid form. On the other hand, the calyces of roselle (*Hibiscus sabdariffa*) were collected when they exhibited intense red coloration, then dried in a hot air oven at 50 ºC for 24 h. The dehydrated roselle calyces were ground into a powder using a mill and mixed with water at a ratio of 1:10 (w/v). Double extraction was performed using the maceration method. The aqueous extracts of roselle calyces were filtered using Whatman® No.1 filter paper. The solvent was evaporated under heat using a stirrer at 50 °C for 6 h to concentrate the extract. The concentrated extract was stored at −80°C overnight and subsequently lyophilized using an Alpha 2-4 LSCplus freeze dryer (An der Unteren SÖse, Germany). The extraction yield of roselle calyx using an aqueous solvent was 44.53%. Both plant extract powders were mixed in a 1:1 ratio and stored in a dark bottle at -20 °C until used. The passion fruit juice with pulp concentrate and roselle calyx extracts were standardized by measuring active compounds following the methods described in our previous publication [14].

The polyphenol-rich (PR) jelly drink was prepared using a dried mixture of passion fruit juice with pulp and roselle calyx as the key ingredients. To enhance the drink's taste, additional quantities of other fruits, including papaya and jujube, were added. Jujube was sourced from Sila Loi, Prachuap Khirikhan province, Thailand, while papaya pulp was obtained from Khlong Luang fresh market in Pathum Thani province, Thailand. To summarize the preparation process, dried roselle calyx and jujube were combined with water and boiled together with papaya pulp at a temperature of 80 °C for 60 min. The mixture was then supplemented with water, a sweetener (sucralose), gelling agents (carrageenan, locust bean gum, and inulin), a flavoring agent, and a multivitamin. It was heated at 80 - 85 °C for 15 min and subsequently hot-filled into aluminum foil pouches. High-pressure processing (HPP) was applied at 600 MPa for 4 min, followed by rapid cooling to 4 °C.

The final product was analyzed for total phenolic and total flavonoid contents, as well as antioxidant activity. The main active compounds in RP powder, namely gallic acid, quercetin, and ascorbic acid, were determined using the method described by Khongrum *et al.* [21].

Rat tail-cuff blood pressure measurement

The systolic blood pressure (SBP) in rats was monitored using a modified tail-cuff technique from the previous protocol [22]. The rats were tail mounted fitted with a compressed air cylinder (tail-cuff) and pulse sensors connected to the Non-Invasive Blood Pressure (NIBP) system (ADInstruments Pty Ltd., New South Wales, Australia) and LabChart 4 software (ADInstruments Pty Ltd., New South Wales, Australia). The SBP tail-cuff was initiated by the compressed airused to the rear cuff for initiate cuff inflation and cuff deflation at a constant rate. SBP was continuously recorded with using a pressure sensor as previously, following the methodology described in the previous study [22].

Acute toxicity test

Acute toxicity test was performed according to the World Health Organization (WHO) guideline (WHO,1986) and the Organization of Economic Co-operation and Development (OECD) guideline for testing of Chemicals number 423 (OECOD, 2001). Six female rats/compound (a total of 24 rats) were administrated a single oral dose of 2,000 mg/kg body weight (BW). The body weight, signs of toxicity, and mortality were observed after the administration at the half-hour, first, second, third, and fourth hour and once daily for the next 14 days. On day 15, all rats were kept fasted for 16 - 18 h and then sacrificed for necropsy examination.

Lipid profiles determination

After 24 h fasting, the blood was collected for lipid profiles determination. Serum concentrations of total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) were measured colorimetrically with commercial kits (HUMAN Gesellschaft fur Biochemica und Diagnostica GmbH, Wiesbaden, Germany). Low-density lipoprotein (LDL) levels were calculated using as follows: LDL = TC-[HDL-(TG/5)].

Atherogenic index calculation

Atherogenic index was calculated by using the following formulas:

Atherogenic index = (Total cholesterol-HDL)/HDL

Statistical analysis

Results of the toxicity test were expressed as mean ± SEM. Statistical significance was determined by one-way ANOVA followed by a post hoc Least-Significant Difference (LSD) test. The other parts of the data were analyzed using GraphPad Prism 5.01 (GraphPad Software, USA). The data are presented as mean ± SEM of 5 - 7 animals per group. The sample size was calculated using R program, based on achieving 80% of statistical power. An analysis of variance (One-way or Two-way ANOVA) was used to detect differences and Tukey's post hoc analysis was used for multiple comparisons. P-value less than 0.05 was considered statistically significant.

