

DIURETIC EFFECTS OF ETHANOL 96% EXTRACT OF ANNONA SQUAMOSA L. IN WISTAR ALBINO RATS

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ABSTRACT

There are about 1260 species of plants that are known to be efficacious as a drug, one of which is srikaya (*Annona squamosa* L.). The leaves of srikaya have flavonoid content that can work as diuretics. This study aims to determine the effect of diuretic extract of ethanol leaves of srikaya to white male rats wistar strain. The test animals used were 25 male wistar rats, divided into 5 treatment groups, ie aquades treatment (negative control), hydrochlorothiazide treatment 0.45 mg / 200 g BB (positive control), ethanolic extract of srikaya leaves dose 100 mg / kg BW; 200 mg / kg BW and mixture of ethanolic extract of leaves of srikaya dose 100 mg / kg BW with hydrochlorothiazide 0.225 mg / 200 g BW, given orally with volume of 2 ml / 200 g BW. The diuretic effect can be seen by calculating the area under the curve (Area Under the Curve). The diuretic effect can also be seen from cumulative urine volume from the 1st to 6th hours, the urine volume of the 6th to the 12th hour and the urine volume of the 1st hour to the 24th hour. The experimental data were analyzed by one-way variant (ANAVA), and if there was a significant difference was continued LSD (Least Significant Difference) test with 95% confidence level. The results of cumulative urine volume of urine volume 6-12 at the time of observation showed that ethanol extract of leaf of srikaya dose 100 mg / kg BW with hydrochlorothiazide 0,225 mg / 200 g BW significantly ($p < 0,05$) with negative control, so it can be said that ethanol extract srikaya leaves can be used as diuretics. AUC results for 1 to 24 hours also showed significant differences ($p < 0,05$) indicating a diuretic effect of ethanol extract of 96% of srikaya leaf.

Keywords: Ethanol extract, srikaya leaf (*Annona squamosa* L.), diuretic.

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INTRODUCTION

There are about 1,260 plant species that are known to be medicinal (Zuhud, 1994). One of them is srikaya (*Annona squamosa* L.). Srikaya leaves can be used as antioxidants, antidiabetic, hepatoprotective, antitumor activities, and so forth. The content of secondary metabolite compounds in srikaya is glycosides, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols, and amino acids. Srikaya leaves contain compounds such as saponins, flavonoids and tannins, but do not contain alkaloids (Barve, 2011). Flavonoids have biological activities such as aromatics, diuretics, analgesics, muscle relaxants, antioxidant and antiinflammation. The mechanism of action of flavonoids as diuretics by increasing glomerular velocity rate and inhibiting reabsorption of Na⁺ and Cl⁻ thus increasing the increase of Na⁺ and water in tubules (Jouad, 2001). Based on these considerations, it is necessary to conduct a research on the effect of diuretic extract of ethanol extract of srikaya leaves on male white rats Wistar strain.

METHODS

The materials used in the study were srikaya leaves, aquadest, 96% ethanol, 25 mg / tablet of hydrochlorothiazide and white rat Wistar strain. The tools used in this experiment are measuring glass, beaker, measuring pipette, sieve, filter paper, mortar, disposable syringe 1, 3 and 5 mL, oral sonde, rotary evaporator, analytical

scale, waterbath, oven, blade, blender, stainless container, animal testing cage, metabolite cage modification, urine container, mask and gloves. Experiment begins by determining the plants. Determination is done at the Faculty of Biology of Muhammadiyah University of Surakarta, Central Java to obtain assurance that the plant is srikaya. The next step is the collection of srikaya leaves. Srikaya leaves that are used in this experiment have to meet criterias such as not old, healthy, and fresh srikaya plants obtained from Rombo Village, Bangil, East Java.

