

EVALUATION EFFECT OF POLARITY OF THE COMPOUND FROM *Sonchus arvensis* (Linn.) LEAVES AS HYPERTENSION INHIBITOR

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ABSTRACT

Sonchus arvensis (Linn) was empirically used for the treatment of hypertension. The extract has a good effect on reducing blood pressure. The compound in *S. arvensis*, a role in controlling the blood pressure still unknown. This experiment evaluated the antihypertension activity of the compound from *S. arvensis* dependent on the polarity of the compound. Evaluation of blood pressure was carried out using rats with a tail-cuff method, and the mechanism evaluated using the frog's heart by observing changes in cardiac contraction. Epinephrine was used as acute inducer hypertension. The result showed that polar and nonpolar compounds did not show antihypertension activity, it was characterized by high of the systolic and diastolic blood pressure, and the semi-polar fraction contains semi-polar compounds were able to inhibit epinephrine-induced hypertension. The result showed that the dose of 16 mg/kg could prevent as much as 68.24% and 58.56% of the elevating systolic and diastolic blood pressure. This Result equivalent to atenolol 4.5 mg/kg as a beta-receptors antagonist. The effect was correlated to the lowered impact on heart rate in the *ex vivo* method. The suggested potential mechanism is blocking the beta-receptor in cardiomyocyte, and blood pressure can be decreased because of reducing cardiac output and heart contraction. Semi-polar fraction collected by ethyl acetate as solvent. That fraction was rich in flavonoid and phenol compounds than other fractions. One of the compounds that characterized in the semi-polar fraction is luteolin. It is the potential candidate who has acted as antihypertension.

Keywords: Hypertension, *S. arvensis*, Flavonoids, Luteolin, Polarity

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INTRODUCTION

Hypertension is one of the conditions that significantly increase the risk of stroke, kidney failure, and heart failure. Nowadays, it was significantly caused the premature death. The leading causes of increased blood pressure are high consumption of unhealthy food, lack of physical activity, alcohol consumption, and smoking. World Health Organization (WHO) showed that 1.13 billion people worldwide have hypertension.¹ WHO has a target to reduce the hypertension population by 25% in 2025.² Prevention of hypertension is one of the major processes to reduce the population of hypertension. Developing traditional medicine becomes one of the choices for treating or inhibiting hypertension because it can be a supplement to control blood pressure.

Indonesia is one of the countries with very high biodiversity. Thus, the government encourages researchers to develop traditional medicine or supplements from Indonesian. One of the purposes is helping and to reducing the hypertension population. Because of that reaches as much as 34.1% in 2018. It is higher than the prevalence in 2013.^{3,4} In this study has a focus to develop the potential of *Soncus arvensis* as one of the plants from Indonesia, which has empirically used to control the blood pressure.

Sonchus arvensis (Linn.), perennial sow-thistle is the *Asteraceae* family and known as *tempuyung* in Indonesia, is one of the very easily found plants in Indonesia, India, and China.⁵⁻⁷ It has characteristics of

weeds with stolone which stretch above the ground and are covered by buds, with green leaves and yellow flowers. It is a herbaceous plant that has a very high flavonoid content which has been identified as containing orientin, hyperoside, quercetin, catechin, rutin, myricetin, and kaempferol.⁸ The high flavonoid content may be one reason why leaves tempuyung has many pharmacological activities. However, to ascertain whether the flavonoids have an essential role in the resulting activity should be conducted in-depth research.

Scientifically, *S. arvensis* has been proven that have pharmacological effects. That was a diuretic, anti-inflammatory, immunomodulatory, hepatoprotective, hypoglycemic effect, anticalculi, and xanthine oxidase inhibitor.^{5,9-12} The results of acute and subchronic toxicity tests on ethyl acetate extract disclosed no toxic effects and death in a single high-dose administration and repeated administration for 90 days¹³. *S. arvensis* showing a high diuretic, anti-inflammatory, and antioxidant effect, it possibly has an impact on blood pressure, where one of the causes of increased blood pressure is inflammation in blood vessel endothelial cells due to high contents of ROS (reactive oxygen species), as well as increased contraction of blood vessels and the heart. Another result showed a dose combination *S. arvensis*, and *Androdera cordifolia* (Ten.) v. Steenis was inhibit angiotensin II in vivo method and *S. arvensis* showed activity to inhibit the angiotensin converting enzyme activity in in vitro method.¹⁴⁻¹⁵

