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RESEARCH ARTICLE

Anti-obesity and Antioxidant Effects of Ethanol Extract of Fingerroot Rhizomes *(Boesenbergia pandurata Roxb.)* on High-Carbohydrate Diet-induced Mice

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Objective: Obesity is defined as the abnormal and excessive accumulation of fat. Enlargement of white adipose tissue due to obesity activates the sympathetic nervous system to stimulate lipolysis to break down fat extensively. This causes a lot of free fatty acids to circulate in the body. Excessive circulating free fatty acids affect many cells and produce oxidative stress, which spreads throughout the body. This research purpose is to determine the anti-obesity and antioxidant activities of ethanol extract of B. pandurata rhizome (EEBP).
Methods: Fingerroot rhizomes were extracted using maceration and the extract was used for in vivo, antioxidant, and total flavonoid concentration tests. Anti-obesity test was carried out by dividing mice into 6 groups such as normal controls, obese controls, standard groups, and extract-treated groups (100, 200, 400 mg/kg b.w). The in vivo test parameters observed included measurement of body weight and also liver, kidney, spleen, and retroperitoneal fat index measurement. Furthermore, in this research antioxidant test was performed using the DPPH method.
Results: In vivo test showed that the giving of EEBP at a dose of 400 mg/kg b.w. effectively decreased body weight and retroperitoneal fat, but slightly affected the organ index of mice such as the liver, renal, and spleen. Furthermore, the antioxidant test showed that the IC50 EEBP results obtained were 37,05 µg/ml. In addition, the total flavonoid content found in the EEBP is 15,775 mgQE/g.
Conclusion: The present study showed that EEBP may have a considerable potential anti-obesity agent and also has a very strong antioxidant effect.

Keywords: extract, fingerroot, high-carbohydrate diet, obesity, rhizomes

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Introduction

The prevalence of obesity continues to increase in the world. The increasing prevalence of obesity has an impact on increasing morbidity and mortality rates [1]. Obesity can be defined as the abnormal and excessive accumulation of fat that can disrupt human health. Obesity occurs due to an imbalance in the body's energy intake and expenditure. If a person's Body Mass Index (BMI) greater than 25 kg/m^2 is defined as overweight, and obese when BMI > 30 kg/m^2 [2].

Obesity management can be done with non-pharmacology therapy and pharmacology therapy. Non-pharmacology therapy involves reducing a high-carbohydrate diet and high-fat diet, increasing vegetable-fruit consumption, and also improving physical exercise. Meanwhile, pharmacological therapy for obese patients is administered synthetic drugs. One of the drugs currently still used to treat obesity is orlistat. Orlistat works by inhibiting the pancreatic lipase enzyme. Long-term use of orlistat causes several kinds of side effects such as incontinence, flatulence, and oily stools [3]. Therefore, there is a need for alternative treatments that are expected to have good effectiveness with lower side effects when compared to synthetic drugs. One natural product that can potentially be used for treatment is fingerroot.

Fingerroot (*Boesenbergia pandurata* Roxb.) is a plant that is often found in Southeast Asia. *B. pandurata* in Indonesia is known as Temu kunci. *B. pandurata* is a family of *Zingiberaceae*, it has been used as a cooking spice and herbal medicine mixture [4]. The part of the plant that is often used for disease treatment is the rhizomes. *B. pandurata* rhizomes contain essential oils and several flavonoid compounds that are useful as anti-fungal, antibacterial, and have antioxidant effects [5]. One of the flavonoid compounds in this plant, namely panduratin, has quite strong abilities as an anti-fungal, antibacterial, anti-inflammatory, and anticancer [6]. Apart from that, flavonoids also have the activity of inhibiting the pancreatic lipase enzyme [7]. Based on the previous research, the results of QSAR analysis showed that the active compound of *B. pandurata* shows potential as a treatment for metabolic syndrome, especially obesity, and also has an effect as a free radical scavenger [8]. Therefore, this study aims to determine the anti-obesity activity of *B. pandurata* rhizomes extract on high-carbohydrate diet-induced mice and also evaluate how the antioxidant effect by *in vitro*.

