Roselle (*Hibiscus sabdariffa* L.) calyces tea improves physical fitness of healthy adults

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Abstract. Roselle calyces (*Hibiscus sabdariffa* L.) is a plant that contains anthocyanin and flavonoids, which function as exogenous antioxidants for the human body to counteract excess oxidative stress. Roselle has anti-hypertensive, anti-cancer and anti-diabetic effects. The present study evaluated roselle to see whether it affects physical fitness. The components of physical fitness include strength, balance, right and left hand grip, vertical jump and VO_{2max} (maximum oxygen consumption). A total of 30 subjects received 200 ml rosella tea for 30 days every morning and evening. Every week, subjects were assessed for physical fitness. Data analysis used paired t and Wilcoxon test according to the normality test results. The results showed significant improvements in strength (from 24.9 to 27.3 kg; P=0.025), balance (from 23.3 to 42.2 sec; P=0.004), right (from 31.8 to 35.1 kg; P<0.0001) and left hand grip (from 29.8 to 31.6 kg; P=0.020), vertical jump (from 38.6 to 41.1 m/sec; P=0.008) and VO_{2max} (from 31.1 to 34.3 ml/kg/min; P=0.014). This demonstrated that roselle significantly improved six parameters of physical fitness and may be used as a supplement to improve physical fitness without severe side effects.

Introduction

During the aging process, physical fitness will reduce by 40-80%, usually caused by metabolic disorders or chronic disease that often occur in adults (1). Age is one aspect that affects fitness levels driven by physiological, psychosocial and environmental factors (2). This decrease in physical fitness is caused by excessive oxidative stress. Under these conditions, the body needs additional or exogenous antioxidants. One source of exogenous antioxidant is rosella (*Hibiscus sabdariffa* L.), which contains high levels of anthocyanins and flavonoids, especially in the flower petals (3). Anthocyanins have been proven to have effects on human body systems, one of which is the cardiovascular system: Research in New Zealand demonstrated that the anthocyanin content in blackcurrants can increase vasodilation and cardiac output (4). This can increase peripheral blood flow and potentially improve exercise performance. Anthocyanin compounds also influence muscle regeneration by accelerating the myogenesis process, protecting mitochondria, muscle protein synthesis and protecting against muscle protein degradation (5). A systemic review concluded that *H. sabdariffa* inhibits LDL oxidation and prevent atherosclerosis (6).

To the best of our knowledge, there are no previous studies on the effect of anthocyanins in *H. sabdariffa* on physical fitness (comprising flexibility and musculoskeletal and cardiorespiratory fitness). Therefore, the present study aimed to investigate whether the anthocyanins in *H. sabdariffa* influence strength, balance, left and right hand grip, vertical jump and VO_{2max} (maximal oxygen consumption), which are the parameters of physical fitness.

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Materials and methods

Materials. Dried rosella (*H. sabdariffa*) was purchased in Bandung and identified by a certified biologist. A total of 100 g dried calyces was boiled with 10 l water for 15 min, as previously described but without adding sliced orange, pineapple or lime (7). The resulting *H. sabdariffa* tea (HST) was cooled at 35˚C and packaged in bottles containing 200 ml HST. HST was prepared twice/day to maintain quality when consumed.

The contents HST, such as proximate content and levels of vitamin C, were determined based on the Indonesian National Standard SNI 01-2891-1992 (8). Anthocyanin content in HST was determined by the differential pH method using ultraviolet spectroscopy or Ultraviolet–visible (UV-VIS) spectrophotometry (9) The anthocyanin structure can be transformed reversibly with changes in pH which can be seen from the absorbance spectra (colored oxonium compounds at pH 1.0 and colorless carbinol compounds).

Study design. The present study was conducted in December 2019 at the Faculty of Pharmacy, Padjadjaran University, and the Health Engineering Unit in Universitas Padjadjaran, Sumedang, West Java, Indonesia, and was approved by the Research Ethics Commission of the Faculty of Medicine, Padjadjaran University (approval no. 35/UN6.C.10/PN/2018; kep.unpad.ac.id/).

The present study used the pre/post observation method on 30 subjects selected from 36 prospective research subjects Inclusion criteria were as follows: i) Subjects were declared healthy by a health worker; ii) subjects provided written informed consent to participate and iii) subjects scored low to moderate on the Global Physical Activity Questionnaire (10). Exclusion criteria were as follows: i) Subjects categorized as obese according to World Health Organization criteria and ii) subjects who did not complete routine physical fitness checks every week. The subjects were categorized as obese (≥30 kg/m^2) or non-obese based on BMI and waist circumference (male, ≥90 cm; women ≥80 cm) (11). Subjects were advised to wear as little clothing as possible and took off their shoes during the examination. Blood pressure was assessed according to the American Heart Association's Recommendation guidelines using a sphygmomanometer and stethoscope (12). Percentage body fat was determined using the 3-site skinfold thickness method using Lange skinfold calipers (13).

