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Antihypertensive Effect of *Brucea javanica* (L.) Merr. Fruit Extract

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Abstract

Ethnopharmacologically, the fruit of *Brucea javanica* (L.) Merr. is acknowledged in the Indonesian community to lower blood pressure. This study assessed the antihypertensive effect of *B. javanica* fruit extract using adrenaline-induced hypertensive Sprague Dawley rats. Hypertensive rats were divided into 4 groups: group A was given *B. javanica* water fraction and adrenaline, group B was given *B. javanica* hexane fraction and adrenaline, group C was given bisoprolol and adrenaline and group D was given adrenaline solely. Systolic blood pressure was regularly measured using the tail-cuff method. Treatment of adrenaline-induced hypertensive rats independently given *B. javanica* water fraction, the hexane fraction, and the bisoprolol group proved to significantly reduce blood pressure by 72.75 mmHg (-34%), 58.5 mmHg (-28%) and 23.25 mmHg (-12%) respectively, while there was an increase of 15.00 mmHg (+9%) SBP in the negative control group given solely adrenaline. The water fraction contains flavonoid and alkaloid. The hexane fraction of this fruit contains alkaloid. Our study suggest that the flavanoid and alkaloid content in *B. javanica* fruit work synergistically to alleviate hypertension, possibly through β1-adrenergic receptor-related mechanism.

Abstrak

Efek Antihipertensi Ekstrak Buah *Brucea javanica* (L.) Merr. Secara etnofarmakologi, *B. javanica* (L.) Merr. dikenal sebagai salah satu tanaman obat yang berhasiat menurunkan tekanan darah pada penderita hipertensi. Pada penelitian ini dilakukan pengujian efek antihipertensi ekstrak buah *B. javanica* menggunakan tikus yang mengalami hipertensi akibat pemberian adrenalin. Tikus hipertensi dibagi menjadi 4 kelompok, diberikan fraksi air *B. javanica,* fraksi heksana *B. javanica*, bisoprolol, serta kontrol yang tidak diberikan apapun kecuali adrenalin. Tekanan darah sistolik diukur menggunakan prosedur *tail-cuff*. Setelah mengalami hipertensi, tikus yang diberikan fraksi air, fraksi heksana dan bisoprolol menunjukkan penurunan tekanan darah berturut-turut sebesar 72.75 mmHg (-34%), 58.5 mmHg (-28%) and 23.25 mmHg (-12%), sementara ada peningkatan sebesar 15.00 mmHg (+9%) pada kelompok kontrol. Fraksi air diketahui mengandung flavonoid dan alkaloid, sementara fraksi heksana mengandung alkaloid. Berdasarkan hasil penelitian ini, kami menduga bahwa flavonoid dan alkaloid yang terkandung dalam buah *B. javanica* berkerja secara sinergis menurunkan tekanan darah, kemungkinan melalui jalur terkait reseptor β1-adrenergik.

Keywords: adrenaline, antihypertensive, β1-adrenergic, Brucea javanica, hypertension

1. Introduction

Cardiovascular disease is the number 1 killer in Indonesia and the world. Hypertension is one of the triggers, and it is experienced by 20-30% of the world's population [1]. Approximately 66% of cardiovascular disease sub-types in 15 countries in the Asia Pacific region, including Indonesia, stem from hypertension. The prevalence of hypertension in the region ranges from 5-47% in men and 7-38% in women [2]. In its early stages, hypertension usually manifests no symptoms, so it is only detected when complications arise [3]. This condition makes Indonesian people at risk of stroke, heart attack, aneurysms and chronic kidney disease.

In the meantime, drugs currently used to lower blood pressure have some drawbacks. Bisoprolol, as an example of a blood pressure lowering drug, has negative side effects such as bradycardia, depression, hallucinations, psoriasis and impotency [4]. For that reason, it is necessary to search for drugs derived from

nature that are more potent but have fewer negative side effects. One of the natural ingredients known to reduce the risk of cardiovascular disease is grape flavonoid. Research by Cosentino and Volpe showed that a lower prevalence of cardiovascular disease in the population of France, in comparison to other developed countries, is due to their higher quantities of wine consumption (known as the French paradox) [5]. Consumption of grape juice for 14 days on human subjects demonstrated positive effects in the form of *endothelium-dependent vasodilation* [6], as one antihypertensive mechanism [7]. This shows how great the potential of natural products is to reduce the risk of cardiovascular disease by preventing hypertension.