Methods

Chemical

All reagents in this research were of analytical grade. Orlistat was bought from a Local Pharmacy, in Tangerang, Indonesia. DPPH was purchased from Sigma-Aldrich.

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Plant source and collection of plant

B. pandurata rhizomes were obtained from Balai Penelitian Tanaman Obat dan Rempah (BALITTRO), Bogor, Indonesia. Plant identification was identified in *Herbarium Bogoriense*, Research Center for Biology, BRIN, Cibinong, Indonesia.

Preparation of extract

B. pandurata rhizomes were obtained from Balai Penelitian Tanaman Obat dan Rempah (BALITTRO), washed to remove impurities, chopped to reduce the particle size, and then dried in the sun for 4 days. Once dry, *B. pandurata* grind it using a grinder. The powder was obtained, then weighed, put in a container, stored at room temperature, extracted with ethanol by maceration method, and evaporated by *Rotary Evaporator* (EYELA). Next, the percentage of yield of extract was calculated. Determination of yield served to determine the levels of secondary metabolites carried by the solvent but was not able to determine the type of compounds carried by solvents [9].

Phytochemical screening of the ethanol extract of *B. pandurata*

Phytochemical screening was done to observe phytochemical constituents in *B. pandurate* involving alkaloids, flavonoids, saponins, tannins, quinones, and steroids/triterpenoids.

Anti-obesity activity against mice

Anti-obesity activity of ethanol extract from *B. pandurata* has been tested in male mice (Swiss-Webster). This research has received an acceptance decision by the Faculty of Medicine and Health Research Ethics Committee University of Muhammadiyah Jakarta, No.58/PE/KE/FKK-UMJ/V/2023. The animal study was conducted in the Laboratory of Pharmacology, Muhammadiyah A.R. Fachruddin University, Tangerang, Indonesia.

Twenty-four *Swiss-Webster* mice with age 2-3 months and body weight of 20-25 g were adapted to room temperature. During adaptation (for seven days), the animals were fed with a standard diet and drinks *ad libitum*. A total of 24 male Swiss-Webster strain mice were randomly divided into 6 groups such as normal group, obese group, standard drug group (orlistat 15.6 mg/kg b.w.), and ethanol extract of *B. pandurata* (EEBP) groups (dose 100, 200, and 400 mg/kg b.w.). Each group consisted of 4 mice.

All experimental groups were orally given a high-carbohydrate diet for twenty-one days to make the obese mice (except, the normal group). The ingredients of the diet are shown in Table 1 and adopted by Sukandar *et al.* [10]. Then therapy was carried out by administering the extract for 14 days as an effort to reduce body weight. At the end of the experiment, the animals were sacrificed using a general anesthetic in a chamber, and the organs were collected including liver, spleen, kidney, and visceral fat including retroperitoneal fat to determine their indexes.

Table 1. Ingredients of the experimental diet

Component	Normal (kg)	High Carbohydrate (kg)
Wheat Flour	1,7	1,7
Rice flour	0	3
Corn starch	1,25	1,25
Fish flour	0,8	0,8
Green bean flour	0,7	0,7
Beef tallow	0,5	0,5

Antioxidant activity using the DPPH method

The antioxidant activity of the ethanol extracts of *B. pandurata* was measured by DPPH [11]. The Radical scavenging activity of ethanol extracts of *B. pandurata* against DPPH was determined by spectrophotometry at wavelength 516 nm. Ascorbic acid was used as standard. The percentage of DPPH scavenging activity was calculated by the formula:

$$\% \text{ Inhibitory} = A0 - A1/A0 \times 100$$

Determination of the total flavonoid concentration of the extract

Determination of the total flavonoid concentration of *B. pandurata* rhizomes extract was performed using UV-visible spectrophotometry. Quercetin solution as a standard. The 1000 ppm of standard solution was made into concentrations of 60, 80, 100, and 120 ppm. the absorbance was measured at a wavelength of 416. The absorbance of extracts containing flavonoids was entered into the linear regression equation y = bx + a.

