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Inhibition Of Uric Acid Formation By *Mimosa pudica* L. Herb Extract Tablets.

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ABSTRACT

Mimosa pudica Linn has been known to have various pharmacological effects. Among these are, as antidiabetic activity, antitoxin, antihepatotoksin antiinflammatory, antioxidant, wound healing, analgesic, and blood diseases (S.Varnika et. Al, 2012). Extracts and fractions with concentrations of 250, 125 and 62.5 µL effective inhibit of xanthine oxidase. The ethanol extract had the highest activity. Tablet formulation of the ethanol extract of Mimosa pudica Linn. herb. In this work we developed Mimosa pudica L. tablets using Vivapur®PH102 and studied the inhibitory activity of the tablets on uric acid formation. Extraction herbaceous Mimosa pudica Linn and evaluation of the active ingredient, drying viscous Mimosa pudica Linn extract. The tablet formulation. additives used are AEROSIL200 and. VIVAPUR®PH102 than as a binder and filler are also crusher, primojel is functioning as a disintegrator, combination with talc, magnesium stearate and AEROSIL200 also is functioning as absorbent. inhibitory activity assay of the tablets on uric acid formation was performed in vitro by measuring uric acid absorbance at 292 nm. Ex vivo study was performed by measuring the concentration of uric acid in oxonic calcium and chicken liver juice induced-mice blood after the mice were treated with Mimosa pudica L. extract tablets. Allopurinol was used as drug control. Mimosa pudica L. extract tablets containing the highest Vivapur®PH102 (86%) showed the shortest disintegration time (1 minute 40 seconds). The tablets inhibited uric acid formation (IC50 of Mimosa pudica tablet = 5.381 ppm, IC50 of the extract = 3.5202 ppm). Its inhibitory activity is weaker than allopurinol (IC50 of allopurinol = 2.181 ppm). Ex vivo study showed that Mimosa pudica L. extract tablets 125 mg/kg of body weight reduced 36% of uric acid level in hyper-urisemic mice (α=0.05). Mimosa pudica tablet 125 mg/kg of body weight inhibited uric acid formation in hyper-uricemic mice, therefore this pharmaceutical dosage form could be proposed as anti-hyperurisemic drug.

Keywords: allopurinol, gout, hyperurisemic, xanthine, xanthine oxidase, Mimosa pudica Linn

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INTRODUCTION

Mimosa pudica L. (Family Mimosaceae or Fabaceae), known as *putri malu* in Indonesia or lajvanthi or chuimui in India [1], has been popular lately and invited many researchers to explore its pharmacological activities. This plant contain mimosine, adrenalin-like substance, crocetin dimethyl ester, tubuline and a new class phytohormone turgorines, quercetin-7-rhamnoside, luteolin-glycoside, acacetin-7-rutinoside, tannin up to 10 %, d-xylose, d-glucuronic acid, mimosainic acid, mimosinamine, green yellow fatty oil up to 17 % and many other chemical constituents [2, 3]. *M. pudica* has been proven to show anti-ulcer [4], anti- inflammatory [5], anti-microbial (in combination with *Tridax procumbens*) [6], anti-toxin against Naja naja and *Bangarus caerulus* venoms [7], wound healing [8], anti-convulsant [9], anti-oxidant [10], anthelmintic [11], aphrodisiac [12], hepatoprotector [13, 14], and xanthine oxidase inhibitory activities [15].

Xanthine oxidase (XO) is an enzyme that catalyses the oxidation of xanthine and hypoxanthine into uric acid. According to the work of Oettl and Reibnegger who studied the inhibitory action of pteridine and its analogues on XO, the most characteristic features of an inhibitor are aromaticity and no substitution at position 7 of the pteridine ring [16]. XO inhibitors which block the terminal step in uric acid biosynthesis, can lower the plasma level of uric acid hence are generally employed for the treatment of gout, as proven by Ishibuchi and colleagues, who studied the structure and activity relationships of 1-phenylpyrazoles as XO inhibitors [17].

Studies of the pharmacological activities of *M. pudica* were mainly performed on the extracts, whilst the pharmacological activity of this plant's pharmaceutical product has not been provided. Therefore, in this work we manufactured *M. pudica* L. tablets using Vivapur®PH102 and studied the inhibitory activity of the tablets on uric acid formation.

MATERIALS AND METHODS

Plant materials:

Dried leaves of *M. pudica* L., were extracted 3 x 24h using ethanol 70%. The extracts were collected and the solvent was evaporated in a water-bath apparatus until a viscous extract was obtained.

Experimental animals:

The experimental animals used were male rats (*Rattus norvegicus)* weighing 150-250 g obtained from rats were quarantined for one week and fasted for 18-20 hours before blood sampling, with water provided *ad libitum*. The experiment was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Padjadjaran No: 265/NN6C 132/KEPK/PN/2015

Chemical materials:

ethanol 70% (BrataChem-Indonesia), Vivapur®PH102, sodium starch glycolate (Primojel®), magnesium stearate, aerosil®, dimethylsulfoxide, xanthine oxidase assay kit (Worthington Biochemical Corporation), Allopurinol tablets.

Instruments:

Spectroscopy UV-Vis

Methods:

Procedure 1:
Formulation:
Six formulas for direct tableting (omitting granulation) using various percentages of Vivapur®PH102 were showed in Table 1.