Results

Acute toxicity

Each substance studied, including roselle calyx extract, passion fruit with pulp concentrate, mixed RP powder, and RP product, was orally administered to rats at a single dose of 2,000 mg/kg BW. No signs of toxicity or rat deaths were observed during the 14-day experimental period. Toxicity evaluation was further conducted by monitoring the body weight gain. Additionally, gross examinations of the internal organs of the treated rats showed no pathological abnormalities (data not shown).

The effect of the extracts on anthropometric parameters and cardiometabolic profiles in HFD-fed rats.

After 8 weeks of treatment, the HFD-fed rats showed significant increase in body weight with 112% increase in weight gain when compared with the control group, whereas rats in all treated groups (passion fruit, roselle, RP powder and RP product) exhibited lower weight gain when compared with the HFD group as shown in **Table 1**. Furthermore, rats in HFD groups had a significantly higher adiposity index than the control ($p < 0.05$). All of the treated groups showed decreased adiposity index when compared with the HFD group.

Similarly, a significant increase in atherogenic index was found in the HFD group when compared with the control group ($p < 0.001$). HFD rats treated with passion fruit, roselle, RP powder, RP product, and simvastatin displayed a significant decrease in atherogenic index when compared with the HFD rats ($p < 0.001$) (**Table 1**). Atherogenic index is a parameter for the prediction of atherosclerosis and coronary artery disease risk. Therefore, it can be concluded that the extracts namely passion fruit, roselle, RP powder, RP product, and simvastatin exhibited anti-obesity properties and reduced the risk occurrence of cardiovascular disease in HFD-fed rats.

Table 1 The effect of the extracts on anthropometric parameters and cardiometabolic profiles in HFD-fed rats**.**

	Control	HFD	Passion fruit	Roselle	RP powder	RP product	Simvastatin
Final body weight (g)	548.80 ± 16.70	629.80 ± 18.17**	547.60 ± 8.94$^{\#\#}$	544.40 ± 22.02$^{\#\#}$	555.6 ± 14.84$^{\#}$	561.20 ± 10.18$^{\#\#}$	575.20 ± 14.46
% Body weight gain	91.514 ± 7.454	112.93 ± 2.69**	83.46 ± 4.67$^{\#\#}$	82.62 ± 6.60$^{\#\#}$	84.54 ± 5.58$^{\#}$	109.50 ± 5.00$^{\tau,\delta,\sigma}$	89.20 ± 5.84$^{\#}$
Adiposity index	1.089 ± 0.026	1.380 ± 0.100*	1.026 ± 0.075$^{\#\#}$	0.998 ± 0.064$^{\#\#}$	1.043 ± 0.067$^{\#}$	0.954 ± 0.061$^{\#\#\#}$	1.003 ± 0.062$^{\#\#}$
Atherogenic index	0.078 ± 0.025	0.486 ± 0.040***	0.124 ± 0.053$^{\#\#\#}$	0.166 ± 0.045$^{\#\#\#}$	0.093 ± 0.013$^{\#\#\#}$	0.175 ± 0.053$^{\#\#\#}$	0.107 ± 0.030$^{\#\#\#}$

The effect of the extracts on anthropometric parameters and cardiometabolic profiles in HFD-fed rats. Control diet fed-rats treated with distilled water (Control, n = 7), HFD-fed rats treated with distilled water (HFD, n = 7), HFD-fed rats treated with passion fruit extract (Passion fruit, n = 5), HFD-fed rats treated with roselle extract (Roselle, n = 5), HFD-fed rats treated with roselle extract and passion fruit extract powder (RP powder, n = 5), HFD-fed rats treated with a product from roselle and passion fruit extract (RP product, n = 6), HFD-fed rats treated with simvastatin (Simvastatin, n = 5). *, **, *** statistically different at p-value < 0.05, 0.01, and 0.001, respectively, when compared to the control group. $^{\#, \#\#, \#\#\#}$ statistically different at p-value < 0.05, 0.01, and 0.001 when compared to the HFD group. $^{\tau}$ statistically different at p-value < 0.05 when compared to the Passion fruit group. $^{\delta}$ statistically different at p-value < 0.05 when compared to the Roselle group. $^{\sigma}$ statistically different at p-value < 0.05 when compared to the RP powder group.

The effect of the extracts on body weight.