Statistical analysis

The data obtained in this research was then analyzed statistically using SPSS version 25. The results of anti-obesity activity were performed using the one-way ANOVA, coupled with the post-hoc Tukey's test. A value of $p < 0.05$ was used to explain statistical significance.

Results

Result of phytochemical screening of the ethanol extract of *B. pandurata*

The percentage of yield of ethanol extract of *B. pandurata* is 9,15%. Phytochemical screening of ethanol extract of *B. pandurata* showed the presence of flavonoids and saponins (in Table 2). Whereas, alkaloids, tannins, quinones, and steroids/triterpenoids are undetectable.

Result of ethanol extract of *B. pandurata* against body weight

Based on Figure 1, all groups were induced by a high-carbohydrate diet for twenty-one days (except, normal groups), showed an increase in body weight if compared to the normal group and there was an increase in body weight >20% from the initial weight.

Table 2. Result of phytochemical screening of the ethanol extract of *B. pandurata*.

Group	Results
Alkaloids	-
Flavonoids	+
Saponins	+
Tannins	-
Quinones	-
Steroids/Triterpenoids	-

Description: (+): detected; (-): non detected

Result of ethanol extract against organ and fat index

In this study, organ and fat index were also calculated, aiming to determine the effect of administering ethanol extract of *B. pandurata* on reducing fat accumulation in body organs. To calculate the organ index and fat index, the formula below was used [12], and the results are presented in figures 2, 3 and 4.

$$\text{Organ Index}(\%) = \frac{Actual\ organ\ weight\ (g)}{Body\ weight\ of\ mice\ on\ the\ day\ they\ were\ sacrificed\ (g)}\ x\ 100\%$$

Result of antioxidant activity using the DPPH method

Table 3 shows the antioxidant activity of EEBP using the DPPH method. The concentrations used in antioxidant testing were 20, 40, 60, 80, and 100 µg/ml, respectively. The IC_{50} EEBP results obtained were 37,05 µg/ml. In addition, the IC_{50} of the ascorbic acid is 5.26 µg/ml.

The total flavonoid concentration of the extract

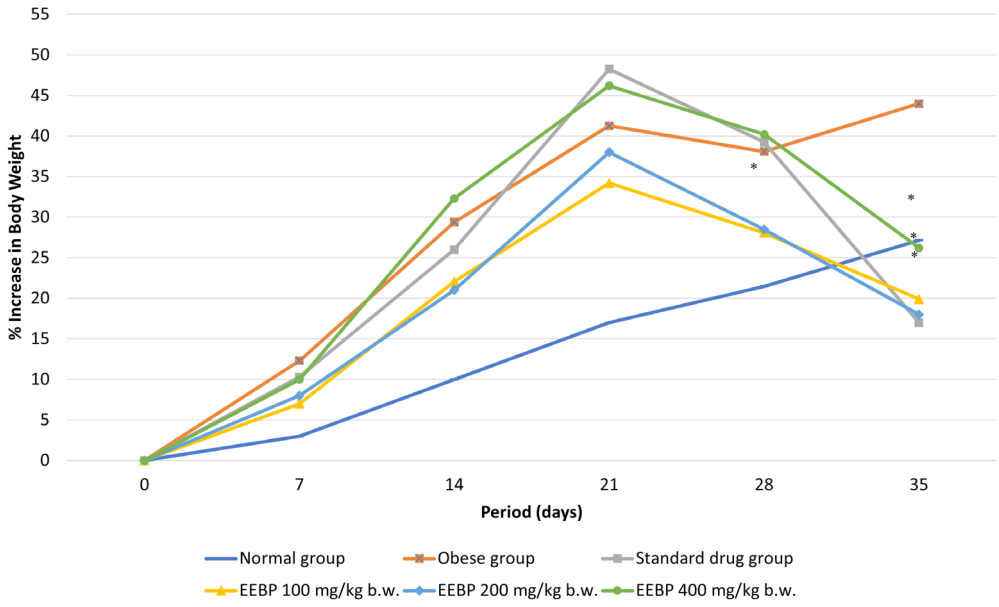
The determination of total flavonoid ethanol extract of *B. pandurata* rhizome was measured using a UV-visible spectrophotometer by colorimetric method, which is based on color formation. Flavonoids can be analyzed using the UV-Vis spectrophotometry method because flavonoids contain a conjugated aromatic system which is a chromophore group and has an Auxochrome group such as –OH. The conjugated double bond present in the aromatic circumference and the –OH group will absorb ultraviolet light and visible light.