Subjects comprised 20 males and 10 females aged 24-56 years (Fig. 1). The subjects received 200 ml HST daily in the morning and in the evening for 30 days. On days 0, 7, 14, 21 and 30, physical fitness was assessed.

Strength. Strength of both arm extensor muscles was performed using a dynamometer. The subject stood with the dynamometer in front of the chest. Withdrawals are made in one pull and repeated three times. Strength was also assessed by right and left grip using a hand grip dynamometer, recommended by the American Society of Hand Therapy (14,15).

Balance. Balance was assessed by single leg balance test. Subjects stood on a Takei 5407c force plate with eyes closed. The subjects were asked to raise one leg and maintain their balance for 20 sec. The test was conducted three times, as previously described (16).

Vertical jump. Subjects were asked to jump as high as possible on a Takei 5414 force plate. The test was conducted three times starting with a warm-up (10 bodyweight walking, five body weight squat jumps, 10 body weight squats, and three maximal body weight countermovement jumps) and resting for 15 sec between tests, as previously described (17).

VO_{2max}. Measurements were taken by conducting the Young Men's Christian Association 3-min step test (18). The subjects rested for 2 min on a chair in a room with good air quality before stepping on and off a box 30 cm high 75 times in 3 min (24 cycles of four steps). This test can show oxygen consumption by see in indirect calorimetry (18).

Table I. Nutritional properties of Hibiscus Sabdariffa tea.

Constituent	Value
Water, %	94.82
Protein, %	0.09
Fat, %	0.28
Ash, %	1.47
Carbohydrate, %	3.34
Vitamin C, %	0.02
Anthocyanin, mg/l	26.44
Calorie, kcal	27.71
Calcium, mg/l	167.10

Water, protein, fat, ash, carbohydrate and vitamins levels were obtained on the Indonesian National Standard SNI 01-2891-1992. Anthocyanin content was determined by the differential pH method.

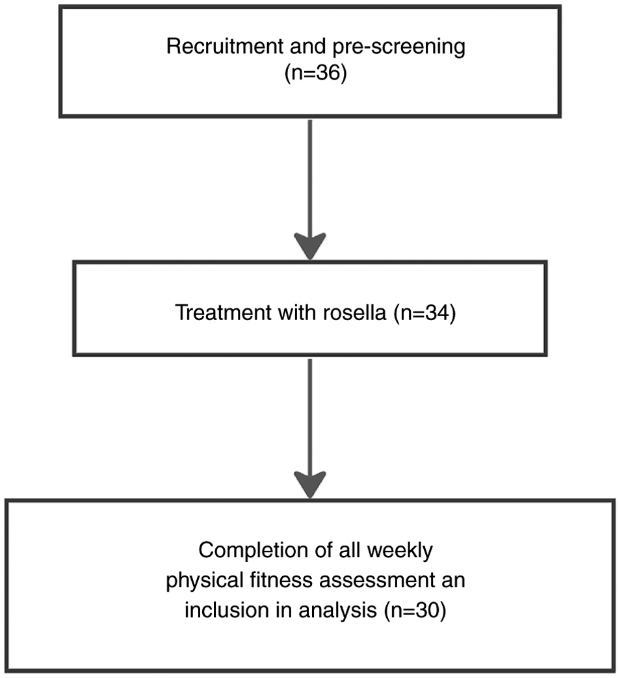

Figure 1. Subject recruitment process.

Statistical analysis. Data are presented as the mean ± SD and analyzed using paired t (normal distribution) and Wilcoxon (abnormal distribution) test using SPSS 25 (IBM Corp.). Normality was assessed using Shapiro-Wilk's test Balance and VO_{2max} were non-normally distributed. $P<0.05$ was considered to indicate a statistically significant difference.

Results and Discussion

All subjects received rosella tea; nutritional content is shown in Table I. Subject characteristics are presented in Table II. HST had significantly improved strength (24.9 to 27.3 kg;

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

Characteristic	n	Mean ± standard deviation
Sex		
Male	20	
Female	10	
Age, years		39.3±9.2
21-40	16	
>40	14	
Body mass index[a]		23.6±3.1
Normal	20	
Overweight	10	
Body fat %		24.9±7.6
Systolic blood pressure		109.0±12.1
Diastolic blood pressure		78.0±9.0
Electrocardiogram		
Normal	30	
Abnormal	0	
Global Physical Activity Questionnaire[b]		
Low	11	
Moderate	10	
High	9	

[a]Based on WHO classification. [b]Developed by WHO. WHO, World Health Organization.

Table III. Pre- and post-physical fitness parameters of subjects.

Parameter	Mean	t-value	P-value
Strength, kg		-2.361	0.0250[a]
Pre	24.9		
Post	27.3		
Balance, sec		-2.901[b]	0.0040[b]
Pre	23.2		
Post	42.2		
Right hand grip, kg		-4.815	<0.0001[a]
Pre	31.8		
Post	35.1		
Left hand grip, kg		-2.454	0.0200[a]
Pre	29.8		
Post	31.6		
Vertical jump, m/sec		-2.868	0.0080[a]
Pre	38.6		
Post	41.1		
VO_{2max}, ml/kg/min		-2.458[b]	0.0140[b]
Pre	31.1		
Post	34.3		

[a]Paired t test. [b]Wilcoxon-test.

