Advances in Pure and Applied Chemistry (APAC)
Vol. 2, No. 1, 2012, ISSN 2167-0854

www.worldsciencepublisher.org

Uric Acid Inhibition Activity of *Annona muricata* L Leave Extract in Hyperuricemia induced Wistar Rat

Sri-Wahjuni, Putra-Manuaba, I. B., Rahayu-Artini, N. P., and Wahyu-Dwijani, S.

Chemistry Department, Faculty of Math and Science, Udayana University,
Bali-Indonesia

Abstract-This research aims to find a cure of gout, base on the utilization of *Annona muricata*. The research was started with descriptive study to explore active components of *Annona muricata* leaf and followed by an experimental study to investigate uric acid inhibition activity of the leaf extract in hyperuricemia induced wistar rat. We observed three dominant components, i.e. 2,3-dihidrobenzofuran; 3-ethoxy-1,4,4a,5,6,7,8,8a-octahydroisoquinoline; 2-cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl) which were probably active to inhibit uric acid formation in hyperuricemic induced wistar rat. In this study, n-buthnol was applied for partitioning the relatively pure compound. The n-buthanol extract obtained was then applied to cure hyperuricemic rat induced by a mixture of chicken livers and *Gnetum gnemon* a high purine diet. It was obtained, that the highest extract dose of 400 mg/kgBW was able to inhibit the formation of uric acid in hyperuricemic rat. It can be concluded that *Annona muricata* leaf extracted with n-buthanol in a dose of 400 mg/kg BW has an ability to inhibit further formation uric acid in hyperuricemic rat. Therefore, this natural plant is potent to develop for hyperuricemic medicine.

Keywords: gout, *Annona muricata*, hyperuricemic rat, active components

Introduction

Uric acid is a metabolic product of exogenous (brought in with food) or endogenous purine bases. This acid in most physiologic fluids is an end product of purine degradation. The serum urate level in a given patient is determined by the amount of purines synthesized and ingested, the amount of urate produced from purines, and the amount of uric acid excreted by the kidney (and, to a lesser degree, from the gastrointestinal tract).[1,2] Gout is an inflammatory arthritis caused by the deposition of monosodium urate crystals in tissues.[1] This condition typically occurs after years of sustained hyperuricemia. It is estimated to affect 5.1 million people in the United States according to the most recent National Health and Nutrition Examination Survey (NHANES III).[2] Gout affects approximately 2% of men older than 30 years and 2% of women older than 50 years, and is the most common form of inflammatory joint disease in men older than 40 years. Serum uric levels are, on average, 0.5 to 1.0 mg/dL higher in men than women, making male sex a risk factor for hyperurisemia and gout. Lower serum uric levels in women are associated with the presence of estrogen, which is thought to act as an antihyperuricemic.[3] In Indonesia, based on Health Survey in the year of 2005, there were around 10-20% men and postmonopause women have a higher levels of uric acids than normal person.[4] It was proven that, celery seed is often used in treating this condition, as it possesses many anti-inflammatory compounds. Other helpful herbs include turmeric, boswellia, cayenne, colchicum and hyssop were also potent to treat hyperurisemia.

Clearly, uric acid is produced by purine nucleoside metabolism through hipoxanthin, xanthin, and guanin basic purine. Distortion of this metabolism leads to elevate level of uric acid and known as hyperuricemia.[5]

Annona muricata L is a traditional plant in Bali known as *sirsak*, empirically in Balinese traditional medicine was proven as a cure of hyperuricemia. This study was carried in order to investigate the component active of the plant that have an ability to inhibit further uric acid formation in the hyperuricemia wistar rat.

Methods

This study employs two research methods, i.e. descriptive explorative to determine the active components of *Annona muricata L* leaf extracted with n-buthanol and followed by experimental study to observed their hyperuricemia activity.

Leaf extract was obtained through maseration process using methanol and followed by partition using n-buthanol. Crude extract obtained was the identified by applying GS-MS instrument.

Post only control group design was applied for experimental study, in which a number of 20 Wistar Rat 1.5 month age and 70-75 g of weight recruited in this

study and devided into 5 groups. First group is a negative control group in which rat fed with a mixture of 4 g/kg BW of *Gnetum gnemon* and 50 mL/kg BW of chicken-liver juice in *ad libitum* manner. The second group is simillar to first group instead of delivering an anti-hyperuricemia medicine, allopurinol in a dose of 10 mg/kg BW oraly. The third group is simillar to the first group instead of delivering *Annona muricata L* extract in a dose of 100 mg/kg BW oraly. The fourth and five groups have similar treatment to the first group, instead of have extract of *Annona muricata L* in dose of 200 mg/kgBW and 400 mg/kgBW, respectively.