Determination of total flavonoid levels of ethanol extract of *B. pandurata* rhizomes was carried out by entering the absorbance value of the sample into the equation of the quercetin standard curve is y = 0.004x – 0.0311. The total flavonoid content found in the ethanol extract of *B. pandurata* rhizomes is 15,775 mgQE/g.

Discussions

Obesity contributes to several diseases involving diabetes mellitus, hypertension, dyslipidemia, insulin resistance, and atherosclerosis. Factors that influence obesity include genetic, environmental, socio-economic factors and the influence of inactivity habits. This is related to an increase in mortality and morbidity rates. Genetic factors have an important role in influencing individuals to become obese because they are inherited. Genes such as agouti, melanocortin, and leptin are all linked to obesity. Damage or disruption of these genes is responsible for obesity in individuals. Food intake is increasing due to the availability of a variety of fast food, affordability of access to it, as well as the support of technological advances and the decreasing physical activity of modern society which also underlies the occurrence of obesity [13].

Enlargement of white adipose tissue due to obesity activates the sympathetic nervous system to stimulate the lipolysis process to break down fat extensively. This causes a lot of free fatty acids to circulate in the body. Excessive circulating free fatty acids affect many cells and produce oxidative stress, which spreads throughout the body. This oxidative stress mechanism occurs in the endoplasmic re-



(*) the mean ± SD of observations from 4 mice, significantly different from the obese group ($p < 0.05$).

Fig. 1. Effect of ethanol extract of *B. pandurata* against body weight.

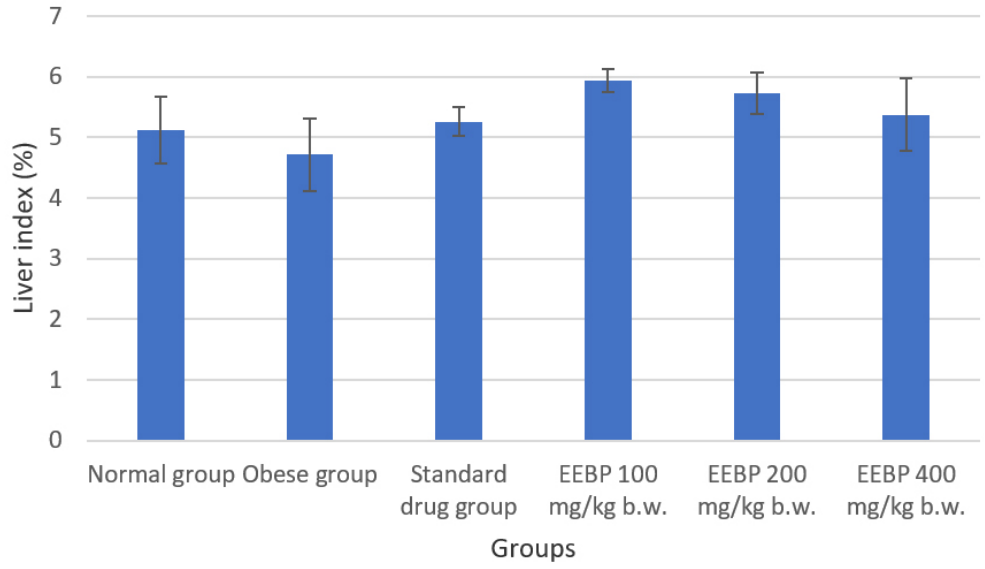


Fig. 2. Impact of EEBP on liver index.

