

Effect of the roselle (*Hibiscus sabdariffa* L.) calyces drink on the physiological parameters of healthy adult subjects

AJENG DIANTINI¹, SYAMSUL RAHMAT^{1,2}, ANNISA ALPIANI^{1,3},
SRI ADI SUMIWI¹, LEONARDO LUBIS⁴ and JUTTI LEVITA¹

¹Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Bandung, West Java 45363; ²Faculty of Health, Universitas Qamarul Huda Badaruddin Bagu, Central Lombok Regency, West Nusa Tenggara 83371; ³Faculty of Health, Universitas Nahdlatul Ulama, Mataram, West Nusa Tenggara 83123; ⁴Department of Anatomy, Physiology, and Cell Biology, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java 45363, Indonesia

Received July 13, 2021; Accepted August 17, 2021

DOI: 10.3892/br.2021.1465

Abstract. The calyx of roselle (*Hibiscus sabdariffa* L.) has been the focus of attention of several studies, and although earlier studies on this plant showed it to possess antilipidemic and antidiabetic activities, none of these studies have assessed the effects of this plant on the physiological parameters of healthy subjects. The present study determined the effects of roselle calyx beverages on blood pressure, abdominal circumference, body mass index (BMI), hematological and urinary parameters, and the lipid profile of healthy subjects. Healthy subjects (n=30) were treated with a 200 ml roselle calyx beverage each morning and evening for 30 consecutive days. Every week the subjects were asked to perform some physical activity by following the Young Men's Christian Association step test. This study used a pre-post quasi-experimental design. Blood samples were taken before (day-0) and after the intervention (day-30). There was an increase in the levels of high-density lipoproteins (from 47.0 to 49.5 mg/dl; P=0.015), and a significant decrease in both systolic and diastolic (P=0.036 and P=0.030, respectively), and the abdominal circumference of the subjects (P=0.005). However, total cholesterol levels (191.2 to 191.9 mg/dl; P=0.821) and low-density lipoprotein levels (129.8 to 133.5 mg/dl; P=0.249) were slightly increased, although not significantly. A lower level of triglycerides (114.3 to 107.4 mg/dl; P=0.269) and very-low-density lipoprotein levels (22.8 to 21.4 mg/dl; P=0.681) was observed. No significant differences were detected in the BMI or the urinalysis parameters were identified. Based on these results, roselle

(*Hibiscus sabdariffa* L.) may be used as a supplement to prospectively improve the health status of subjects. No serious adverse effects were observed during this trial.

Introduction

In Asia, particularly Indonesia, the dried calyces of roselle (*Hibiscus sabdariffa* L.) flower have been commonly utilized as hot and cold beverages. A study of the effect of the dried *H. sabdariffa* calyces extracts in hypertensive rats indicated it possessed antihypertensive activity (1).

A previous randomized-control cross-over study on 25 British male participants with 1-10% cardiovascular risk disease at the Hugh Sinclair Unit of Human Nutrition (University of Reading, UK), reported that the consumption of 2-meal *H. sabdariffa* calyces extracts increased the flow-mediated dilatation of the branchial artery, urinary and plasma nitric oxide (NOx) levels, and decreased the systolic (SBP) and diastolic blood pressure (DBP), as well as serum glucose, plasma insulin, serum triacylglycerol and C-reactive protein levels (2). A double-blind, placebo-controlled clinical trial revealed that daily consumption of *H. sabdariffa* tisane significantly lowered SBP and DBP in 65 non-smoking adults (70% Caucasians) recruited from the greater Boston area who were diagnosed with pre to mild hypertension (3). *H. sabdariffa* consumption also significantly lowered plasma angiotensin-converting enzyme activity and serum sodium levels without altering potassium levels in Mexican adult patients with stage I or II hypertension (4). Furthermore, twice daily consumption of *H. sabdariffa* tea in 60 Iranian diabetic patients with mild hypertension in Yazd, Iran, for 1 month resulted in a decrease in SBP, but no effect on DBP (5). A study on 32 African male subjects (aged 21-32) in Cameroon, Central Africa, showed that consuming 500 ml *H. sabdariffa* drink twice a day for 2 weeks resulted in a significant increase in erythrocyte counts, hemoglobin levels, packed cell volume, mean platelet volume, high-density lipoprotein (HDL), triglycerides and creatinine levels, as well as significantly reducing leucocytes counts, MID cells, low-density lipoprotein (LDL) and total cholesterol levels. It