S. arvensis has potential as an antihypertensive agent with a variety of mechanisms of action, including diuretics, ACE inhibitors, and angiotensin receptor blockers but requires further study to confirm this. Previous studies have not been able to determine whether the content of *S. arvensis* which has activity on blood pressure so that in this study an activity evaluation will be carried out in the leaves as an initial step in determining compounds that have antihypertensive activity. This experiment was evaluated the antihypertensive effects based on the compound polarity in *S. arvensis* leaves, this study aimed to detect the main compound that has an activity to regulate the blood pressure and predicting the occurring working mechanism in the heart.

EXPERIMENTAL

Plant Material

S. arvensis obtained from Kimia Farma Corp. Herbal plantations, Bandung, West Java, Indonesia. The plants had been identified at Herbarium Bandungense, School of Life Science and Technology, Institut Teknologi Bandung, Indonesia.

Extraction Preparation

The plants were selected, dried, and mashed by Kimia Farma, Corp. The extraction method was maceration at room temperature, and the solvent was ethanol 96% (1:8, plant: solvent). The compounds in the extracts that have been dried were separated based on the compound polarity using n-hexane, ethyl acetate, and water (1:1) as a solvent and the method was liquid-liquid extraction. The sample was concentrated with a rotary evaporator and dried in an oven at 50°C.¹⁶

For the pharmacological effect test, the sample was dissolved in carboxymethyl cellulose-Na (CMC-Na) 5% solution for oral administration, dimethyl sulfoxide (DMSO) 1% and deionized water.

Total Phenolic Content

Total phenolic content in the compound was determined by Folin-Ciocalteu Colorimetric method with gallic acid as a standard. Gallic acid solution was made with a concentration of 50-150µg/mL, while the test sample made with a concentration of 2000µg/mL in methanol (Merck, Corp.). The Folin-Ciocalteu reagent solution diluted with aqua dest at 1:10. The sample volume of 0.5 ml was added 5 ml of the reagent and 4 ml of Na₂CO₃ (Merck, Corp.) 1M and incubated for 15 minutes at room temperature. The changes in color reactions that occurred were measured using a UV-VIS spectrophotometer at a wavelength of 739 nm. The determination of the total phenol content in the test sample was calculated based on the equivalence of the amount of gallic acid per gram of the test sample (mg GA/g).¹⁷

Total Flavonoid Content

Total flavonoid content in the compound was determined by the colourimetric method. Quercetin solution as a standard made in methanol with a concentration of 20-100µg/mL and the test compound was made

with a concentration of 1000 µg/mL. 0.5 sample was added 1.5 ml of methanol (Merck, Corp.), 0.1 ml of AlCl₃ 10%, 0.1 ml of sodium acetate 1M, and 2.8 ml of distilled water. The solution incubated for 30 minutes at room temperature and absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 435.5 nm. Total flavonoid content was shown in equivalence to mg quercetin/gram sample (mg Q/g).¹⁸⁻¹⁹

Identification of Marker

Identification of marker compounds in *S. arvensis* was carried out using a thin-layer chromatography method using the stationary phase was Silica Gel GF 254 (Merck Corp.), and the mobile phase was toluene: ethyl acetate: formic acid= 6:4:0,1. The spot appearance was citroborat. The standard compound used was Luteolin (Sigma Aldrich, Corp.).

Densitometry Analysis

Marker compound assay in *S. arvensis* was performed using densitometry method. Luteolin standard curve was made with a concentration range (0,1-2 mg/mL) on the plate of Silica Gel GF 254, and the mobile phase was toluene: ethyl acetate: formic acid= 6:4:0,1.

The process of staining the sample on the TLC plate was done automatically and detected using a densitometer at a wavelength of 365 nm. The calculation of contents was based on the results of the sample density area on the standard linear regression equation and expressed in the mgLut/g sample.

Evaluation of Blood Pressure

Male Wistar rat aged 10-12 weeks with weights of 200-250 grams were used, purchased from Animal Laboratory, Inter-University Research Center, Institut Teknologi Bandung. The rats were acclimatized for two weeks with temperatures of 22-25°C, cycles of 12 hours dark/light, food and drinks ad libitum.