The test animals used here are white male rats Wistar strain, age 2-3 months, weighing 150-260 grams of 25 tails. Furthermore, the extract of ethanol leaves srikaya were made by maceration. Maceration is done by simplicia of srikaya leaves (1.5 kg) with a suitable fine degree is inserted in stainless container. Then poured with 75 parts ethanol solvent 96% (4 liters) and left for five days with occasional stirring, to prevent the occurrence of saturation. After five days, then filtered to obtain the dregs and filtrate (liquid extract). The dregs plus the fluid of the dancers sufficiently stirred and filtered, so as to obtain the whole essence of 100 parts. Closed, left in a cool place, away from the light, deposited for two days to avoid the possibility of continued pollination powder after filtrate precipitated separated from precipitate. The next is the evaporation process. Evaporation is done by using a rotary evaporator and water bath. Evaporation lasts until a thickened extract

is obtained. This condensed extract will be used in the diuretic test. After evaporation process, diuretic test was made. All 25 rats were adapted in the laboratory for 7 days and administered for 12-18 hours before treatment, but were still given *ad libitum*. The rats were divided into 5 groups of randomized treatments. The treatment for each animal group test is as follows:

- a. Group I, aquades given orally with volume of 2 ml / 200 g BB, used as a negative control;
- b. Group II, treated with single-dose hydrochlorothiazide 0.45 mg / kg body weight (2 ml / 200 g BW) orally, was used as a positive control;
- c. Group III, treated with ethanol extract of srikaya leaves dose 100 mg / kg BW (2 ml / 200 g BB), orally;
- d. Group IV, treated with ethanol extract of srikaya leaves dose 200 mg / kg BW (2 ml / 200 g BB), orally;
- e. Group V, treated with ethanol extract of srikaya leaves dose 100 mg / kg BW and hydrochlorothiazide single dose 0,225 mg / 200 g BB (2 ml / 200 g BB), orally.

The results analysis was performed with the following steps: the test animal's urine volume data after 1, 2, 3, 6, 9, 12 and 24 hours were collected, based on statistical tests. The data obtained is then searched for the area under the curve (Area Under the Curve). Then the AUC data tested its normality distribution by Kolmogorov-Smirnov test, while its uniformity variant was tested by Levene test using 95% confidence level. When the data is

normally distributed and homogeneous, ANAVA (Varian analysis) is performed one way and if it is significantly different, it is continued with a Least Significant Difference (LSD) test with a 95% confidence level. The presence of the overall diuretic effect of the test material can be determined by comparing AUC observation times versus urine volume from the test group with negative control AUC. The same test was performed on cumulative urine volume data at 1st hour to 6th hour, urine volume data at the 6th hour to 12th hour and clock data volume 1st hour to 24th hours.

RESULTS AND DISCUSSION

Determination of plants

The results of the determination of srikaya leaves are as follows:

1b, 2b, 3b, 4b, 6b, 7b, 9b, 10b, 11b, 12b, 13b, 14a, 128b, 129b, 135b, 136b, 139b, 140b, 142b, 143b, 146b, 154b, 155b, 156b, 162b, 163a, 164b, 165b,

166a, Annonaceae

1b, Annona

1b, 2b, Annona squamosa L.

Results of Srikaya Leaves Ethanol Extract

Srikaya leaf powder weighing 1.5 kg with ethanol 96% and obtained ethanol extract of srikaya leaf of 158.4 gram, its mean the rendement is 10,56%.

Test of Diuretic

The measured data were the volume of urine at 1st, 2nd, 3rd, 6th, 9th, 12th and 24th hours. From the urine volume data each time can be calculated cumulative urine volume. Urine volume data per observation time (mean \pm SD) can be seen in Table 1.

The increase in overall urine volume during observation time can be seen from cumulative urine volume data. Data on cumulative urine volume at 1-6 hours, cumulative urine at 6-12 hours and cumulative urine hours 1-24 hours per observation time (mean \pm SD) can be seen in Table 2. The increase in urine volume at 6-12 hours may be indicated by cumulative urine 6-12. There was a significant difference ($p = 0.028$) indicated by the one-way ANOVA test. Result of LSD test, negative control significantly different with positive control and ethanolic extract of srikaya leaves dose 100 mg / kg BW plus hydrochlorothiazide 0,225 mg / 200 g BW. Comparison of the AUC (Area Under the Curve) volume of urine per observation time can be used to determine the overall diuretic effect of ethanol extract of srikaya leaf in each treatment group of ethanol extract of srikaya leaves with negative control AUC. The larger the AUC means the

greater the volume of urine produced. From the comparison of AUC values can be known whether the extract of ethanol leaves srikaya have the ability to raise the volume of urine as a whole or not. AUC 1-24 can be used to see the diuretic effect of ethanol extract of srikaya leaves at 1-24. A significant difference ($p = 0.031$) was indicated by one-way ANOVA results. The result of LSD test obtained showed that treatment group with ethanolic extract of srikaya leaves dose 200 mg / kg BW, and dosage of ethanolic extract of srikaya leaves dose 100 mg / kg BW with hydrochlorothiazide 0,225 mg / 200 g BW was significantly different with negative control, meaning that both treatments have diuretic effect at 1-24, since the AUC value is significantly greater than the negative control.