Correspondence to: Professor Jutti Levita, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang km 21, Sumedang, West Java 45363, Indonesia
E-mail: jutti.levita@unpad.ac.id

Key words: anthocyanins, antioxidants, flavonoids, *Hibiscus sabdariffa*, roselle

was reported that there was no significant change in blood glucose, L-aspartate aminotransferase, L-alanine aminotransferase and urea levels (6).

However, none of these studies assessed the effects of *H. sabdariffa* on healthy Indonesian subjects. The present study examined the effects of the *H. sabdariffa* beverage (HSB) on the physiological parameters of healthy Asian male and female subjects. The aim of this study was to: i) Assess hematological and urinary parameters; and ii) measure the lipid profile of healthy Indonesian male and female subjects, after twice daily consumption of HSB for 30 days.

Materials and methods

Study area. The present study was performed at the Faculty of Pharmacy, Universitas Padjadjaran, and the Health Technical Unit of Universitas Padjadjaran, (West Java, Indonesia) in 2019.

Identification of plant material. Dried roselle (*H. sabdariffa*) calyces, purchased in Bandung (West Java, Indonesia), were taxonomically identified (document no. 187/05/HB/2018) by Dr Joko Kusmoro, a certified biologist, at the Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran.

Preparation of HSB. HSB was prepared by following the boiling method described by Salami and Afolayan (7) with some modifications (without the addition of sliced fruits pineapple, oranges or lime with peel). In total, 100 g dried calyces were boiled in 10 l potable water for 15 min. A small amount of sugar 500 g/10 l was added to neutralize the sourness.

Proximate analysis, and determination of vitamin C and anthocyanins in HSB. Proximate content (moisture, ash, protein, fat and carbohydrates) and vitamin C levels in the HSB were strictly determined according to the protocol of the Indonesian National Standard SNI 01-2891-1992 (8). The determination of anthocyanin content in the HSB was carried out by following the pH differential method as described by Giusti and Wrolstad (9). This method measures the difference in the absorbance spectra of the colored oxonium form of anthocyanin at pH 1.0 and the colorless hemiketal form at pH 4.5. The results are presented in Table I.

Subject recruitment and ethical considerations. A total of 30 subjects aged 21-55 (39.33±9.18) years old, 17 male and 13 female, were recruited by an advertisement spread. The subjects had agreed to participate in the present study by signing the informed consent form according to the Ethical Principles for Medical Research involving Human Subjects of the WMA Declaration of Helsinki (wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). All subjects were confirmed healthy (Table II) after being examined by a certified medical practitioner at the Health Technical Unit of Universitas Padjadjaran. The present study was approved by the Research Ethics Committee of Faculty of Medicine, Universitas Padjadjaran (approval no. 35/UN6.C.10/PN/2018; kep.unpad.ac.id/), which is recognized by the FERCAP-Forum of Ethics Review Committee in Asia & Western Pacific Region, since 2014).

Table I. Nutritional properties of HSB.

Constituent	Result	Method ^a
Water, %	94.82	SNI 01-2891-1992
Ash, %	0.09	SNI 01-2891-1992
Protein, %	0.28	SNI 01-2891-1992
Fat, %	1.47	SNI 01-2891-1992
Carbohydrate, %	3.34	SNI 01-2891-1992
Vitamin C, %	0.02	Titrimetric/iodimetric
Anthocyanin, mg/l	26.44	pH differential method
Calorie, kcal	27.71	-
Calcium, mg/l	167.10	Atomic Absorption Spectroscopy

^aAll methods are based on the Indonesian National Standard SNI 01-2891-1992 (8).