The Research Ethics Committee of Padjadjaran University, Bandung approved the research protocol, for animal subjects in research with number 667/UN6.KEP/EC/2018.

After the protocol had been approved, the experiment began with animal habituation on the blood pressure measuring device (a tail-cuff method, CODA noninvasive Blood Pressure system, Kent Scientific Corporation) and was continued with measurement of the initial blood pressure. Only the animals that had blood pressure below 120/70 mmHg would be used in the study.

Evaluation of the antihypertension effect was used epinephrine dose of 0.25 mg/kg as hypertension inductor. The compound test dose was based on the yield produced in the extraction process. The test animals were grouped into seven test groups (n=5). Each test animal was measured at baseline (T0), and given the test compound per orally, the positive control group was given CMC-Na 0.5% and atenolol 4.5 mg/kg as a reference drug. Epinephrine was given intraperitoneal at 30 minutes after the sample administration. At the minute 60 (T60), the final blood pressure measurements were taken with the systolic blood pressure, diastolic, and mean as the measurement parameters.

Identified the Mechanism of Action Using the Frog's Heart.

This research used a 50-75gram male frog heart (*Duttaphrynus melanostictus*) from Pharmacology Laboratory, University of Jenderal Achmad Yani, Cimahi, Indonesia. The animals had been identified at Padjadjaran University, Indonesia. The testing was divided into 7 test groups (n=5).

The frog was anaesthetized using ketamine at a dose of 100 mg/kg. Then, they were put on the fixation board and cut in the part of the pericardium that wrapped the heart with an inverted Y slice, and the heart was removed, then the frenulum cordis was hooked using a hook connected to kymograph. The heart contraction was recorded for 90 seconds with a rotation speed of 2.5 mm/s with the test parameters in the form of frequency and amplitude of the heartbeat. Krebs's solution was used to keep the heart alive. Norepinephrine 2.9 x 10⁻³ mM was the contraction inductor. The recording of the heart contraction was performed in each frog in the normal state and continued with the administration of epinephrine by droplets. It is together with the test compound directly to the heart. Observations were made about changes in the frequency and amplitude of the heart contraction, which would be analyzed as heart rate and echocardiogram pattern. Bisoprolol was used as comparative medicine (negative control).²⁰

Statistical Analysis

The data were illustrated in mean \pm deviation (SD) and analyzed using the GraphPad Prism Software with a one-way ANOVA statistical test with significance $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Compounds, Concentration of Phenol and Flavonoids Content on the *Sonchus arvensis* Leaves Fraction

Dried *S. arvensis* leaves extracted using ethanol 96%. The yield of the extract is 7.67% g/g. Furthermore, the extract was liquid-liquid extracted to produce three types of parts. They were hexane fraction (42.66% \pm 1.9%) as nonpolar fractions, water fraction (29.05 \pm 0.4%) as polar fractions, and ethyl acetate fraction (8.69 \pm 0.21%) as a semi-polar fraction. The contents of phytochemical compounds contained in each fraction were in Table-1. It disclosed that the separation process has succeeded in separating compounds based on the polarity.^{21,22} Each fraction did not contain similar secondary metabolites.

Table 1. Phytochemical Content of Three Fractions of *Sonchus arvensis* (Linn.) Leaves.

Phytochemical	Results		
	Polar fraction	Semi polar fraction	Nonpolar fraction
• Flavonoids	+	+	-
• Saponin	+	-	-
• Quinones	+	+	-
• Tannins	+	+	-
• Polyphenols	+	+	-
• Alkaloids	-	-	-
• Steroid and Triterpenoid	-	+	+
• Mono and Sesquiterpene	-	+	+

Data presented as a result of colourimetric and sedimentation. $n=3$

S. arvensis has been known to have abundant total flavonoid and phenol contents.⁸ Table-2 showed that a semi-polar fraction had a higher concentration of phenol and flavonoid contents than a polar fraction, while the phenol and flavonoid contents in hexane fraction not detected up to 5000 $\mu\text{g/mL}$.

Table-2: Concentration of Phenol and Flavonoid Concentrations of the Fractions of *S. arvensis* (Linn.).