Animal ethical clearance was obtained from a local authority body at Veterinary Faculty Udayana University, Bali-Indonesia. Around 1 mL of blood was taken from rat heart aorta which was anesthesia before proceeding. The blood was then centrifuged for 15 minutes at the rate of 3.000-3.500 rpm. Uric acid reagent, FS TBHBA (2,4,6-tribromo-3-hydroxybenzoic acid) was then added to the serum obtained. The mixture was then incubated for 10 minutes at a temperature of 37^0C. Then, optical density of the mixture was determined using sphectrophotometer at 546 nm of wave number.

ANOVA was performed to determine the different effect amongst treatment with $p<0.05$ was consider significant.

Results

Descriptive study

Around 1,200 g of *Annona muricata L*. leaf powder was macerated with methanol for overnight. From this, a number of 158 g crude extract was obtained. This crude extract was then tested for its antioxidant activity using DPPH test. The tes results was presented in Table 1.

Table 1. Antiozidant Activity Test of *Annona muricata L* Crude Extract

Sample	Time (minutes)	Test	Absorbance 497 nm	517 nm	537 nm	A 517 nm	% inhibition
Crude extract	5	DPPH	0.714	0.785	0.698	0.0790	77.22 %
		Sample	0.635	0.593	0.515	0.0180	
	60	DPPH	0.651	0.704	0.613	0.0720	85.42 %
		Sample	0.527	0.508	0.468	0.0105	

The crude extract was the purified by applying partition using petroleum ether, chlroform, n-buthanol, and water. Amongst them, in this research, it was obtained that partiton with n-buhanol produce the highest anti-oxidant ativity indicates by their DPPH test. Therefore, the n-buthanol extract was then identified Phythochemically using a number of reagent as indicated in Table 2.

Table 2. Phythochemical Test of n-Buthanol Extract

No.	Compounds	Reagent	Coloue Changes	Results
1.	Alkaloid	Meyer	Yellow - orange (without white precipitate)	-
		Wagner	Yellow - chocolate (without chocolate precipitate)	-
2.	Flavonoid	Wilstatter	Yellow - crimson	+
		NaOH 10 %	Yellow - chocolate	+
		H_2SO_4 concentrated	Yellow - crimson	+
		Bate Smith-Metacalf	Yellow - red	+
3.	Triterpenoid	Lieberman-Burchard	Yellow - chocolate	+
		H_2SO_4 10 %	Yellow - chocolate	+
4.	Saponin	Hot water + HCl	No foam formation	-
5.	Phenolate (tannin)	Hot water + $FeCl_3$	Yellow – greenish black	+
6.	Steroid	Lieberman-Burchard	Yellow - chocolate	-
		H_2SO_4 10 %	Yellow - chocolate	

Remarks:
(+) = containing compound tested
(-) = not containing compound tested

The most active extract was then identified by applying GC-MS, the chromatogram obtained was presented in Figure 1.

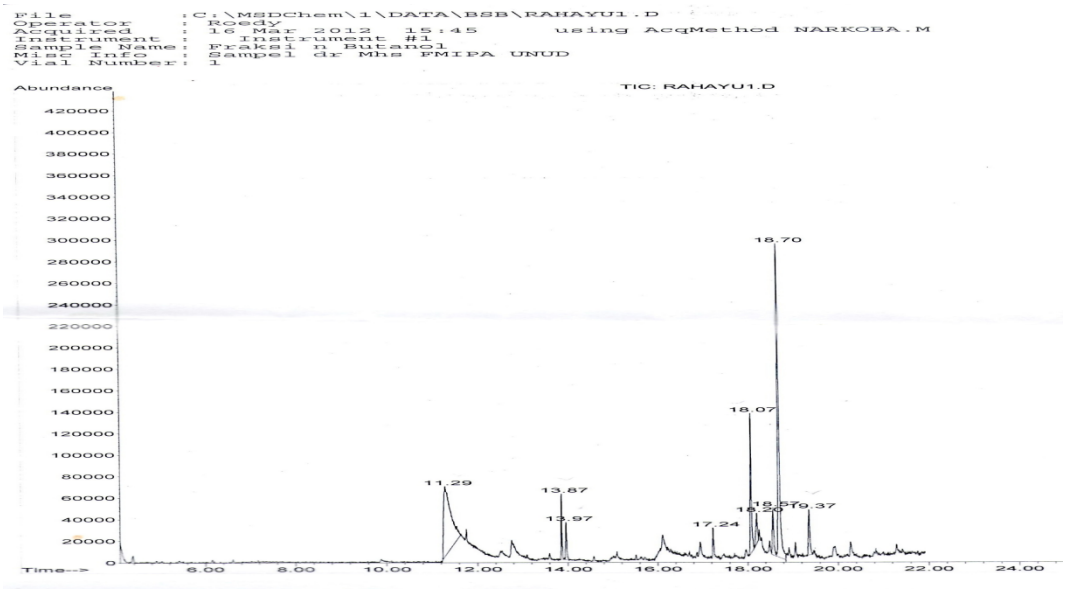

Figure 1. GC-MS Chromatogram of The Most Active Extract of Leaf *Annona muricata L*

Based on library data base of the GC-MS instrument, there were three compound detected as indicated in Table 3.