Table II. Baseline physiological parameters of the subjects (n=30).

Characteristics	n (%)	Mean ± standard deviation
Age, years		39.33±9.18
21-40	14 (47)	
>41	16 (53)	
Sex		-
Male	17 (56.7)	
Female	13 (43.3)	
Body mass index		24.41±3.48
Normal	18 (60)	
Overweight	10 (33)	
Obese	2 (7)	
Abdominal circumference		84.23±9.44
Systolic blood pressure		113.00±9.79
Diastolic blood pressure		79.67±7.53
Education		
Secondary	14 (46.6)	
Higher education	16 (53.3)	
Exercise or other physical activity	21 (70)	
Smoking		
Yes	7 (23.3)	
No	23 (76.7)	

Study design and preparation. The present study was a quasi-experimental study with a pre-post design. A total of 30 healthy subjects were treated with 200 ml HSB each morning and evening for 30 consecutive days. Every week the subjects were asked to perform physical activity by following the Young Men's Christian Association step test (10,11). Urine and blood samples were taken before (day 0) and after completion of the intervention (day 30) at 8 am prompt by the clinicians

Table III. Pre- and post-physiological parameters of the subjects (n=30).

Physiological parameter	Mean	t/Z	P-value
Body mass index, kg/m ²		0.74 ^c	0.338
Pre	24.4		
Post	24.1		
Abdominal circumference, cm		3.004 ^c	0.005 ^b
Pre	84.2		
Post	81.8		
Systolic blood pressure, mmHg		-2.094 ^d	0.036 ^a
Pre	113		
Post	108.3		
Diastolic blood pressure, mmHg		-2.988 ^d	0.030 ^a
Pre	77.3		
Post	73.2		
Cholesterol levels, mg/dl		-0.229 ^c	0.821
Pre	191.2		
Post	191.9		
Low density lipoprotein levels, mg/dl		-1.176 ^c	0.249
Pre	129.8		
Post	133.5		
High density lipoprotein levels, mg/dl		-2.440 ^d	0.015 ^a
Pre	47		
Post	49.5		
Triglyceride levels, mg/dl		1.127 ^c	0.269
Pre	114.3		
Post	107.4		
Very low density lipoprotein levels, mg/dl		-0.411 ^d	0.681
Pre	22.8		
Post	21.4		

^aP<0.05, ^bP<0.01. ^cPaired t-test; ^dWilcoxon test. t, used for testing the mean of one population against a standard or comparing the means of two populations; Z, used for testing the proportion of a certain characteristic vs. a standard proportion, or comparing the proportions of two populations.

at the Health Technical Unit of Universitas Padjadjaran. The subjects were asked to fast for 10 h prior to collection.

Measurement of physiological parameters pre and post-treatment with HSB. Anthropometry data of the subjects: Height, weight and abdominal circumferences, were measured, as they represent the diagnostic criteria for obesity. The subjects were advised to wear light clothing and to take off their shoes before the measurement (12).

BMI for each participant was calculated based on the body weight (kg) and height (cm) using an online BMI calculator (nhlbi.nih.gov/health/educational/lose_wt/BMI/bmi-m.htm).

SBP and DBP. SBP and DBP were measured using a sphygmomanometer and stethoscope based on the American Heart Association's recommendation with three replicates per reading (13).

Urine collection and analysis. Overnight urine was collected and analyzed as described previously (14-16). The subjects

were given direct instructions regarding the morning urine collecting procedure. The first few drops of the urine were discarded, and the collected urine sample was stored in sealed tubes. The urine samples were analyzed qualitatively using urinalysis reagent strips (cat. no. 006T250; Ultimed Products) to determine leukocyte counts, nitrite levels, urobilinogen levels, protein content, pH, blood, ketones, specific gravity, bilirubin and glucose levels.