Sample	Phenol Total (mgGA/g)	Flavonoids Total (mgQ/g)
Polar fraction	6.77 \pm 1.80	5.21 \pm 0.79
Semi polar fraction	20.83 \pm 2.20	84.69 \pm 1.65
Nonpolar fraction	0	0

Data showed the mean \pm standard deviation. $n=3$

The Effect of the Polarity Compound of the *Sonchus arvensis* Leaves on Blood Pressure

The principle of this study was a preventive method. Each fraction was given orally before epinephrine injection. The results showed that the administration of epinephrine of 0.25 mg/kg to the positive control was able to induce high blood pressure. It could increase the diastolic blood pressure (DBP) 38.29 \pm 6.67%, systolic blood pressure (SBP) 28.22 \pm 2.47%, and mean blood pressure (MBP) 30.43 \pm 9.00% (Fig.-1.) significantly towards normal blood pressure ($p < 0.05$). The dose of epinephrine 0.25 mg/kg could induce acute hypertension. Atenolol as the reference group showed significantly inhibit hypertension (Fig 1.). Furthermore, semi-polar fraction administration at doses of 8 and 16 mg/kg was able to attenuate the increase of blood pressure induced by epinephrine. It was marked by an increase in blood pressure in the semi-polar fraction group at 8 mg/kg at 20.63 \pm 5.51% for DBP, 24.10 \pm 6.00% for SBP, and 22.67 \pm 5.185% for (MBP) and the dose 16 mg/kg showed a higher activity to control the blood pressure which is only increased 12.28 \pm 5.66% for DBP, 11.44 \pm 3.27% for SBP, and 11.19 \pm 5.69% for MBP. In Figure-1(a-c), epinephrine could induce hypertension for 60 minutes indicated by elevated diastolic and systolic blood pressure on male *Wistar* rats (Key: * = $p < 0.05$) compared to the normal group. Some fraction effects of

inhibiting high blood pressure. It shows (d-f) semi-polar fraction has lowest on the increase of blood pressure after induction with significant effect compared to negative control group (Key: # = $p < 0.001$) and 16 mg/kg of dose of semi-polar fraction has similar effect to standard (atenolol groups) (Key: ** = $p > 0.05$). $n=5$.