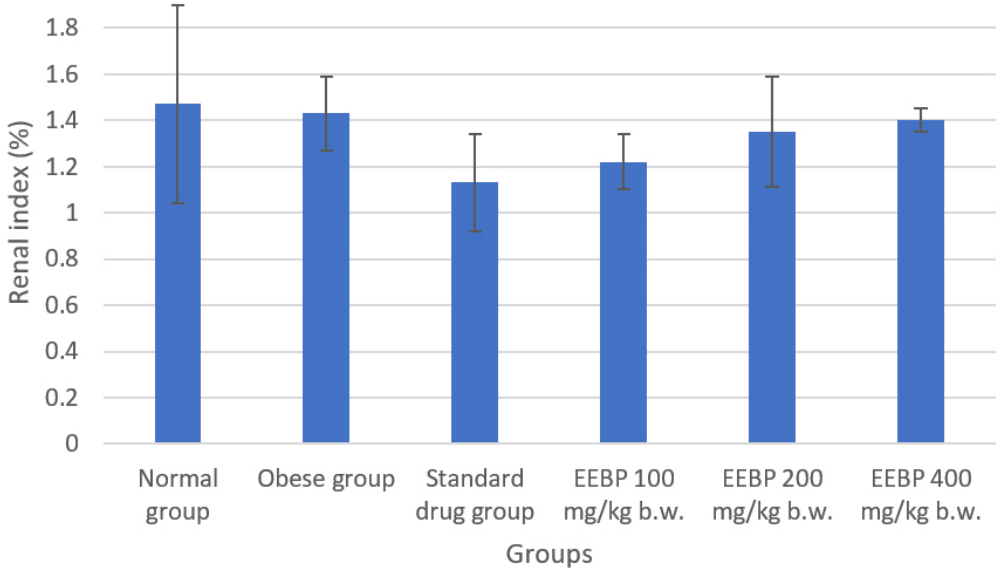


Fig. 3. Impact of EEBP on renal index.

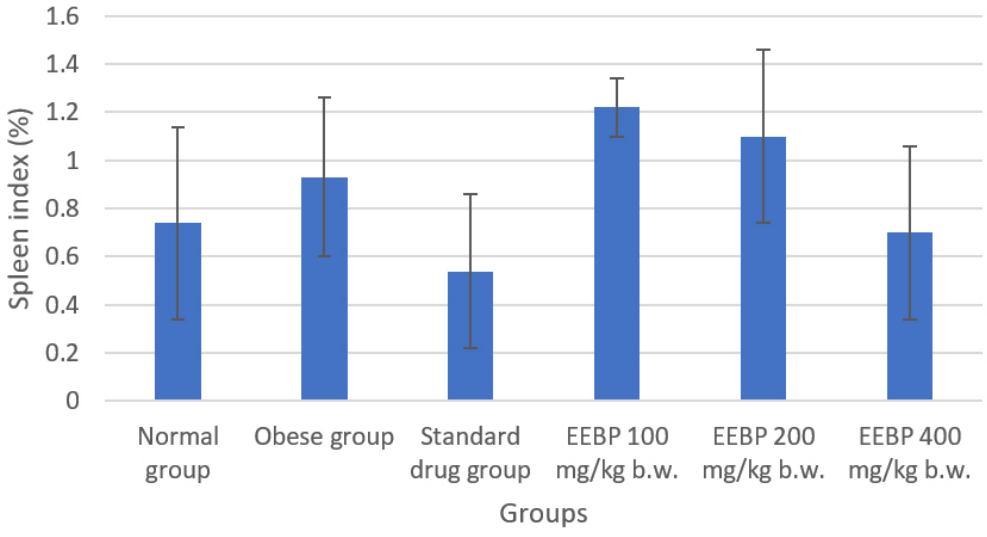


Fig. 4. Impact of EEBP on spleen index.

Table 3. Result of antioxidant activity using the DPPH method.