Table 3. Compound Identified Based on GC-MS Chromatogram

Peaks	Retentiom Time (t_R)	% Area	Compounds identified
Peak 1	11.29 minutes	31.48	benzofuran,2,3-dihidro
Peak 2	18.07 minutes	11.71	3-ethoxy-1,4,4a,5,6,7,8,8a-octahydroisoquinoline
Peak 3	18.70 minutes	30.89	2-cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl

Experimental study

In this study, increase uric acid in wistar rat was achieved by intake of high purine diet. Rat were fed with a mixture of 4 g/kg BW of *Gnetum gnemon* with 50 mL/kg BW of chicken liver *ad libitum*. After hyperuricemia condition was achieved, the rat then was fed with varies dose of leaf *Annona muricata L* extract, i.e 100 me/kgBW, 200 mg/kgBW, and 400 mg/kgBW. Other treatments are positive control using allopurinol and negative control. The uric acid concentration of hyperurisemia rat were presented in Table 4.

Table 4. Uric Acid Levels of Hyperurisemia Wistar Rat

Tratrment group	Uric acid concentration (mg/dL)			
	Day-6	Day-9	Day-14	Day-18
Hyperurisemia				
Control $(H)_1$	3.48	4.05	4.35	4.98
Control $(H)_2$	5.54	5.65	5.87	6.07
Control $(H)_3$	4.48	4.62	5.97	6.23
Control $(H)_4$	4.25	4.65	5.03	5.67
Average	**4.44**	**4.74**	**5.31**	**5.74**
Allopurinol dose 10 mg/kg BW				
Control positive 1	3.81	4.39	3.46	3.42
Control positive 2	3.50	6.23	4.35	3.88
Control positive 3	3.96	6.88	5.77	5.34
Control positive 4	4.38	8.19	4.50	3.08

Average	**3.91**	**6.42**	**4.52**	**3.93**
Extract dose of 100 mg/kg BW				
Treatment I_1	4.81	7.18	3.35	3.08
Treatment I_2	4.19	6.11	3.00	2.65
Treatment I_3	4.50	5.73	4.19	3.96
Treatment I_4	4.92	6.04	3.77	3.50
Average	**4.61**	**6.27**	**3.58**	**3.30**
Extract dose of 200 mg/kg BW				
Treatment II_1	3.00	3,38	2,31	1,61
Treatment II_2	3.27	3,57	3,00	1,69
Treatment II_3	4.11	4,35	2,61	1,69
Treatment II_4	3.73	4,11	3,04	2,73
Average	**3.53**	**3,85**	**2,74**	**1,93**
Extract dose of 400 mg/kg BW				
Treatment III_1	3.69	4.69	3.00	3.00
Treatment III_2	3.81	5.15	3.77	3.00
Treatment III_3	3.77	4.19	2.73	2.31
Treatment III_4	4.04	7.69	5.19	3.96
Average	**3.83**	**5.43**	**3.67**	**3.09**

Anova test indicates there was a significant different between treatment and control groups, indicate by $p < 0.05$.

Discusions

Discriptive study

As can be seen in Table 2, n-buthanol fraction based on phytochemical test was positively containing flavonoid, triterpenoid, and phenolate indicates by the colour changes for all compounds type tested. This is because of n-buthanol is a polar solvent with 3.9 of polarity index.[6] Generally, the present of glucose bind to flavonoid group results in the compound easier to solve on water and polar solvent.[7] Tannin group is a phenolate compound, that has a tendency to solve in water and polar solvent. On the other hand, triterpenoid group of compound is a pentacyclic compound tend to solve in nonpolar solvent. GC-MS analysis confirms three important compounds observed as indicates on Table 3. All of these compounds are benzofuran,2,3-dihidro, 3-ethoxy-1,4,4a,5,6,7,8,8a-octahydroisoquinoline, and 2-cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl. The present of triterpenoid is probably due to the present of hydroxide group on the structure. The extract that contain all these thee compound was then tested for their anti-hyperurisemia activity.