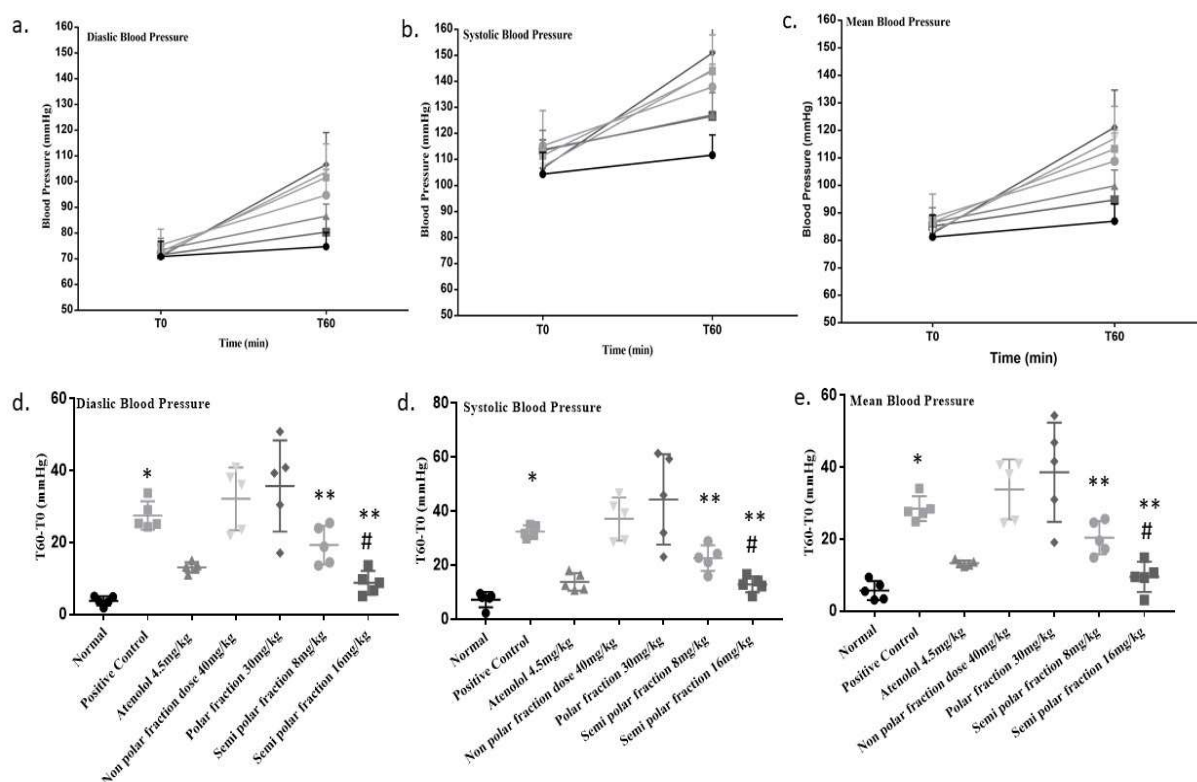


Fig.-1: Effect of Nonpolar, Polar and Semi-polar Compounds of the Fraction of *S. arvensis* (Linn.) to inhibit Hypertension induced by Epinephrine.

On the other hand, the polar fraction showed that the increase in blood pressure was significantly exceeded the positive control group (DBP $47.86 \pm 19.55\%$, SBP $37.01 \pm 14.79\%$, and MBP $43.36 \pm 17.45\%$). Moreover, the nonpolar fraction disclosed results equivalent to the polar fraction in which it could not prevent an increase of blood pressure induced by epinephrine (DBP $47.18 \pm 10.97\%$, SBP $35.78 \pm 5.90\%$, and MBP $42.43 \pm 8.39\%$).

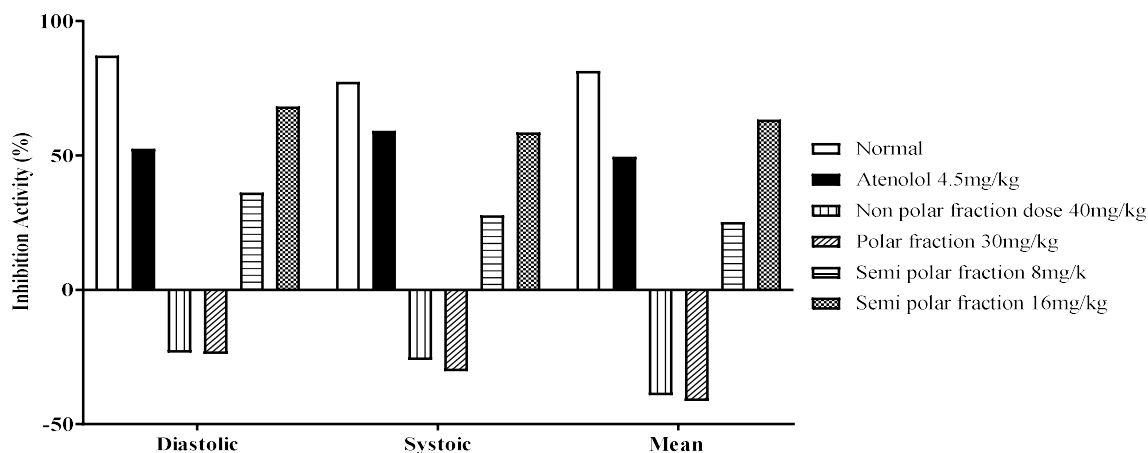


Fig.-2: Percentage of Activity in Inhibiting Hypertension

It compares elevated the blood pressure to the positive control. The data calculated from the mean of each group (n=5). Positive value show activity to inhibit hypertension, and the negative value did not show that activity.

Detection of Mechanism of Action as Antihypertension

In determining the mechanism, the experiment was continued in the *ex vivo* method using the frog's heart. The heart rhythm movements have recorded and displayed in a manual electrocardiogram (ECG) (Fig.-3). The cardiac activity was indicated as the contraction of sinus venosus, it was occurred because of the electrical signal that spreads over the heart. The atrial will active after the sinus venosus, the ventricle moved after the atria, and the aortic trunk active after the ventricle. Each peak in an ECG corresponds to changes in specific regions of the heart, the P wave is atrial depolarization, the QRS complex is atrial repolarization and ventricular depolarization, and T wave is ventricular repolarization.^{23,24}