The body weight of each group was monitored for 8 weeks during the treatment period. A two-way ANOVA with repeated measures revealed significant effects for time ($F(8,264) = 1,230$, $p < 0.001$), treatment ($F(6,264) = 2.45$, $p = 0.046$), and the interaction between time and treatment ($F(48,264) = 3.19$, $p < 0.001$). A post hoc test using Turkey's method indicated that at week 3, the body weight of HFD-fed rats (HFD) was significantly higher than that of the control group (Control) ($p < 0.05$). Furthermore, at week 5, HFD-fed rats consistently showed higher body weight than the control group ($p < 0.001$). At this time point, the body weights of the Roselle, Passion fruit, and RP powder groups were significantly lower than that of the control group ($p < 0.05$). However, at the end of the treatment period, there was no significant difference in body weight among the groups ($F(6,33) = 2.034$, $p = 0.089$) (**Figure 2**).

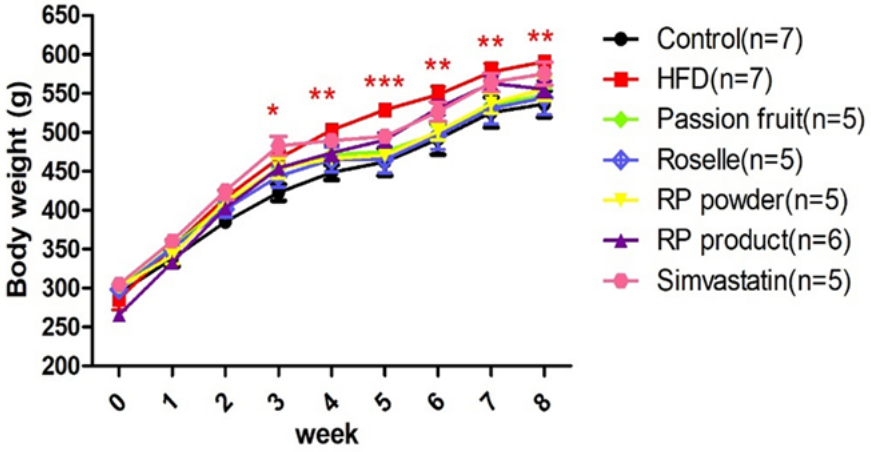

Figure 2 The effect of the extracts on body weight. Control diet fed-rats treated with distilled water (Control, n = 7), high-fat diet fed-rats treated with distilled water (HFD, n = 7), HFD-fed rats treated with passion fruit extract (Passion fruit, n = 5), HFD-fed rats treated with roselle extract (Roselle, n = 5), HFD-fed rats treated with roselle extract and passion fruit extract powder (RP powder, n = 5), HFD-fed rats treated with a product from roselle and passion fruit extract (RP product, n = 6), HFD-fed rats treated with simvastatin (Simvastatin, n = 5). *, **, *** statistically different at p-value < 0.05, 0.01 and 0.001, respectively, when compared to the control group.

The effect of the extracts on blood pressure levels in HFD-fed rats.

Blood pressure was measured in all rat groups at weeks 0, 4, and 8. Caudal blood pressure was measured using a tail-cuff connected to a PowerLab device. SBP was calculated from the recorded graph of the pulse wave displayed on the computer screen. A two-way ANOVA with repeated measures showed no significant effects for time ($F(2,66) = 1.702$, $p = 0.194$), treatment ($F(6,66) = 1.073$, $p = 0.398$), or the interaction between time and treatment ($F(12,66) = 1.342$, $p = 0.217$) (**Figure 3**). These findings suggest that both HFD intake and the different extract formulas do not affect SBP in these rats.

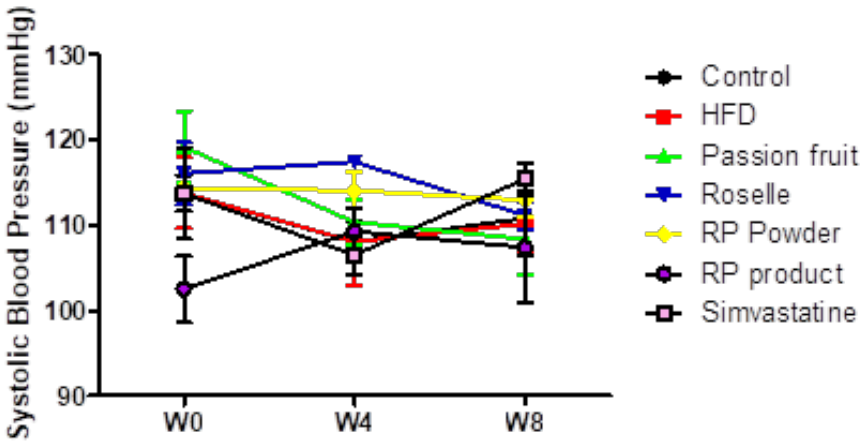

Figure 3 The effect of the extracts on blood pressure levels in HFD-fed rats. Control diet fed rats treated with distilled water (Control, n = 7), HFD-fed rats treated with distilled water (HFD, n = 7), HFD-fed rats treated with passion fruit extract (Passion fruit, n = 5), HFD-fed rats treated with roselle extract (Roselle, n = 5), HFD-fed rats treated with roselle extract and passion fruit extract powder (RP powder, n = 5), HFD-fed rats treated with a product from roselle and passion fruit extract (RP product, n = 6), HFD-fed rats treated with simvastatin (Simvastatin, n = 5).