P=0.043), balance (23.3 to 42.2 sec; P=0.006), right (31.8 to 35.1 kg; P<0.001) and left hand grip (29.8 to 31.6 kg; P=0.016), vertical jump (38.6 to 41.1 m/sec; P=0.014) and VO_{2max} (31.1 to 34.3 ml/kg/min; P=0.021; Table III).

HST that mostly contain water and calcium level, a moderate level of anthocyanin and a small amount of fat, carbohydrate, and vitamin C. The part with the most anthocyanin levels is the rosella calyx. There are two major anthocyanins in rosella, namely delphinidin-3-xylose glucoside or hibiscin and cyanidin-3-xyloside glucoside or gossypicyanin (19). Anthocyanins are natural water-soluble pigments and serve as antitumor, antioxidants and anticarcinogenic. At the cancer formation stage, anthocyanins play a role in various mechanisms, that is inhibiting cellular transformation, cell proliferation and signaling pathways (20). These compounds are derivatives of flavonoids found in phenolics (19).

Anthocyanins improve the performance and maintenance of skeletal muscle by increasing the synthesis of skeletal muscle proteins by several mechanisms (5). First, anthocyanins are directly involved in the PI3K/Akt signaling pathway. Activation of PI3K/Akt signaling activates mTOR, which plays a role in initiating protein synthesis in muscle. Second, anthocyanins are directly involved in the expression of IGF-1, which plays a role in inhibiting C-reactive protein (CRP). CRP inhibits protein synthesis by inhibiting mTOR. Third, anthocyanins serve as angiotensin-converting enzyme inhibitors to improve endothelial function and angiogenesis, increase levels of IGF-1, initiate glucose uptake and inhibit the release of pro-inflammatory molecules such as IL-6 (21). The present study showed difference in results from the left hand (29.8 to 31.6 kg; P=0.016) and the right hand (31.8 to 35.1 kg; P<0.001). This can be because all subjects were right hand-dominant. The dominant hand has higher strength than the non-dominant hand (22).

The vestibular system has a function in detecting changes in the position of the head and also maintaining body balance. One part of the vestibular system is the vestibular hair cell, which serves as a mechanosensory receptor. Anthocyanins protect vestibular hair cells from damage by inhibiting reactive oxygen species production and caspase-3 activation (23).

When jumping, muscles and nerves work together to produce a force and speed, which is called power. This neuromuscular cooperation is called the stretch-shortening cycle. This cycle consists of three phases. The first phase, eccentric, occurs when there is stretching of the target muscle, which causes an increase in energy storage in muscles and tendons. When a muscle lengthens, there is feedback from the central nervous system to produce active movement. This phase is amortization. As a result, the muscles will contract to prevent overstretching (concentric phase) (24).

As aforementioned, anthocyanins increase muscle performance by increasing protein synthesis in muscles. In addition, anthocyanins have a neuroprotective effect by decreasing neuroinflammation and promoting neuronal autophagy to decrease neuronal damage (25). Therefore, anthocyanins can increase power in vertical jumps increasing performance of muscles and nerves.

Maximum oxygen intake (VO_{2max}) is limited by the cardiorespiratory ability to deliver oxygen supply when the muscles contract (26). VO_{2max} is affected by the ability of the heart to pump, blood flow and the ability of skeletal muscles to capture and use oxygen.

Anthocyanins have an antioxidant and anti-inflammatory effect to improve vasodilatory function and blood flow. Blood that flows well can increase tissue perfusion and uptake of oxygen in skeletal muscle (27). In addition, anthocyanins enhance mitochondrial biogenesis and metabolism. Mitochondria have the function of using oxygen to produce energy (5).

In the present study, 200 ml HST given to healthy adults for 30 days in the morning and evening significantly improved physical fitness parameters including strength, balance, hand grip, vertical jump and VO_{2max}. HST, which contains anthocyanins provides antioxidant and neuroprotective effects and improves muscle function. However, there are several shortcomings and limitations, such as the heterogeneous subjects, small sample size, absence of a normal control group and subject physical activities that may affect physical fitness result. In addition, further research is needed to prove whether the levels of physical activity are related to increasing physical fitness when giving HST in healthy adults.

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Availability of data and materials

The datasets used and/or analyzed in the current study are available upon reasonable request from the corresponding author.

Authors' contributions

AD and AP designed the study. LL administered the tests. ANDS, LL, GTD and AA analyzed data and wrote the manuscript. ANDS and LL confirm the authenticity of all the raw data. LL and GTD revised the manuscript. All authors have read and approve the final manuscript.

Ethics approval and consent to participate

All subjects provided written informed consent according to the Helsinki Declaration (2000). This research procedure was approved by the Research Ethics Committee of Padjadjaran University (approval no. 35/UN6.C.10/PN/2018).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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