Experimental study

A number of 20 wistar rat were adapted in a laboratory condition. Then, all of these rats were fed with high purine diet, i.e. a mixture of 4 g/kg BW of *Gnetum gnemon* and 50 mL/kg BW of chicken liver and mix with 100 g pelete a standard diet for rat. On day-6 and day-9, about 1 mL of blood were taken from the heart aorta of the rat to determine the increase of uric acid. Before treatment uric acid serum level of the rats were determined. In this study, uric acid levels of normal rats were in the range of 1.7 – 3.0. After induction with high purine diet the uric acid levels of the rat was increase roughly, in which all rats have uric acid levels above 3 mg/dL, on average of 4.74 ± 0.665 mg/dL. It can be said, that all experimental rats are in hyperuricemia condition.

Rats induced hyperuricemia were achieved during 9 days after feeding with high purine diet. Then, on day-10 all experimental rats receive treatment for decreasing uric acid levels. Five groups of experiment were carried out as mentioned on the method. The treatment was stopped on the day-18 and uric acid leves were detrmined for all experimental rats.

In this study we obtain that for positive control group treated with allopurinol, there is a 51.93% decrease of uric acid levels, their uric acid levels become 3.93 ± 0.995 mg/dL. For the varies extract treatment, i.e. dose of 100, 200, and 400 mg/kg BW, the uric acid decrease levels obtained are 63.98%, 86.29%, and 61.50%, respectively. Therefore, the optimum dose of 200 mg/kg BW produces the highest decrease.

Allopurinol was applied in this study as a positive control, since this medicine is a cure for hyperuricemia case. In low dose this compound has an ability to inhibit the formation of xanthine oxidase enzyme.[8] Allopurinol dose of 10 mg/kg BW applied is on the basis of Zhao et al, (2005), they obtain this dose was effective to decrease uric acid levels until 125.59 ± 1.49 on their mice experimental study.[9]

Conclusion

This study investigates the application of natural plant, *Annona muricata L* as a cure of hyperuricemia on experimental rat. The rat was induced to become hyperuricemia by feeding the animal with high purine diet. Traditionally, in Bali this plant was applied to cure hyperurucemia, therefore, we would like to collect scientific data of this plant. In this study, three

compounds, i.e. benzofuran,2,3-dihidro, 3-ethoxy-1,4,4a,5,6,7,8,8a-octahydroisoquinoline, and 2-cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl) were identified. However, this still need to be further investigated. Our study also gained that the extract leaf of this plant is potent to develop as a cure for hyperuricemia, since we obtain that the dose of 200 mg/kg BW of rat is effective to decrease uric acid levels. This also need to be investigaed further, whether that will give simmilar effect on human.

Akcnowledgment

The authors would like to thank Staff of UPT Lab. Analitik Udayana University for access and aid of their fasilities for managing the research. Thanks also to Mr. Priono from Kristallindo for providing reagents for uric analysis, Mr. Rudy at Forensic Laboratory Police Department for help in assessing the GC-MS data. And special thank to Vetinery Board for providing rat for this experiment.

References

1. Stankov, M., Predrag, D., and Stankov, D. 2003. Determination of uric acid in human serum by an enzymatic method using *N*-methyl-*N*-(4-aminophenyl)-3-methoxyaniline reagent. *J.Serb.Chem.Soc. 68*(8–9)691–698.

2. Mandell, B. F. 2008. Clinical manifestations of hyperuricemia and gout. Cleveland Clinic Journal of Medicine Volume 75, Supplements 5 July 2008.

3. Luk AJ, Simkin PA. Epidemiology of hyperuricemia and gout. *Am J Manag Care*. 2005;11 (15 Suppl):S435-S442; quiz S465-S468 [Review].

4. Saraswati-Sylvia. 2009. Diet Sehat Untuk penyakit Asam Urat, Diabetes, Hipertensi, dan Stroke, Yogyakarta, A-Plus Books.

5. Harn-Shen, C. 2011. Clinical implications of the metabolic syndrome and hyperuricemia. Journal of the Chinese Medical Association 74 (2011) 527-528.

6. Snyder, C. R., et al, 1997. *Practical HPLC Method Development, Second Edition*. New York:John Wiley and Sons, Lnc, Hal 722-723.

7. Markham, K. R., 1988, Cara Mengidentifikasi Flavonoid, ITB, Bandung, Hal 15-17.

8. Tjay, T.H., dan Raharja., 2002, Obat-Obat penting, Khasiat, Penggunaan dan Efek-Efek sampingnya, Edisi V, Cetakan ke-2, Penerbit PT. Eleks Media Komputindo Kelompok Gramedia, Jakarta.

9. Zhao, X., Zhu, X. dan Pan, Y., 2005, *Effects Of Cassia Oil On Serum and Hepatic Uric Acid Levels In* Oksonat*e-Induced Mice and Xantine Dehiydrogenase and Xantin Oksidase Aktivities In Mouse Liver*, Journal Of Ethnopharmacology, (http:/ www.elsevier.com/locate/jethpharm, diakses 15 Agustus 2005