The effect of the extracts on serum triglyceride level in HFD-fed rats.

We further investigated the effect of HFD intake on serum triglyceride levels and evaluated whether the different formulas of extracts could affect the serum triglyceride level in HFD-fed rats. One-way ANOVA for serum triglyceride levels revealed a significant difference between groups ($F(6,33) = 18.400$, $p < 0.001$). A post hoc test using Turkey's method indicated that the HFD group exhibited a significant increase in serum triglyceride levels compared to the control group ($p < 0.001$). Additionally, the passion fruit, roselle, RP powder, RP product, and simvastatin groups showed significant decreases in serum triglyceride levels compared to the HFD group ($p < 0.001$). However, the serum triglyceride levels of the passion fruit group, roselle group, RP product group, and simvastatin group were significantly higher than those of the control group ($p < 0.05$, $p < 0.001$, $p < 0.01$ and $p < 0.01$, respectively). Interestingly, the serum triglyceride levels of the RP powder group did not differ significantly from those of the control group ($p > 0.05$) (**Figure 4**), indicating that roselle and passion fruit powder treatments fully normalize serum triglyceride levels in HFD-fed rats.

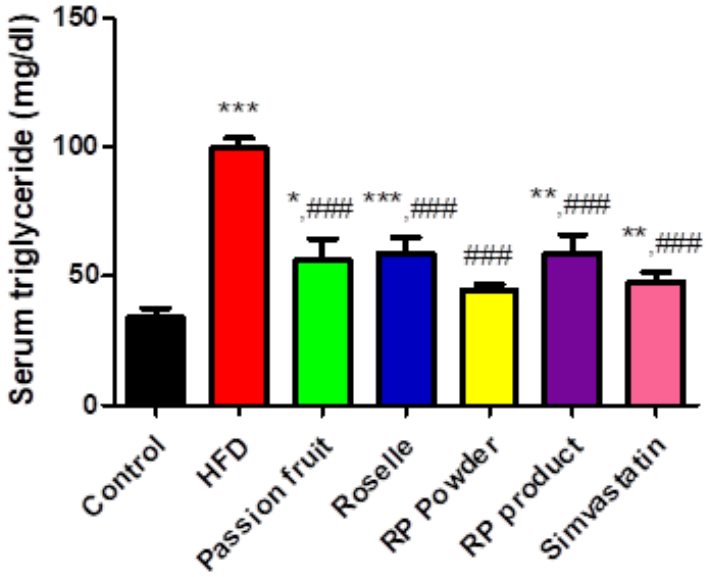

Figure 4 The extracts decreased serum triglyceride levels in HFD-fed rats. Control diet fed rats treated with distilled water (Control, n = 7), HFD-fed rats treated with distilled water (HFD, n = 7), HFD-fed rats treated with passion fruit extract (Passion fruit, n = 5), HFD-fed rats treated with roselle extract (Roselle, n = 5), HFD-fed rats treated with roselle extract and passion fruit extract powder (RP powder, n = 5), HFD-fed rats treated with a product from roselle and passion fruit extract (RP product, n = 6), HFD-fed rats treated with simvastatin (Simvastatin, n = 5). *, **, *** statistically different at *p*-value < 0.05, 0.01 and 0.001, respectively, when compared to the control group. ### statistically different at *p*-value < 0.001 when compared to the HFD group.

The effect of the extracts on serum total cholesterol level in HFD-fed rats.