From the obtained AUC1-24 data, it can be used to calculate the diuretic power percentage of each treatment group against the negative controls shown in Table 3. The significance values of the AUC1-24 intergroup with the LSD test can be seen in Table 4.

Table 1. Data of Urine Volume Each Time Observation (ml)
in each Treatment Group (mean \pm SD) (n = 5)

Animal Test Number	Treatment	The volume of urine every hour to - (ml)						
		1	2	3	6	9	12	24
1	Negative control 2 ml / 200 g BW	0	0	0	0,1	0,2	0	0
2		0,2	0	0,2	0,9	1,2	0,3	1
3		0,5	0	0	0,7	0,6	0,5	0,9
4		0	0	0	0	0,4	0	0
5		0	0	0,1	0	0,2	0	0,3
<i>Mean \pm SD</i>		0,14 \pm 0,22	0 \pm 0	0,06 \pm 0,09	0,34 \pm 0,43	0,52 \pm 0,41	0,16 \pm 0,23	0,44 \pm 0,48
1	Positive control 0,45 mg / 200 g BW	0	0,5	0,3	1,0	0,6	0,3	0,3
2		0	1,0	0	0,3	1,3	0,8	0,5
3		0,5	2,6	1,2	2,5	1,0	0,2	0,2
4		0	0	0	0	0,2	0,8	0,5
5		0	0	0	0	0	0,2	0,4
<i>Mean \pm SD</i>		0,10 \pm 0,22	0,82 \pm 1,08	0,30 \pm 0,52	0,76 \pm 1,05	0,62 \pm 0,54	0,46 \pm 0,31	0,38 \pm 0,13
1	Ethanolic extract of srikaya leaves dose 100 mg / kg BW	0	0	0	0,3	0	0,2	1,7
2		0	1,8	0	0	0	0,2	1,5
3		0,4	0,2	0	2,2	0,9	0	1,1
4		0,8	1,1	0	1,1	0,5	0,1	0,2
5		0	0,7	0	1,1	0,7	0,2	0,7
<i>Mean \pm SD</i>		0,24 \pm 0,36	0,76 \pm 0,72	0 \pm 0	0,94 \pm 0,86	0,42 \pm 0,41	0,14 \pm 0,09	1,04 \pm 0,61
1	Ethanolic extract of srikaya leaves dose 200 mg / kg BW	0,5	0,5	0	1,0	0,3	0,7	0,4
2		0	0	1	0,6	0,3	2,5	0,1
3		0	0	0	1,6	0,6	1,5	0
4		0	1,0	1	0,3	1,0	1,0	0
5		1,6	0	0,3	0	1,3	0	0,8
<i>Mean \pm SD</i>		0,43 \pm 0,69	0,30 \pm 0,45	0,46 \pm 0,51	0,70 \pm 0,62	0,70 \pm 0,44	1,14 \pm 0,93	0,26 \pm 0,34
1	Ethanolic extract of srikaya leaves dose 100 mg / kg BW + HCT	0	1,2	0	2,2	1,7	0,2	0
2		0	3,2	0	1,4	1,8	2,0	0,2
3		0	0,7	0	0,5	0,5	1,3	0,4
4		0	2,3	0	3,5	2,1	0	0
5		0	0,2	0	0,4	0,5	0,7	1,0

	0,225 mg/kg BW							
<i>Mean ± SD</i>	0 ± 0	$1,52 \pm 1,22$	0 ± 0	$1,60 \pm 1,29$	$1,32 \pm 0,76$	$0,84 \pm 0,82$	$0,32 \pm 0,41$	



Table 2. Cumulative Urine Volume 1-6, Cumulative Urine 6-12, Cumulative Urine 1-24, Every Time Observation (ml) (mean \pm SD) (n = 5)