Makassar fruit *(Brucea javanica* (L.) Merr.) is one of the plants used in herbal medicine. Traditionally, the fruit is used to combat fever, treat bleeding, kill parasites, cure malaria and food poisoning and relieve lower back pain [8]. This plant originated in China and Vietnam. It is known as k'u-shen-tzu, kho sam, ko-sam, ku-sheng-tzu, nha dàm tùr, raat cha dat, raat dat, ratchadat, sàu dau rùng, xoan rùng, ya tan tzu, ya-dan-zi and yadãnzi [9].

In ethnofarmacology, the first author reported the traditional usage of *B. javanica* fruit to lower blood pressure. It is important to scientifically study the efficacy of this ethnofarmacology claim, especially as this finding has the potential to be developed as herbal medicine. Bisoprolol is used for comparison because it has been proven to lower blood pressure through the blocking of β1-adrenergic receptors [4]. The drug is also used because it has greater potency in animals, compared to other antihypertensive drugs such as atenolol and metoprolol [10]. It also can compete on the same receptors with adrenaline [4]. The use of adrenaline to induce hypertension is because Zhao *et al*. showed that an increased release of heart adrenaline coupled with an increase of noradrenaline from the heart is one of the features of primary hypertension [11]. To test the claimed benefits of *B. javanica*, we explore the potential antihypertensive effect of *B. javanica* fruit water and hexane fraction using male Sprague Dawley rats with adrenaline-induced hypertension.

2. Methods

Identification, extraction and phytochemical test. Fresh *B. javanica* fruit was taken from the scientist's garden cultivated in Pondok Cabe, North Jakarta, Indonesia. The identity of the plant was verified by several references, in particular the WHO monograph [12].

The fruit was washed, dried and milled. A total of 75.53 g of dry milled *B. javanica* fruit was extracted by modifying a method used by Wijono [13]. The fruit was macerated with 96% alcohol for 24 hours. After the alcohol extract was collected, the residue was macerated again with a new alcohol solvent. This process was repeated 14 times to make sure all the compounds were extracted. The yield of the maceration was then collected, filtered and concentrated.

The concentrated extract was then separated by using the previous method [14]. Briefly, the extract was dissolved in a mixture of hexane: methanol: water with ratio 5:9:1. The fraction was then separated into 2 parts, ie, a hexane fraction and a fraction of methanol: water. The fraction of methanol: water was concentrated and separated with a mixture of chloroform: water. The formed water fractions and fraction of chloroform were then separated and concentrated. Only hexane fractions and water fractions were used in this study. Before being administered to the rats, the hexane fraction was dissolved using 0.1% CMC. Qualitative phytochemical content tests were conducted using the Harbonne method [15]. This is done to determine the content of alkaloids (using Dragendorf, Meyer and Wagner reaction), flavonoids, triterpenoids and steroids.

Animal testing. Male Sprague Dawley rats (200-300 g) were purchased from the National Veterinary Research Agency, Ministry of Agriculture, Bogor, Indonesia. Rats were acclimatized and monitored for 30 days after purchase. Systolic Blood Pressure (SBP) was then measured with a Rat Tail Blood Pressure Monitor (Harvard) using the *tail-cuff* method. Animals used for testing are given food chow (PT. Indofeed) and drink *ad libitum*. Rats are kept in individual cages at room temperature (25 $\pm$ 1 °C) and exposed to 12 hour cycles of light/dark. All animal testing was conducted according to the national and international regulations on handling animals, in particular in accordance with the Declaration of Helsinki. Rats only experienced oral and intra peritonial induction without using any other invasive approaches.

Preliminary test. This test is performed to determine the blood pressure response to treatment at any given time. Firstly, the rats's blood pressure were measured. Various treatments, such as dispensing adrenaline (Merck) intra peritonially, bisoprolol orally (DexaMedica), hexane fraction *B. javanica* orally, and the water fraction *B. javanica* orally were then administered separately to different rats. After that, blood pressure was measured regularly every 20 minutes for 80 minutes. This is to find the time when the highest levels of SBP respond to the adrenaline dose, also the time of the lowest SBP resulting from the separate administration of hexane fractions, water fractions and bisoprolol.

Antihypertensives test. Antihypertensives test protocol refers to Fidrianny *et al*. [16]. A total of 16 rats were

randomly divided into 4 groups: (1) the adrenalin group: only given adrenaline, (2) the bisoprolol group: given adrenaline and bisoprolol, (3) the water fractions group: given adrenalin and water fraction, and (4) the hexane fractions group: given adrenaline and hexane fraction. The amount of adrenaline dispensed to each rat was 1.2 μg adrenaline/kg body weight (BW). Dosage of water fractions and hexane fractions were equal to 0.0714 mg/kg BW, equivalent to the dose of bisoprolol.