We investigated the effect of HFD intake on serum total cholesterol levels and evaluated whether the different formulas of extracts affect the serum total cholesterol level in HFD-fed rats. One-way ANOVA for serum total cholesterol levels revealed a significant difference between groups ($F(6,33) = 25.820$, $p < 0.001$). A post hoc test using Turkey's method indicated that the HFD group exhibited a significant increase in serum total cholesterol levels compared to the control group ($p < 0.001$). Interestingly, the passion fruit, roselle, RP powder, RP product, and simvastatin groups showed significant decreases in serum total cholesterol levels compared to the HFD group ($p < 0.001$). However, the serum total cholesterol levels of the

passion fruit group, roselle group, RP product group, and simvastatin group were significantly higher than those of the control group ($p < 0.05$, $p < 0.001$, $p < 0.01$, and $p < 0.01$, respectively). Remarkably, the serum total cholesterol levels of the RP powder group did not differ significantly from those of the control group ($p > 0.05$) (**Figure 5**), indicating that roselle and passion fruit powder treatments fully normalize serum total cholesterol levels in HFD-fed rats.

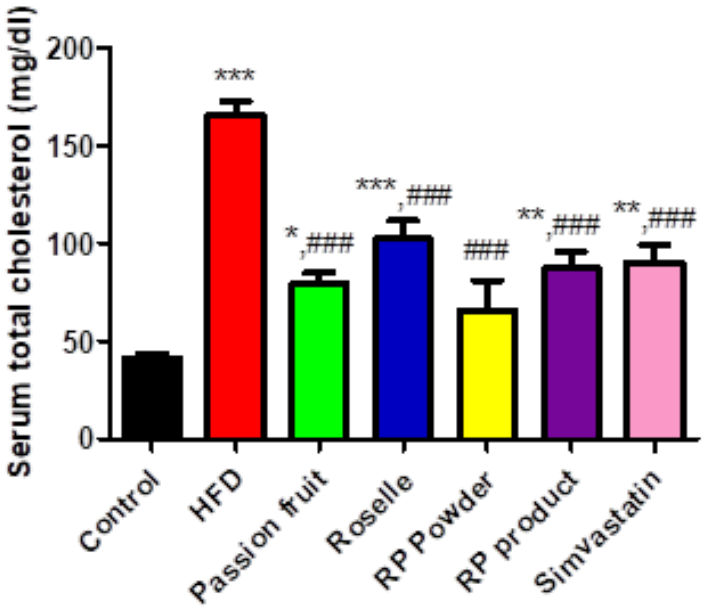

Figure 5 The extracts decreased serum total cholesterol levels in HFD-fed rats. Control diet fed-rats treated with distilled water (Control, n = 7), HFD-fed rats treated with distilled water (HFD, n = 7), HFD-fed rats treated with passion fruit extract (Passion fruit, n = 5), HFD-fed rats treated with roselle extract (Roselle, n = 5), HFD-fed rats treated with roselle extract and passion fruit extract powder (RP powder, n = 5), HFD-fed rats treated with a product from roselle and passion fruit extract (RP product, n = 6), HFD-fed rats treated with simvastatin (Simvastatin, n = 5). *, **, *** statistically different at *p*-value < 0.05, 0.01, and 0.001, respectively, when compared to the control group. ### statistically different at *p*-value < 0.001 when compared to the HFD group.

The effect of the extracts on serum low-density lipoprotein (LDL) level in HFD-fed rats

The effect of HFD intake on serum LDL levels was investigated and compared among the groups. The HFD group exhibited a significant increase in serum LDL levels compared to the control group ($p < 0.001$). Interestingly, the passion fruit, roselle, RP powder, RP product, and simvastatin groups showed significant decrease in serum LDL levels compared to the HFD group ($p < 0.05$ and $p < 0.001$). In addition, the serum LDL levels of the RP powder and passion fruit groups were not different when compared to those of the control group ($p > 0.05$) (**Figure 6**). This indicates that the combination of roselle and passion fruit could normalize serum LDL levels in HFD-fed rats.