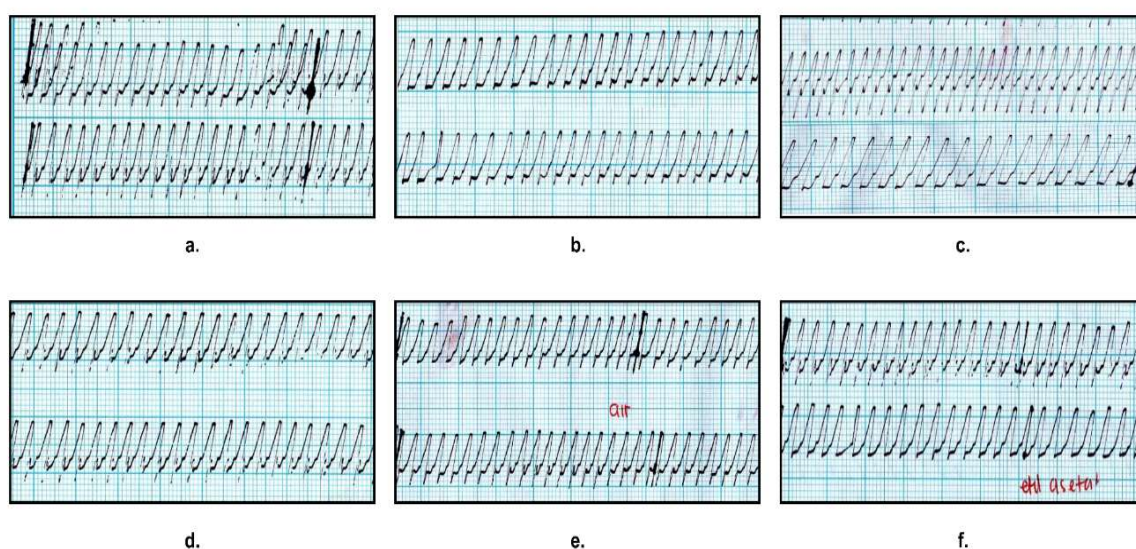


Fig.-3. Frog's Heart Electrocardiogram Pattern with Defined P, QRS, and T Waves.

In Fig.-3, groups consist of norepinephrine (positive control) showed elevation of the ST segment (a), DMSO as solvent did not give significant effect to the pattern ECG compare to a positive control (b), bisoprolol showed positive T and decreased the peak and longer of ST-segment (c), nonpolar fraction showed positive T wave and ST-segment (d), polar fraction increase RS and ST-segment (e), and semi-polar fraction negative T wave and decrease ST segment(f).

The parameters are heart rate and amplitude of the heart activity. In this study, norepinephrine reduced the amplitude (distance between two of the peaks in an ECG) $46.12 \pm 3.74\%$ (Fig.-4a) and increased the heart rate (heartbeat/min) $28.31 \pm 1.46\%$ (Fig.-4b).

Marker of *Sonchus arvensis* Leaves

Table-1 showed that semi-polar fraction contained phytochemical compounds in the classes of flavonoids, quinones, tannins, polyphenols, steroids, and terpenes. The concentration of phenol and flavonoid total was content on ethyl acetate fraction were 20.83 ± 2.20 mg equivalent to Gallic acid per gram fraction and 84.69 ± 1.65 mg equivalent to quercetin per gram fraction. The monitoring of the chromatogram patterns revealed that semi-polar fraction had the same rf value as luteolin which was 0.34 and was not found in the other two fractions. The result of densitometry analysis in a semi-polar fraction of *S. arvensis* contains luteolin 0.65 ± 0.059 mg luteolin per gram fraction.