Biochemical analyses. Blood samples were taken by a clinician. The blood was placed in K₂EDTA BD vacutainer plastic blood collection tubes (Thermo Fisher Scientific, Inc.). Plasma was separated by centrifuging at 2,040 x g for 15 min and stored at -20°C until required. The lipid profile was measured by enzymatic assays using an ARCHITECT c4000 clinical chemistry analyzer (Abbott Pharmaceutical Co. Ltd.).

Statistical analysis. A Shapiro-Wilk test for normality was used and data are presented as the mean ± standard deviation. Data were compared using a paired t-test or Wilcoxon test as

based on the normality of distribution in SPSS version 13.0 (SPSS, Inc.). $P < 0.05$ was considered to indicate a statistically significant difference.

Results and Discussion

Proximate analysis, and determination of vitamin C and anthocyanin content in the HSB. The nutritional properties (proximate, vitamin C and anthocyanin) of HSB are presented in Table I. HSB has been shown to contain high calcium levels, a moderate amount of anthocyanin, and a small amount of protein, fat, carbohydrate and vitamin C.

The presence of anthocyanin in dried roselle calyces has been reported previously (17,18). Moreover, the vitamin C content was also confirmed (18-20). Delphinidin-3-sambubioside, cyanidin-3-sambubioside, delphinidin-3-glucoside and cyanidin-3-glucoside are four anthocyanins that have been shown to be present in the *H. sabdariffa* calyces and are hypothesized to play a role in its antioxidant property (21,22).

It was reported that anthocyanins, due to their phenolic structure, function as an antioxidant via different mechanisms. Anthocyanins capture free radicals, inhibit xanthine oxidase and chelate metal ions (23). The phenolic moiety in the anthocyanins molecule contributes to the transfer of a proton to a free radical, eventually regenerating the acyl glycerol molecule, and stopping the oxidation reaction (24). The substitution of hydroxyl with sugars in anthocyanins molecule reduces xanthine oxidase activity. Moreover, the planarity of aromatic rings of anthocyanins, (the B ring that is connected by conjugation to A and C rings) may be responsible for this inhibitory effect (25).

Characteristics of the subjects and the baseline physiological parameters (pre-treatment with HSB). The subjects, aged 21-55 years, with a BMI between 18-30, were recruited in the present study. Of these, 7 of the subjects (23.3%) were smokers, and none consumed alcohol; 18 subjects (60%) had a normal BMI, and the average abdominal circumference was 84.23 ± 9.44 cm. The BP of the subjects was in the normal range. Additionally, 21 of the subjects (70%) confirmed their weekly exercise, such as walking and biking, or other physical activity (Table II).

The subjects were confirmed healthy by a physician's examination and urinalysis at the Health Technical Unit of Universitas Padjadjaran (unpad.ac.id/universitas/fasilitas/kesehatan/). Electrocardiographic examination of the 30 healthy subjects (encoded as R1 to R30) revealed normal heart condition.

The color (colorless to pale yellow) and pH of urine of the subjects ranged between 5.0-6.5 (the mean pH value was 6.07), which was considered normal. One subject (R20) had a high leucocyte (white blood cells, WBC) number in the urine. The presence of abnormal of WBC counts was also detected in the urine of 3 subjects (R7, R8 and R16), which was likely associated with inflammation of the urinary tract or the use of steroid drugs (16). Moreover, no erythrocytes, nitrites, ketones, bilirubin or glucose were detected in the urine of any of the subjects.

Physiological parameters of the subjects post-treatment with HSB. Daily two-meal consumption (morning and evening) of 200 ml HBS for 30 consecutive days, significantly lowered the

Table IV. Pre- and post-urinalysis parameters (n=30).

