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Effect of pH increasing of Wuluh star fruit (*Averrhoa bilimbi* L.) juice on vasodilatation activity

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Abstract. Wuluh star fruit (*Averrhoa bilimbi* L.) is Oxadiliaceae family which can be used as an antihypertensive drug by ethnomedicine. *A. bilimbi* fruit juice is proven to lower blood pressure in preclinical testing and limited phase 1 clinical trials, where the mechanism of action is through vasodilatation of blood vessels. The taste of juice fruit is very sour, but there is no information about the effect of pH increasing of juice on vasodilatation activity in blood vessels. In this research, *A. bilimbi* fruits were made into juice, filtered, centrifuged to get the supernatant. The pH of juice was increased by NaOH and KOH addition. Vasodilatation activity of juice was tested on isolated organs of the aortic ring endothelium. The control that used was Kreb's-Henselheit solution with a pH approaching the tested juice solution. The result showed that an increase in the pH of *A. bilimbi* fruit juice caused a decrease in vasodilatation activity. The type of solution to increase the pH of juice also affected the changes in vasodilatation activity. It can be concluded that the pH increasing on juice of *A. bilimbi* fruit can decrease vasodilatation activity on isolated organs of the aortic ring endothelium.

1. Introduction

Averrhoa bilimbi L., belongs to Oxalidaceae and known as Wuluh star fruit, is an Indonesian endemic plant thought to originate from Maluku [1]. Based on ethnobotany, this fruit is used as herbal ingredient, such as an antihypertensive agent [2]. Previous studies has proven that *A. bilimbi* fruit juice can reduce the blood pressure in rats and can acts as antihypertension [3]. In other research, also stated that the use of *A. bilimbi* fruit juice in small amount also can reduce the blood pressure [4, 5].

Recent research showed that *A. bilimbi* fruit juice can cause vasodilatation in separate blood vessels of the aortic ring endothelium given directly [6], this can be a proof of mechanism of action of blood pressure reduction through direct effects on vessel bloods. *A. bilimbi* fruit juice is organoleptically felt very acidic with a low pH, this can cause metabolic disorders and stomach disorders in patients with a history of peptic ulcer if taken in large and long-term to control blood pressure.

The acidity level of this fruit juice needs to be increased close to the body pH towards neutral or slightly alkaline, but the effect of increasing pH on blood vessel vasodilation activity is not yet known.



2. Materials and methods

2.1. General information

Chemicals that used in this research were the solution of Krebs-Henselheit, NaOH GR (Merck), KOH GR (Merck), Phenylephrine (Sigma-Aldrich), Methacoline (Sigma-Aldrich), Carbogen gas (Various Gas). The equipment used were a pH meter (Horiba), 10 mL chamber with circulation (IKA), octal bridge amplifier and power lab / 16SP (AD Instrument), isometric transducer (IKA), juice extractor (Cosmos) and Chart ver. 5.0.

2.2. Animals

Wistar male rats, aged 3-4 months, weighing 150-200 g, healthy, and active, from the Pharmacology Laboratory of Faculty of Medicine, Mulawarman University, maintained in a controlled environment according to standard animal care testing at the Mulawarman University Medical School with free access to food and drink. All procedure concerning animals were carried out in an ethically proper way and the experiments were approved by the Ethics Committee of the Faculty of Medicine, Mulawarman University.

2.3. Plant material

The fruit of *A. bilimbi* was taken from the area of Samarinda, East Kalimantan, in March, during the rainy season. Type identification was assisted by taxonomists from the Faculty of Forestry, Mulawarman University.

2.4. Preparation of sample

The fruit of *A. bilimbi* were washed and drained, the juice by using juice extractor, the juice solution was then filtered with a flannel cloth and then centrifuged to take the supernatant. Before being tested, *A. bilimbi* juice is stored in a refrigerator of -20°C in a small package. *A. bilimbi* fruit was taken from Samarinda area, East Kalimantan Province in March, during the rainy season. Type identification is assisted by taxonomists from the Faculty of Forestry, Mulawarman University. The fruit taken is ripe fruit which is yellowish and not rotten. After washing and draining the juice using extractor juice, the juice solution is then filtered with a flannel cloth and then centrifuged to get the supernatant. Before being tested, *A. bilimbi* juice is stored in the refrigerator -20°C in a small package. At the time of testing *A. bilimbi* juice as much as 60 mL measured its acidity with a pH-meter (referred to as JBA), and neutralized with a few drops (4-8 drops) saturated solution of NaOH (referred to as JBA + NaOH) and KOH (referred to as JBA + KOH), then note the acidity level. For comparison, the Krebs-Henselheit solution was used as pH 7.4 as a control (referred to as K) and the Krebs-Henselheit solution with acidic pH (referred to as K + HCl).