Shortly before adrenaline is dispensed, the SBP of the rats is measured (this period is called T1 and is set as minute-0). After the adrenaline is administered, the rats exhibited highest SBP values at time T2. After settling for 60 minutes to allow the adrenaline effect to *wash-off*, the treatment (in the form of adrenaline with/ without the provision of water fractions, hexane fractions and bisoprolol) is repeated. The SBP of the rats is measured at T3 period occuring at different times. T3 represents the time at which the maximum effects of hypertension (caused by artificially inducing adrenaline) and the maximal hypotensive effect (due to the administration of water fractions, hexane fractions and bisoprolol separately) overlap. T3 is necessary to observe the interaction between these two effects simultaneously on β1-adrenergic receptors, as is the case in real conditions. This occurrence is illustrated in Figure 1 using the hexane fractions group as an example. Because the time it takes for each treatment is

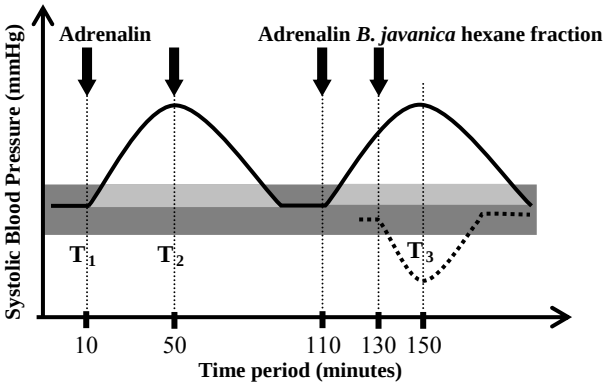

Figure 1. The Antihypertensive Protocol for Hexane Fractions Group. Adrenaline given in the 10th Minute Causes Maximum SBP in the 50th Minute. After the Wash-off Period, the Adrenaline is Given at Minute 110, while Hexane Fractions is Given at Minute 130. The Maximum Effects of Adrenaline Hypertension and the Effect of SBP Reduction in Response to Hexane Fractions is Expected to be Achieved, Overlap and Interact at Minute 150. Striped Black Curve Shows the Effect of SBP Caused by Adrenaline, Dashed Curve Represents the Antihypertensive Effect of Hexane Fractions and the Gray Area Represents the Range of Normal SBP

different, there are differences in the timing for dispensing adrenaline and countering with water fractions, hexane fractions and bisoprolol in each group. Data analyzed used covariance analysis (ANCOVA) using SPSS 16.0. Differences between the groups were analyzed by Duncan test ($p < 0.05$).

3. Results and Discussion

Extraction and phytochemical analysis. The maceration yield of ethanol, water fractions and hexane fractions is equal to 27.78%, 4.38%, and 6.11% respectively. The greater yield of hexane fractions is probably due to the dominance of the non-polar compounds in the fruit pericarp and fruit seeds [17]. Phytochemical analysis showed that the water fractions contains alkaloids (based on the Dragendorf and Wagner reaction) and flavonoids. Meanwhile, hexane fractions only contains alkaloids. A negative Meyer reaction result indicates the absence of alkaloid tannins in both fractions. Furthermore, neither fractions contains triterpenoid despite Liu *et al.* finding showing that *B. javanica* contains brucejavanin C, which is a triterpenoid [18]. The absence of triterpenoid is most likely due to the small concentration of the sample used and the differences in the plants used. This research only extracted 75.53 g of dried fruit extract with ethanol, while Liu *et al.* used ethanol (with reflux) to extract 10 kg of *aerial parts* from *B. javanica* [18].

Preliminary test. The mean initial blood pressure of all rats was 173.58 $\pm$ 24.46 mmHg. Vogel suggests that the SBP in this range (170-200 mmHg) classifies as hypertension, while normal rats have SBP in the range of 100 mmHg [19]. The cause of hypertension occurring naturally in rats is most likely triggered by the excessive salt content in foods [20], even though the food is not labeled as having salt content. Nevertheless, these shortcomings were overcome by using the negative control of rats that were only given adrenaline to specifically observe the effect of hypertension. Rats given adrenaline and bisoprolol were also used to observe the antihypertensive interaction effect. Figure 2 shows the mean initial SBP and response to the induction of bisoprolol, water fractions and hexane fractions.

Adrenaline was able to induce a hypertensive effect at a maximum of 40 minutes after induction. This is indicated by an increase of 48.00 mm Hg from the initial SBP value of 144.00 mmHg. For 80 minutes after induction, SBP decreased but did not match the initial value.