Physiological parameter	Mean	t/Z ^a	P-value
White blood cell count		-1.054	0.292
Pre	23.67		
Post	6.61		
Nitrite		0	1
Pre	0		
Post	0		
Urobilinogen		0	1
Pre	0.2		
Post	0.2		
Protein content		-1.508	0.132
Pre	6.96		
Post	4.29		
pH		-1.794	0.073
Pre	6.06		
Post	5.86		
Red blood cell count		0	1
Pre	0		
Post	0		
Specific gravity		-0.617	0.537
Pre	1.017		
Post	1.018		
Ketones		0	1
Pre	0		
Post	0		
Bilirubin		0	1
Pre	0		
Post	0		
Glucose		0	1
Pre	0		
Post	0		

^aAll data were compared using a Wilcoxon test. t, used for testing the mean of one population against a standard or comparing the means of two populations; Z, used for testing the proportion of a certain characteristic vs. a standard proportion, or comparing the proportions of two populations.

abdominal circumference ($P=0.005$) and both SBP and DBP ($P=0.036$ and $P=0.030$, respectively) of healthy adults. There was an increase in the levels of high-density lipoproteins (47.0 to 49.5 mg/dl; $P=0.015$). However, the total cholesterol (191.2 to 191.9 mg/dl; $P=0.821$) and low density lipoprotein levels (129.8 to 133.5 mg/dl; $P=0.249$) were slightly increased, although not significantly. A lower level of triglycerides (114.3 to 107.4 mg/dl; $P=0.269$) and very low-density lipoprotein (VLDL) (22.8 to 21.4 mg/dl; $P=0.681$) was observed (Table III). No significant differences were observed in the BMI (Table III) or the urinalysis parameters (Table IV).

These findings are in line with previous similar studies in 25 British male participants with 1-10% risk of cardiovascular disease (2), in 65 non-smoking pre- to mild hypertensive

patients (70% Caucasians, aged 30-70 years) (3), in 60 Iranian diabetic patients with mild hypertension (5) and in 32 African male subjects (aged 21-32) (6). Interestingly, a *H. sabdariffa* isotonic drink was reported to be capable of increasing the physical fitness index of healthy respondents (25).

In conclusion, healthy subjects treated with 200 ml roselle calyx beverage each morning and evening for 30 consecutive days resulted in an increase in HDL levels. A significant decrease of both SBP and DBP and the abdominal circumference of the subject was also noticed. However, the total cholesterol and LDL levels were slightly increased, although not significantly. Lower levels of triglycerides and VLDL were observed. No significant difference was detected in the BMI and the urinalysis parameters. *H. sabdariffa* L. may thus prospectively improve the health status of subjects. No serious adverse effects were observed during this trial. However, the data of this study is still limited due to the lack of a normal control group, the heterogeneity of the subjects and the small sample size. Nonetheless, the present study provides evidence of the value of *H. sabdariffa* L for healthy subjects.

Acknowledgements

We would like to thank Professor Tri Hanggono Achmad, the Rector of Universitas Padjadjaran via the Directorate of Research and Community Engagement for facilitating this work by granting the Academic-Leadership Grant (ALG) 2019 of Universitas Padjadjaran.

Funding

This work was funded by the Academic-Leadership Grant (grant no. 1373i/UN6.O/LT/2019).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Author's contributions

AD and JL were principally responsible for the conception and design of the study. SAS and LL supervised and monitored the recruitment and selection of the subjects. SR and AA participated in the preparation of the roselle beverage and measurement of other clinical parameters. AD, JL and SAS contributed equally to the acquisition, and interpretation of the reported data. AD, JL and LL contributed to the processing and analysis of the data. JL contributed to the writing, and revising of the manuscript. All authors read and approved the final manuscript. SR and AA confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Written informed consent was obtained from all the participants. The Research Ethical Committee of Universitas Padjadjaran, Indonesia approved the procedures (approval no. 35/UN6.C.10/PN/2018). The Declaration of Helsinki (2000) and the applicable national standards as they

relate to the involvement of human subjects in the research were enforced in the design and conduct of the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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