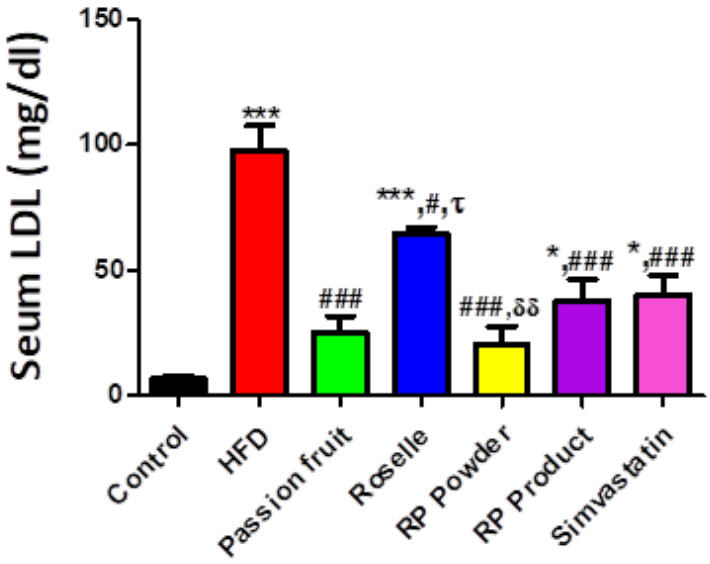

Figure 6 The extracts decreased serum low-density lipoprotein (LDL) levels in HFD-fed rats. Control diet fed-rats treated with distilled water (Control, n = 7), HFD-fed rats treated with distilled water (HFD, n = 7), HFD-fed rats treated with passion fruit extract (Passion fruit, n = 5), HFD-fed rats treated with roselle extract (Roselle, n = 5), HFD-fed rats treated with roselle extract and passion fruit extract powder (RP powder, n = 5), HFD-fed rats treated with a product from roselle and passion fruit extract (RP product, n = 6), HFD-fed rats treated with simvastatin (Simvastatin, n = 5). *, *** statistically different at p-value < 0.05 and 0.001, when compared to the control group. $^{\#, \#\#\#}$ statistically different at p-value < 0.05 and 0.001 when compared to the HFD group. $^{\tau}$ statistically different at p-value < 0.05 when compared to the Passion fruit group. $^{\delta\delta}$ statistically different at p-value < 0.01 when compared to the Roselle group.

The effect of the extracts on serum high-density lipoprotein (HDL) level in HFD-fed rats.

The serum HDL levels were evaluated in this study to compare the effect of extracts in various groups in HFD-fed rats. The HFD group exhibited a significant decrease in serum HDL levels compared to the control group (p < 0.001). Interestingly, the passion fruit, roselle, RP powder, RP product, and simvastatin groups showed significant increase in serum HDL levels compared to the HFD group (p < 0.001) (**Figure 7**). These findings indicate that all the extracts enhance serum HDL levels in HFD-fed rats.

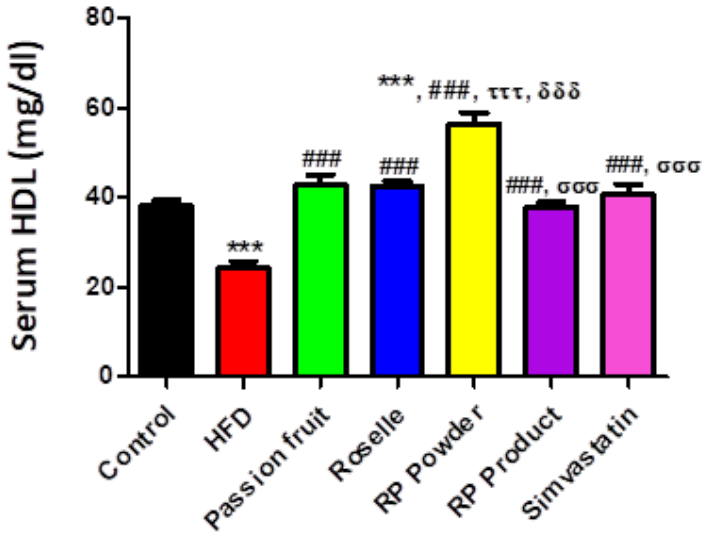

Figure 7 The extracts increased serum high-density lipoprotein (HDL) levels in HFD-fed rats. Control diet fed rats treated with distilled water (Control, n = 7), HFD-fed rats treated with distilled water (HFD, n = 7), HFD-fed rats treated with passion fruit extract (Passion fruit, n = 5), HFD-fed rats treated with roselle extract (Roselle, n = 5), HFD-fed rats treated with roselle extract and passion fruit extract powder (RP powder, n = 5), HFD-fed rats treated with a product from roselle and passion fruit extract (RP product, n = 6), HFD-fed rats treated with simvastatin (Simvastatin, n = 5). *** statistically different at p-value < 0.001, when compared to the control group. ### statistically different at p-value < 0.001 when compared to the HFD group. τττ statistically different at p-value < 0.001 when compared to the Passion fruit group. δδδ statistically different at p-value < 0.001 when compared to the Roselle group. σσσ statistically different at p-value < 0.001 when compared to the RP Powder group.