Sample	Concentration (µg/ml)	Inhibition (%)
EEBP	20	47.927
	40	50.450
	60	52.792
	80	55.135
	100	58.198

ticulum and cell mitochondria. The occurrence of oxidative stress due to free fatty acids is known as *Lipotoxicity* and causes disorders in many organs such as the liver, pancreas, digestive tract, kidneys, and reproductive organs [14,15]. Based on this, we carried out an antioxidant and total flavonoid concentration test. The hope is that if EEBP has good activity, thus it can help improve free radical problems in organs.

Based on Figure 1, all experimental groups (except, the normal group) were induced by a high-carbohydrate diet for three weeks, showed an increase in body weight if compared to the normal group and there was an increase in body weight >20% from the initial weight. Based on the previous research, experimental animals are said to be obese if their body weight increases by >20% compared to their initial body weight. These results are in line with the previous research where high-carbohydrate feeding can increase the body weight of experimental animals [10].

The period 28-35 showed the phase of therapy. It can be seen from Figure 1 that there was a decrease in body weight in the standard drug, the extract treatment group if compared to the obese group. The decrease in mice's body weight is thought to be due to the content of bioactive compounds in ethanol extract of *B.pandurata* rhizomes. Based on Iswantini *et al.* [16] showed that phytochemical compounds such as flavonoids, saponins, alkaloids, tannins, and steroids/triterpenoids have a contribution against the anti-obesity effect. Based on the data, there was a decrease in body weight in the extract group compared to the obese group. Based on statistical results, the EEBP dose of 400 mg/kg body weight decreased better than the EEBP doses of 100 and 200 mg/kg body weight.

The results of statistical analysis on the liver, renal, and spleen showed no significant difference ($p > 0.05$). It can be said that the administration of ethanol extract of *B. pandurata* did not affect the organ index. Many factors influence how EEBP does not seem to have such a strong effect in reducing lipid accumulation in several organs, but one of the possible factors is the low penetration ability of EEBP to reach organs so that a decrease in lipid accumulation does not occur.

Table 3 shows the antioxidant activity of EEBP using the DPPH method. The concentrations used in antioxidant testing were 20, 40, 60, 80, and 100 µg/ml, respectively. The IC_{50} EEBP results obtained were 37,05 µg/ml. According to Blois, if the IC_{50} of a substance is below 50 ppm, it is said to have strong antioxidant activity. This shows that EEBP has very strong antioxidant activity. The research results obtained are in line with previous research conducted by Yuniarto [8], which shows that fingerroot rhizomes have strong antioxidant activity. In addition, the IC_{50} of the ascorbic acid is 5.26 µg/ml.

The DPPH method was delivered by Blois in 1958 and is widely used to evaluate the ability of a compound to work as a radical scavenger. The IC_{50} parameter is used to interpret the outcome of the DPPH method and is defined as the concentration that can inhibit 50% of DPPH free radicals. The advantage of using the DPPH method is that it is fast and simple when compared to several other methods [11].

In this study, apart from antioxidant tests, total flavonoid concentration was also measured. The relationship between measuring total flavonoid and antioxidant testing is that the higher the total flavonoid content of a material, the higher is the antioxidant activity [9]. Based on the obtained results, the total flavonoid in EEBP was 15.775 mgQE/g, which means that it is likely that the content influences the antioxidant effect of EEBP to be very strong.

Conclusion

It can be concluded that ethanol extract of *B. pandurata* rhizomes (EEBP) has anti-obesity activity at a dose of 400 mg/kg b.w. as the most effective dose in decreasing body weight and excessive retroperitoneal fat of mice. The present study showed that ethanol extract of *B. pandurata* rhizomes may have a considerable as potential anti-obesity agent and also has a very strong antioxidant effect. In the next studies, it is necessary to carry out toxicity tests to assess its safety and consider appropriate formulations so that the penetration ability of EEBP is even more effective.

Authors' contribution

AY (Conceptualization, extraction process, methodology, data validation, and writing of original draft).
AAS (Conceptualization, check for final manuscript)
WS (Investigation, data collecting from In vivo)
ERT (Antioxidant test)

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Conflict of interest

No conflict of interest

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