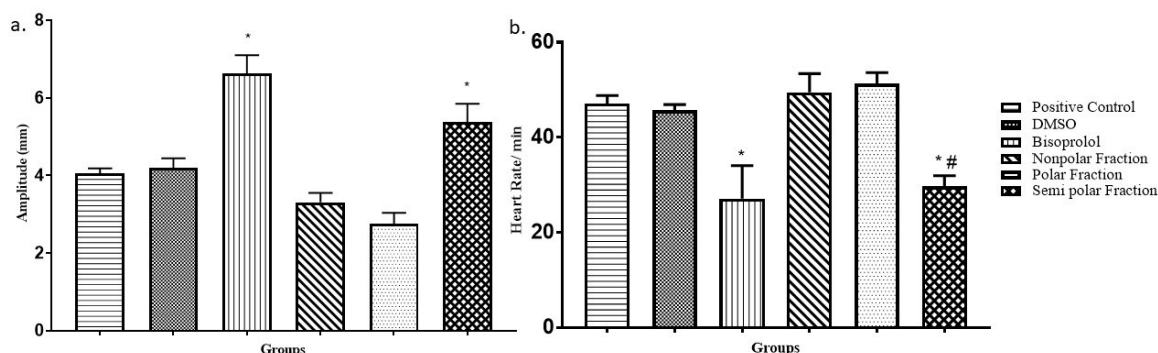


Fig.-4. Effect of *Sonchus arvensis* Fraction on Amplitude (a) and Heart Rate (b) of Frog's Heart induced by norepinephrine. Data are shown as mean \pm SD. N=5, * means $p < 0.05$ compared to the positive control, # means $p > 0.05$ compared to bisoprolol

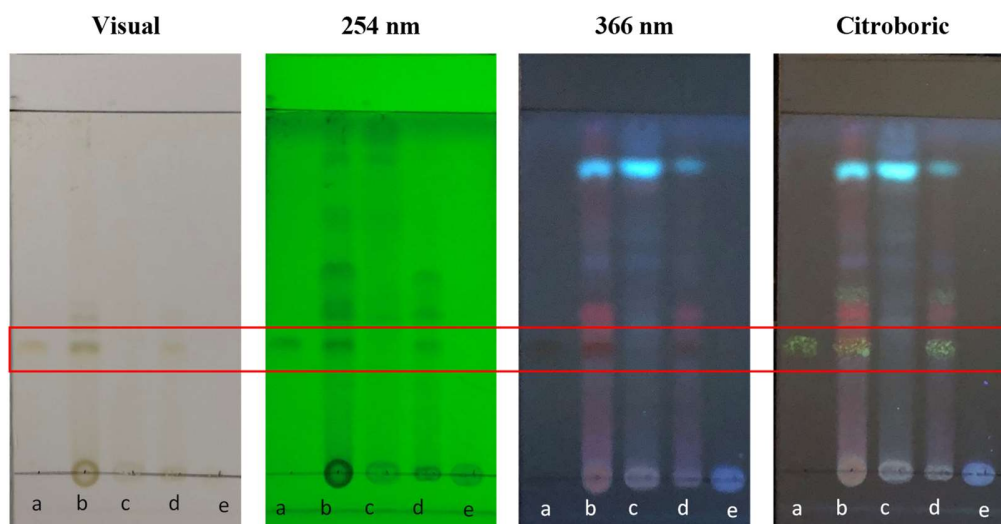


Fig.-5: Thin-layer Chromatography Plates for the Pigment of Fractions of *Sonchus arvensis* Leaves. (a) Luteolin as Standard, (b) Ethanol Extract, and (d) Semi-polar Fraction showed High Fluorescence after sprayed by Citrobolic. It indicated that contain high Flavonoid Compound than (e) Polar Fraction and (c) Nonpolar Fraction

Discussion

In this study, epinephrine was used to induce acute hypertension in male Wistar rats by stimulating pre-junctional β -adrenergic receptors that would facilitate exocytotic norepinephrine from sympathetic nerve endings.²¹⁻²⁴ The acute hypertension rat can make in 60 minutes after induction of epinephrine 0.25 mg/kg. It has significant ($p < 0.05$) elevated the diastolic (DBP), systolic (SBP) and mean arterial blood pressure (MBP) of normotensive rats (Fig.-1). The mean normotensive systolic and diastolic the blood pressure of rat is $107.92 \pm 3.26 / 72.82 \pm 4.83$ mmHg, and the epinephrine can increase the SBP as much as 31.18 ± 3.01 mmHg and the DBP as much as 27.82 ± 4.64 mmHg (Fig.-2d and e). This method success in making the hypertension rat. The approach of this experiment is used the beta-blocker mechanism because of that atenolol was used for reference groups. Atenolol is a cardioselective beta-adrenoreceptor antagonist that is an active antihypertensive agent.²⁷ The results showed that atenolol was sufficient to inhibit the activity of epinephrine, and it has significant to prevent the enhancement of the DBP, SDP, and MBP. The normal, positive control and atenolol groups were used to validating the method. This method was great to be one of the choices to check the activity of the new compound as an antihypertension based on beta-blockers activity.