Discussion

The present study demonstrated that consumption of a HFD could induce obesity, all HFD-fed rats, especially the group that received only HFD were increased body weight and became obese. Comparing to the rats fed with standard diet, the HFD rats showed 14% body weight than the control group and were classified as obese. According to the previous study, the rats received HFD regimen in which body weight increased higher than 10% when compared to the normal diet were considered obese [23]. Interestingly, treatment with passion fruit extract, roselle extract, a combination of roselle and passion fruit extract powder, the product derived from roselle and passion fruit extract, and simvastatin for 8 weeks was found to decrease the levels of serum triglyceride, total cholesterol, LDL and increased HDL in rats fed HFD. In addition, HFD rats treated with different formulas exhibited lower weight gain over the experimental period compared to the HFD group. Therefore, the extracts demonstrated the anti-obesity and anti-dyslipidemic effects. However, these treatments did not affect SBP in the HFD-fed rats.

Our study found that rats fed a HFD exhibited dyslipidemia, characterized by hypercholesterolemia and hypertriglyceridemia. These findings align with previous studies demonstrating that prolonged HFD intake can induce dyslipidemia in rats [14,24]. Furthermore, treatment with roselle extract alone, at a dose of 500 mg/kg BW for 8 weeks, resulted in decreased levels of serum triglyceride, total cholesterol, and LDL in HFD-fed rats. The reductions observed in serum triglyceride, total cholesterol, and LDL levels in HFD-fed rats treated with the roselle extract were similar to those observed in HFD-fed rats treated with simvastatin, a commonly prescribed cholesterol-lowering medication [9]. These results are consistent with previous studies reported the hypolipidemic effect of roselle extract in animal models of obesity [13,14]. Interestingly, we also found that the levels of serum triglyceride, total cholesterol, and LDL in HFD-fed rats

treated with a mixture of roselle and passion fruit extract powder (RP powder group) were comparable to those of the control group. This suggests that the roselle and passion fruit extract in powder formula completely normalizes the serum triglyceride, total cholesterol, and LDL levels in HFD-fed rats.

Roselle has a long history of use in traditional medicine due to its well-known medicinal properties, including anti-inflammatory, hepatoprotective, antioxidant, anti-hypercholesterolemic, anti-triglyceridemic, and anti-hyperglycemic activities [25,26]. Several mechanisms have been proposed to explain the anti-hyperlipidemic effect of roselle, such as the modulation of fatty acid synthase (FASN), acetyl-CoA carboxylase (ACC), and sterol element-binding protein (Srebp-1c) expressions, as well as the activation of the energy sensor AMP-activated protein kinase (AMPK) [13,26]. Regarding bioactive compounds found in roselle, key constituents responsible for their pharmacological and therapeutic effects have been identified. These include organic acids such as hydroxycitric acid and hibiscus acid, anthocyanins like delphinidin3-sambubioside and cyanidine-3-sambubioside [25], phenolic acids including protocatechuic acid and caffeic acid, flavonoids like epigallocatechin gallate and catechin, as well as volatile compounds [27,28]. Antioxidant capacity and anti-hyperlipidemic effects of the polyphenolic fraction of roselle extract have also been reported [25]. Notably, it has been demonstrated that the phenolic extract of roselle (HPE) is more potent than the crude extract in reducing serum LDL cholesterol, decreasing hepatic lipid content, and improving LDL uptake in hepatocytes [29]. Furthermore, treatment with HPE at a dose of 200 mg/kg has been shown to reduce plasma triglyceride and cholesterol levels in both HFD-fed rats and rats with type 2 diabetes induced by HFD combined with streptozotocin injection [30]. Clinical studies have also reported the beneficial effects of consuming roselle extract on lipid profiles in patients with metabolic syndrome [31]. A randomized crossover study involving individuals with elevated cholesterol levels demonstrated the dose-dependent cholesterol-lowering effect of roselle extract [32]. Similarly, a randomized clinical study reported that daily consumption of roselle capsules (containing 450 mg of roselle extract) for 12 weeks effectively reduced total cholesterol, triglycerides, and serum fatty acids in obese subjects [15]. These anti-hyperlipidemic effects of roselle may be attributed to its anthocyanin, flavonoid, and polyphenol contents.

P. edulis, commonly known as passion fruit, has been recognized for its various biological and pharmacological properties. The extract derived from its leaves has been reported to possess anti-inflammatory, antioxidant, and anti-hypertensive effects [33,34]. Our study observed that rats fed a HFD exhibited elevated levels of serum triglyceride, total cholesterol, and LDL and the treatment with passion fruit extract for 8 weeks resulted in a reduction of serum triglyceride, total cholesterol, and LDL levels in HFD-fed rats. These findings are consistent with a previous study reporting the hypolipidemic effect of passion fruit leaf extract [35].