No.	Treatment	The cumulative urine volume every hour to- (<i>mean</i> \pm <i>SD</i>)		
		1-6	6-12	1-24
1	Negative control 2 ml / 200 g BW	0,54 \pm 0,15	1,02 \pm 0,18	1,66 \pm 0,20
2	Positive control 0,45 mg / 200 g BW	1,98 \pm 0,35	1,84 \pm 0,15	3,44 \pm 0,26
3	Ethanolic extract of srikaya leaves dose 100 mg / kg BW	1,94 \pm 0,44	1,50 \pm 0,41	3,54 \pm 0,41
4	Ethanolic extract of srikaya leaves dose 200 mg / kg BW	1,88 \pm 0,17	2,54 \pm 0,25	3,98 \pm 0,31
5	Ethanolic extract of srikaya leaves dose 100 mg / kg BW + HCT 0,225 mg/kg BW	3.12 \pm 0,90	2,87 \pm 0,29	5,60 \pm 0,70

Table 3. AUC 1-24 Urine Each Time Observation (mean \pm SD) and Percent Diuretic Power (n = 5)

No	Treatment	AUC 1-24 (<i>mean</i> \pm <i>SD</i>)	% Diuretic Power
1	Negative control 2 ml / 200 g BW	33.4 \pm 7,05	-
2	Positive control 0,45 mg / 200 g BW	56.95 \pm 5,62	70.51%
3	Ethanolic extract of srikaya leaves dose 100 mg / kg BW	61.85 \pm 12,37	85,18%
4	Ethanolic extract of srikaya leaves dose 200 mg / kg BW	79.75 \pm 5,21	13.88%
5	Ethanolic extract of srikaya leaves dose 100 mg / kg BW + HCT 0,225 mg/kg BW	92.5 \pm 6,19	17.69%

Table 4. Data on Significance of AUC1-24 Between Groups With LSD Test

Multiple Comparisons

LSD

(I) group	(J) group	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
		(I-J)			Lower Bound	Upper Bound
k -	k +	-4.71000	3.51793	.196	-12.0483	2.6283
	ext 100	-5.69000	3.51793	.121	-13.0283	1.6483
	ext 200	-9.27000*	3.51793	.016	-16.6083	-1.9317
	ext + HCT	-11.82000*	3.51793	.003	-19.1583	-4.4817
k +	k -	4.71000	3.51793	.196	-2.6283	12.0483
	ext 100	-.98000	3.51793	.783	-8.3183	6.3583
	ext 200	-4.56000	3.51793	.210	-11.8983	2.7783
	ext + HCT	-7.11000	3.51793	.057	-14.4483	.2283
ext 100	k -	5.69000	3.51793	.121	-1.6483	13.0283
	k +	.98000	3.51793	.783	-6.3583	8.3183
	ext 200	-3.58000	3.51793	.321	-10.9183	3.7583
	ext + HCT	-6.13000	3.51793	.097	-13.4683	1.2083
ext 200	k -	9.27000*	3.51793	.016	1.9317	16.6083
	k +	4.56000	3.51793	.210	-2.7783	11.8983
	ext 100	3.58000	3.51793	.321	-3.7583	10.9183
	ext + HCT	-2.55000	3.51793	.477	-9.8883	4.7883
ext + HCT	k -	11.82000*	3.51793	.003	4.4817	19.1583
	k +	7.11000	3.51793	.057	-.2283	14.4483
	ext 100	6.13000	3.51793	.097	-1.2083	13.4683
	ext 200	2.55000	3.51793	.477	-4.7883	9.8883

* = significantly different

K - = Negative control 2 ml / 200 g BW

K + = Positive control 0,45 mg / 200 g BW

Ext 100 = Ethanolic extract of srikaya leaves dose 100 mg / kg BW

Ext 200 = Ethanolic extract of srikaya leaves dose 200 mg / kg BW

Ext + HCT = Ethanolic extract of srikaya leaves dose 100 mg / kg BW + HCT 0,225 mg/kg BW

CONCLUSION

Ethanol extract 96% of srikaya leaves have diuretic effect in Wistar albino rats. Dose of ethanolic extract of 96% of srikaya leaves that can cause diuretic effect in wistar albino rats is 100 mg / kg BW and 200 mg / kg BW with power of diuretic 85.18% and 13.88% respectively. Dose 100 mg / kgBW ethanolic extract 96% of srikaya leaves added hydrochlorothiazide 0.225 mg / 200 g BW gives greater diuretic effect than hydrochlorazid 0.45 mg / 200 g BW (dose which is believed can have diuretic effect to rat).

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