2.5. Vasodilatation activity

Separate organ preparation of the rat aortic ring and its installation technique as described by Ismail et al (2017) and Ismail & Yuniati (2016) [7, 8]. Here thoracic aorta is used, inserted in a 10 mL organ chamber containing a solution of Krebs-Henselheit pH 7.4 at 37°C and gassed with carbogen, then the solution is replaced every 10 min. The composition of the Krebs-Henselheit is as described by Ismail et al (2013) [9]. After 90 minutes of acclimation, the integrity test of contractility with 10⁻⁶ M Phenylephrine (PE) solution, if a contraction response occurs, then endothelial integrity test with metacholin solution (META) 10⁻⁵ M. Contraction response arises after PE administration and vasodilatory response after META as a sign of good aortic contractility and intact endothelium so that it is ready for testing. After acclimation and integrity tests, the aorta is contracted with 10⁻⁶ M PE, after reaching a horizontal contraction given 0.3 mL of juice solution or Control and waited for 300 seconds

then washed out. The percentage of vasodilation or vasoconstriction activity was calculated using formula (1).

$$\text{Percentage of Vasodilation or Vasoconstriction Activity} = \frac{[(APE - \frac{AJ}{\text{control}}) - APE]}{APE} \times 100\% \quad (1)$$

Notes:

APE = peak tonus value after administration of phenylephrine

AJ = peak tonus value after administration of juice

Negative values indicate vasodilation activity, a positive value indicates vasoconstriction activity. The results are tabulated in the Mean + SD value, then tested statistically using SigmaStat ver. 2.0, significantly different if $p < 0.05$. In this study 6 animals were tested using Wistar rats for replication 6 times and pH measurements were carried out three times.

3. Results and discussion

The results of pH measurements of *A. bilimbi* fruit juice and control in various treatments with pH meters and bioassay testing of vasodilation activity in separate organs of rat aorta with endothelium can be seen in table 1. It showed that *A. bilimbi* fruit juice has acidic pH values and causes vasodilation in separate organs of the aortic ring of mice with endothelium. The increase in pH in *A. bilimbi* fruit juice with the addition of alkaline solvents resulted in a decrease in vasodilation activity. The type of alkaline solution to neutralize this juice affects its vasodilatory activity, here it is seen that the KOH solution looks better for increasing the pH of *A. bilimbi* fruit juice than NaOH solution. In this study it was also known that the solution of Krebs-Henselheit in an acidic atmosphere as in *A. bilimbi* fruit juice can also cause vasodilation in blood vessels. Loutzenhisser et al (1999) had clearly demonstrated that acidic conditions can induce vasodilation in rat aorta through modulation of sequestration of intracellular calcium [10].

Table 1. The value of pH and vasodilation activity in various treatments of *A. bilimbi* fruit juice and control.

	The value of pH			Percentage of effect		
	Mean	±	SD	Mean	±	SD
JAB	2.35	±	0.03	-50	±	5**
JAB+NaOH	7.42	±	0.02	-4	±	1
JAB +KOH	7.43	±	0.03	-11	±	2
K	7.41	±	0.03	5	±	2*
K+HCl	2.32	±	0.03	-18	±	1

Notes: Performed in triplicate in determining the value of pH and 6 wistar rat for vasodilation percentage. JAB = Juice of *A. bilimbi*; JAB +NaOH= Juice of *A. bilimbi* with NaOH; JAB +KOH= Juice of *A. bilimbi* with KOH; K= *Krebs-Henselheit* solution; K+HCl= *Krebs-Henselheit* solution with HCl. The negative value of effect percentage showed the vasodilation activity, while the positive value showed vasoconstriction activity. Statistical test with One-way Anova, different significance with $p < 0.05$. *Significantly different if compared with all of groups. ** Significantly different if compared with JAB +NaOH.

In *A. bilimbi* fruit juice, neutralizing the acidic atmosphere can lead to a decrease in vasodilation activity as in this study, where the mechanism of action is still unclear. It is noteworthy, the salting

reaction between acids and bases can inactivate the active ingredient so that it becomes insoluble because the acidic atmosphere changes to neutral or towards the base, this needs further verification.

Another thing to consider is the change towards a neutral atmosphere or towards the base in *A. bilimbi* fruit juice can cause other secondary metabolites in the juice to become easily dissolved and then activate the endothelium in the blood vessels to remove various vasoconstrictor mediators. It is necessary to note that endothelium can produce a mediator of nitric oxide (NO) through activation of endothelial nitric oxide synthase (eNOS) and various mediators of endothelium-derived hyperpolarizing factors (EDHF) which both cause vasodilation in blood vessels, and endothelium-derived contracting factors (EDCF) such as endothelin, prostaglandin F₂ α and thromboxane A₂ which can cause vasoconstriction in blood vessels [11-13]. The role of the endothelium in the occurrence of vasoconstriction after being given *A. bilimbi* fruit juice with neutral pH or alkaline direction needs to be further proven in the bioassay using separate organs of the aortic ring of rats with endothelium denuded, then the results are compared with the aorta with endothelax.

4. Conclusion

The pH increasing on juice of *A. bilimbi* fruit can decrease vasodilatation activity on isolated organs of the aortic ring endothelium.

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