Passion fruit comprises various components, including polyphenols, triterpenes, glycosides, carotenoids, cyanogenic glycosides, polysaccharides, amino acids, essential oils, and microelements [16,36]. Polyphenols, triterpenes, and polysaccharides are considered the key active constituents responsible for the biological and pharmacological activities of passion fruit. Passion fruit pulp, in particular, is known to be rich in flavonoids, with approximately 158 mg/ml of total flavonoids [37]. Major flavonoids identified in passion fruit include vitexin, isovitexin, isoorientin, apigenin, quercetin, luteolin, and their derivatives, contributing to its diverse biological and pharmacological properties [16]. Triterpenoids have also been identified as significant constituents of passion fruit. Notably, cycloartane triterpenoids have demonstrated protective effects against glutamate-induced excitotoxicity in PC12 cells [38]. Furthermore, *in vivo* studies have reported the antidepressant-like effect of cycloartane triterpenoids [39].

Hyperlipidemia is a long-time well-documented parameters associated with cardiovascular diseases including atherosclerosis, myocardial infarction, stroke, and many others [40,41]. The preventive approach, fast diagnosis, and proper treatment and management of dyslipidemia can reduce the risk of premature death from cardiovascular diseases [42]. Several studies reported that adiposity index and atherogenic index are directly associated with cardiometabolic risk [43,44]. From the present study, roselle, passion fruit, and a mixture of roselle and passion fruit in the forms of

powder and jelly exhibited the potential benefit to reduce adiposity index and atherogenic index, which can be implied for the good predictor for reducing cardiovascular disease risk.

It has been demonstrated that hyperglycemia plays a significant role in determining concentrations of total cholesterol, VLDL, and triglycerides [45]. Therefore, further studies should investigate whether treatment with passion fruit extract and roselle extract would impact plasma glucose levels in HFD-fed rats. Additionally, both clinical and animal studies have reported the detrimental effects of obesity and long-term HFD intake on brain function, particularly in the areas of learning and memory processes [46-48]. Consequently, it would be interesting to examine whether passion fruit extract and roselle extract affect cognitive function and brain plasticity in HFD-induced obese animals.

To expand potential molecular pathways in order to explain the hypolipidemic effects of different formulas, 5′-adenosine monophosphate (AMP)-activated protein kinase (AMPK) is one of the target proteins of interest.

It has been shown that AMPK inhibits fatty acid synthesis by inducing the inhibitory phosphorylation of acetyl-coA carboxylase 1 (ACC1) and sterol regulatory element-binding protein 1c (SREBP1c), a transcription factor that induces several lipogenic enzymes expression, including ACC1 and fatty acid synthase [49,50]. AMPK also inhibits cholesterol synthesis by inducing the inhibitory phosphorylation of the rate-limiting enzyme HMG-CoA reductase [51]. Further studies should investigate whether different formulas would affect the expression of AMPK levels in different cells including liver cells, adipose cells, and skeletal muscle cells.

The low-density lipoprotein receptor (LDLR) plays a crucial role in clearing LDL from the bloodstream via receptor-mediated endocytosis. This process involves LDL binding to the LDLR on the cell surface, internalization of the complex, and subsequent degradation of LDL in lysosomes, while the receptor is recycled back to the cell surface [52]. Thus, LDLR recycling to the plasma membrane is crucial in the LDL clearance from plasma and in reducing the levels of circulating LDL. Therefore, further study is needed to investigate the LDLR levels in this context.

The present study has some limitations. First, only male rats were used then it may not account for sex-specific effect. Therefore, future studies should be conducted to evaluate the hypolipidemic potential of roselle extract and passion fruit juice with pulp concentrate formulations in female HFD-fed rats. Second, the treatment duration should be extended to reflect the long-term effect of these formulations. Lastly, unequal group sizes were used due to budget constraints and limited availability of the treatment samples.

In term of human application, our study provides valuable insights for the commercial development of functional ingredients or foods and functional beverages with anti-dyslipidemic properties, utilizing *H. sabdariffa* and *P. edulis* as sources of functional bioactive compounds. However, the bioavailability and the dosage of these active compounds should be investigated in human subjects.

Conclusions

Our study demonstrates that passion fruit juice with pulp concentrate, roselle extract, RP powder, and RP product, all of these formulas possess anti-dyslipidemic properties. Moreover, the use of these formulas is safe and their efficacy in reducing serum lipid levels is compatible with simvastatin treatment. These findings suggest that passion fruit juice with pulp concentrate and roselle extract could be considered as potential alternative therapies for preventive approaches or treatment of individuals with hyperlipidemia conditions.

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