Meanwhile, the minimum SBP effected by administering bisoprolol, was reached in 80 minutes, when the SBP decreased by 30 mmHg from 162 mmHg to 132 mmHg. There is a tendency for the SBP to continue declining if the observation time is extended.

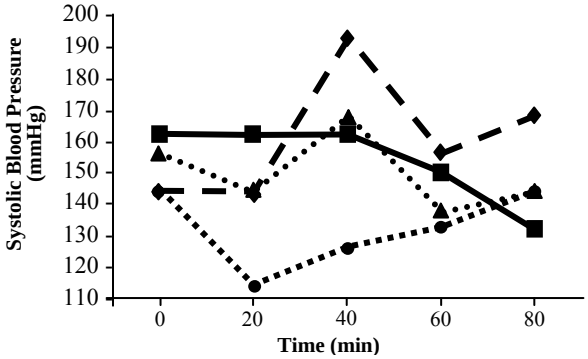

Figure 2. The Lowest Fall in Systolic Blood Pressure Occurred in the Hexane Fractions Group (▬✕▬), Followed by the Bisoprolol Group (▬■▬) and then Water Fractions (▬▲▬). Hexane Fractions also Showed a Rapid Decline in Blood Pressure, while the Adrenaline only Group (▬◆▬) Showed an Increase in this Parameter

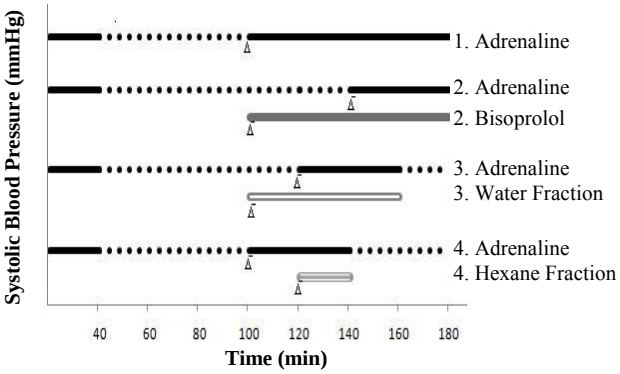

Figure 3. Protocol of Antihypertensive Test Showed Differences in Induction Time (△) between Adrenaline to Bisoprolol, Water Fractions and Hexane Fractions. The Thick Black Line (▬) Indicates the Period when the Adrenaline Affects Hypertension, while the Other Thick Lines Show the Time Periods that Bisprolol (▬), Water Fractions (▬) and Hexane Fractions (▬) Have an Antihypertensive Effect. The Dotted Line (••••) of Adrenaline Shows the Effects of the *Wash-off*

This 80 minutes period is chosen as T2 point because any longer than that could result in the antihypertensive effect of extract *B. javanica* disappearing thus an interaction between *B. javanica* and adrenaline can not be measured.

Water fractions reaches a maximum SBP reduction at 60 minutes. SBP dropped as much as 18 mm Hg from its initial SBP value (156 mmHg). Meanwhile, administering hexane fractions made the SBP decrease to its maximum after 20 minutes with a decline from 144 mmHg to 30 mmHg. SBP then gradually returned to normal after 80 minutes. The pattern of decline in SBP from hexane fractions was faster than with bisoprolol. A drastic reduction in SBP is clinically dangerous because it can lead to hypotensive shock [21]. Bisoprolol tended to reduce SBP more gradually, making it more secure physiologically. Further testing needs to be done to determine the behavior of hexane fractions and adrenaline on the same receptors in adrenaline-induced hypertension.

Antihypertensives test. Antihypertensive test were conducted to observe the interactions between the compounds contained in *B. javanica* and adrenaline on β1-adrenergic receptors simultaneously. Fraction *B. javanica* and bisoprolol were given at specific times to allow overlapping of the antihypertensive effect (*B. javanica* and bisropolol) on hypertension (artificially adrenaline induced) at the same time. The time difference of the induction treatment on each group is illustrated in Figure 3.

The results showed that water fractions and hexane fractions are equally able to reduce SBP quite significantly compared to bisoprolol which statistically is more moderate. An antihypertensive effect was achieved despite the fact that rats in the bisoprolol group initially had lower SBP than those in the water fractions and hexane fractions groups. Administration of hexane fractions, bisoprolol and water fractions to hypertensive rats can reduce SBP as much as 72.75 mmHg (-34.40%), 58.5 mmHg (-27.66%) and 23.25 mmHg (-11.67%) respectively, while rats given only adrenaline show 15.00 mmHg SBP increase (+8.93%), as described in Table 1. However, a significant reduction in SBP in the short term as a result of administering water fractions and hexane fractions should be studied further, because the condition is prone to cause clinical hypotensive shock [21]. The ANCOVA statistical test method was chosen because there were differences of initial SBP between the groups before treatment. If the differences were analyzed by the Annova test, there is the possibility of bias.