The result showed various part of the compound in the *S. arvensis* leaves, which has separated based on the polarity divided into the three-part of fraction has a different effect on the blood pressure. The polar and

nonpolar fraction did not have an activity to prevent the activity of epinephrine on inducing hypertension because that has no difference significantly ($p < 0.05$) compare to positive control groups. Another result was the polar fraction increased the blood pressure more than positive control groups. It was showed the polar compound of the *S. arvensis* has another potential to have a synergistic effect with the epinephrine. The semi-polar fraction has activity in lowering the blood pressure. It has inhibited the elevating of the diastolic, systolic, and mean blood pressure (Fig.-1). Two variety of doses of semi-polar fraction showed a great activity to inhibit hypertension. The highest dose of 16 mg/kg has a similar effect to the atenolol, and it was inhibiting the elevated blood pressure as much as 68.24% for DBP, 58.56% for SBP and 63.25% for MBP. The atenolol could inhibit hypertension as much as 52.48% for DBP, 59.10 for SBP and 49.52% for MBP. Overall, the results indicated that the candidate of compounds in *S. arvensis* leaves that had anti-hypertension effects contained on the semi-polar polar fraction, and it was possible compounds.

The activity to prevent hypertension observed with epinephrine. It is a beta-receptor agonist with potent to promote high blood pressure.²⁵⁻²⁷ Epinephrine was exposed to the heart to increase heart rate by stimulating β_1 adrenoreceptor and vasoconstriction.³⁰ In this study showed adrenaline was increasing the contraction and relaxation, it changes the configuration of the action potential in heart muscle. In the frog heart, the adrenaline induced elevating of the slow inward. It is an effect on the prolongation of action potential and potentiation of contraction.³²

The semi-polar fraction at a concentration of 0.9 mg/ml could decrease heart contraction. It shows by decrease the RS and ST-segment (Fig.-3f). The effect of this condition was changed the ventricular depolarization and ventricular repolarization, which has capable of reducing the heart rate (Fig. 4b). It is correlated to activity semi-polar fraction to inhibit hypertension on *in vivo* tests. The heart rate and cardiac output were suppressed in a dose-dependent manner. The highest dose has the most significant effect on depressing the high blood pressure through the beta-receptor.

Polar fraction showed an increased heart rate and reduced the distance between two R segments. It means the polar fraction contains a compound that effects to increase the atrial depolarization and repolarization and the other effect is increased ventricular depolarization and repolarization. It showed a polar compound on *S. arvensis* has potency as cardioprotective for heart failure to increase the strength of muscular contraction.³²

The pharmacology effect from traditional medicine has correlated to the compound that contains. *S. arvensis* is one of the plants that abundant in the flavonoid contain. The separated compound based on the polarity affects the pharmacology activity. Semi-polar compound on the fraction with ethyl acetate as solvent shows the best activity to the blood pressure than other fractions. The effect from that because of semi-polar fraction contains a high of total phenol and flavonoids⁸. The flavonoid compounds were alleged to have an essential role in the pharmacological effects produced by *S. arvensis*.

This study was identified luteolin as one of the types of flavonoids contained in *S. arvensis*. It was seen in the thin layer chromatography method. Luteolin was a candidate for the compound that affected the effective inhibition of epinephrine in inducing high blood pressure. Besides, luteolin was a compound that had a direct effect on NO production, which played a role in vasorelaxant, and has excellent activity as an antioxidant.³³⁻³⁵

This study only determines the group of compounds that have a role in controlling blood pressure, so the results indicate that it was the semi-polar compounds that have activity on blood pressure, so to ensure the specific compounds that play a role in blood pressure further study is needed. At present, it is suspected that luteolin has activity on blood vessels produced by *S. arvensis*

CONCLUSION

The antihypertension effect of the *Sonchus arvensis* is useful for developing traditional medicine for reducing the prevalence of hypertension. The semi-polar compound on *S. arvensis* has the potential in controlling the blood pressure. The candidate compound which has correlated to the effect is flavonoid groups. The candidate of the semi-polar compound from *Sonchus arvensis* is luteolin. It is a compound that has a good pharmacology effect of regulating blood pressure, but it was needed to determine the specifics of active compounds in further research to carry out the process of isolating the compounds followed by screening for activity tests

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