The hexane fractions antihypertensive effect could be caused by the alkaloids it contains. However, the alkaloids are not the only compounds in water fractions that may cause antihypertension because this fraction also contains flavonoids. We suspect alkaloid and flavonoid compounds work synergistically to lower blood pressure through the β1-adrenergic receptor. The competition at this receptor could be observed through the ability of the compounds contained in *B. javanica* to combat hypertension caused by adrenaline that also works on the β1-adrenergic receptor.

Previously, Maccha and Mustafa showed several groups of flavonoid compounds such as quercetin also produce antihypertensive effects through *endothelium-dependent aortic relaxation* [22]. The mechanism of the

Table 1. Result Showed that the Antihypertensive Effect of Water Fractions Indicated the Largest Decline in SBP, followed by Water Fractions and Bisoprolol, Respectively, while Adrenaline Experienced Increasing SBP. Description: T1 = Initial Conditions of SBP (none given), T2 = Highest SBP Point after Administering Adrenaline, T3 = SBP Interaction between Adrenaline and Test Compounds (Bisoprolol/Hexane Fraction/Water Fraction). SBP Values Written as the Mean ± SD

Group	Period Systolic Blood Pressure (SBP) Measurement *				Decrease (%)
	T1	T2	T3	ΔT	
Adrenaline	157.75 ± 23.24	168.00 ± 29.80	183.00 + 25.22	-15.00 ± 6.00 [a]	-8.93
Bisoprolol	182.25 ± 25.62	199.50 ± 19.67	176.25 ± 23.41	23.25 ± 9.91 [b]	11.67
Water Fraction	180.75 ± 22.23	211.50 ± 20.57	138.75 ± 17.73	72.75 ± 11.32 [c]	34.40
Hexane Fraction	160.50 ± 24.19	211.50 ± 26.10	153.00 ± 14.28	58.50 ± 28.72 [c]	27.66

The meaning of the superscript letters are: (a) no significant difference, (b) significant difference, (c) very significant difference. ΔSBP value is the sum of T2 minus T3.

antihypertensive alkaloid is gauged to be through the antioxidants because antioxidants are known to relieve hypertension through several related mechanisms [23], although this notion does require further testing. *Brucea javanic*a fruit is known to contain compounds derived from quercertin, namely quercetin-3-O-beta-D-galactoside, and alkaloid 4-ethoxycarbonyl-2-quinolone [24].

Brucea javanica also contains several quassinoid compounds such as bruceoside, bruceantinol, and yadanziolides [8], brucein D and E, several type of yadanziosides, javanicolide A and B, and javanicoside A and brusatol [17]-[18]. Further research needs to examine which of these compounds have the greatest antihypertensive potential along with research on how their antihypertensive mechanism.

Experiment model selections. These models are non-invasive models free from ethical issues compared to the more invasive methods, for instance like the arterial chronically-implanted catheters. It also require less skills. Measurements can also be carried out repeatedly over a period of time. This model allows the evaluation of adrenaline with possible antihypertensive drugs. The tendency of high initial SBP in all groups of rats, even after adaptation, is the potential for the development of primary hypertension rat strain. Although the initial SBP values were higher than rats consuming high-fat feed (+10 mmHg) and high salt feed (+5 mmHg), the value is still lower than the hypersensitive rat model *2-kidney 1-clip* (+20 mmHg), DOCA salt (+20-35 mmHg) and angiotensin infusion (+45-60 mmHg) [25].

Tail-cuff method does not measure the diastolic blood pressure value directly but it is estimated through electronic calculations. This is not a problem because SBP is a better predictor of cardiovascular disease and more important than the diastolic blood pressure, especially for person over 50 year olds. Nevertheless, some small sub-populations have shown that diastolic blood pressure has a greater correlation as a predictive factor of cardiovascular disease than SBP.

4. Conclusions

The water and hexane fraction from *B. javanica* fruit have great potential as antihypertensive compounds because they show a more significant reduction in SBP compared to bisoprolol. This is likely caused by the synergistic effect of the content of flavonoid and alkaloids compounds. Nevertheless, the rapid antihypertensive effects of the water and hexane *B. javanica* fraction may also cause hypotensive shock, so it requires careful evaluation. Identification of active compounds that may act as a single antihypertensive agent is necessary. In addition, the detailed mechanism and the long term impact of antihypertensive effects of this plant needs to be studied.

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