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Table 1. Formulas of *M. pudica* L. Tablets

Formula	A	B	C	D	E	F
M. pudica extract	11.5%	11.5%	11.5%	11.5%	11.5%	11.5%
Vivapur®PH102	81.0%	82.0%	83.0%	84.0%	85.0%	86.0%
Primojel	5.0%	4.0%	3.0%	2.0%	1.0%	--
Magnesium stearate	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
Talc	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
Aerosil	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%

In vitro study: This study was performed on the best-evaluated tablets. 20 tablets were weighed and powdered. 50 mg of the powder (equals to 11.5 mg of the extract) was added with 3 drops of DMSO and phosphate buffer to the final volume of 100 mL. This solution was diluted into 5 concentrations: 11.50; 23.00; 34.50; 46.00; 57.50 ppm. To 1 mL of each solution was added 1.9 mL of phosphate buffer 50 mM pH 7.5 and 1 mL of xanthine solution 0.15 mM. The mixture was added with 0.1 mL of xanthine oxidase and was measured its absorbance at 292 nm. The same procedure was carried out on the extract and the standard drug (allopurinol).

Ex vivo study:

25 Swiss-Webster mice (*Mus musculus*) were treated according to the Ethics Approval Committee. Their blood was taken to measure the uric acid level for baseline. The mice were kept fasted (only drink-water was allowed to be given) for 18 h then were separated into 5 groups:

I. PGA 2% only
II. This group was induced to hyper-uricemic with calcium oxonic 300 mg/kg of body weight and chicken liver juice 0.5 mL/25 g of body weight. This group was used as negative-control.
III. This group was induced to hyper-uricemic with calcium oxonic 300 mg/kg of body weight and chicken liver juice 0.5 mL/25 g of body weight, and treated with allopurinol 13 mg/kg of body weight. This group was used as positive-control.
IV. This group was induced to hyper-uricemic with calcium oxonic 300 mg/kg of body weight and chicken liver juice 0.5 mL/25 g of body weight, and treated with *M. pudica* extract 125 mg/kg of body weight
V. This group was induced to hyper-uricemic with calcium oxonic 300 mg/kg of body weight and chicken liver juice 0.5 mL/25 g of body weight, and treated with *M. pudica* tablet 125 mg/kg of body weight

Their blood was taken during 1, 2, 3, 4, and 5 hours after treatment, and were measured using UA Sure®. The inhibition percentage of xanthine oxidase activity was calculated according to the formula:

$$(A_{control} - A_{sample}) / A_{control} \times 100\%$$ [18].

Data Analysis

Statistical analysis was performed with Anova method with SPSS software tools.

hypothesis:
H0: $\tau\_1 = \tau\_2 = \cdots = \tau\_4 = 0$ (There are 4 treatment on the formation of uric acid)
H0: At least one sign $\neq 0$ (There is a difference of 4 treatment on the formation of uric acid).

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Tabel 4 Varians of Analisis

Diversity	Sum of Squares	Db	Central Square	F	Sig,
Treatment	11727,987	3	3909,329	630,837	0,000
Block (Concentration)	131,116	4	32,779	5,289	0,011
Error	74,365	12	6,197		
Total	11933,467	19			

According to the table F the F value [0.05: 3: 12] = 3.49. In a test analysis of variance, reject the hypothesis if F count> F table (α: k, (n-k)) or if the value sig <α, in other cases accept the null hypothesis. Therefore the value of F (630 837)> F table (3.49) and sig (0000) <α (0.05), the null hypothesis is rejected. So it can be concluded that there 4 treatment is difference on the inhibition of the formation of uric acid.

Duncan test

Based on testing Anava Table 4, resulted in the value of H0 is rejected, we can do further testing to determine which treatment gives different effect on the inhibition of the formation urat. Results test is as follows:

Tabel 5 Duncan Test Advanced Treatment

Treatment	Subset		
	1	2	3
Alopurinol	53,1264		
Mimosa pudica extract Tablet		57,2866	
Mimosa pudica extract		59,2594	
Negative Control			112,247

The results shows that the difference in treatment effect on the inhibition of the formation of uric acid can be divided into three groups: the first group is a positive control, the second group is extracts tablet and extract and third group is a negative control

RESULTS AND DISCUSSION

Table.2 Granule and tablet evaluation

Evaluation parameters	A	B	C	D	E	F
Water content (%)	2.10	2.60	3.10	3.90	4.10	4.90
Compressibility (%)	16.95	17.73	20.30	21.22	24.32	26.66
Density (g/mL)	1.79	1.62	1.54	1.50	1.44	1.42
Flow rate (g/sec) (with vibration)	6.86	6.76	6.48	6.49	6.48	6.50
Repose angle (º) (without vibration)	-	-	-	-	21.51º	20.65º

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Repose angle (°) (with vibration)	19.61º	16.18º	26.04º	25.54º	25.07º	28.14º
Weight uniformity (mg)	499.33	497.14	498.49	499.88	498.65	497.03
Thickness (mm)	4.35	4.40	4.52	4.60	4.91	5.34
Diameter size (mm)	12.10	12.10	12.10	12.10	12.10	12.00
Hardness (N)	82.45	83.00	84.40	85.40	86.50	84.20
Friability (%)	1.02	0.71	0.42	0.33	0.14	0.13
Disintegration (minute)	4.40	3.48	3.30	3.40	3.50	1.40

Table 2 showed that all formulas fulfill USP requirements. Furthermore we calculated the correlation between Vivapur®PH102 concentration and disintegration time (Fig.1). An increasing of Vivapur®PH102 and decreasing of Primojel in a mixture of tablet, resulted a lower crushing strength, shorter disintegration time.

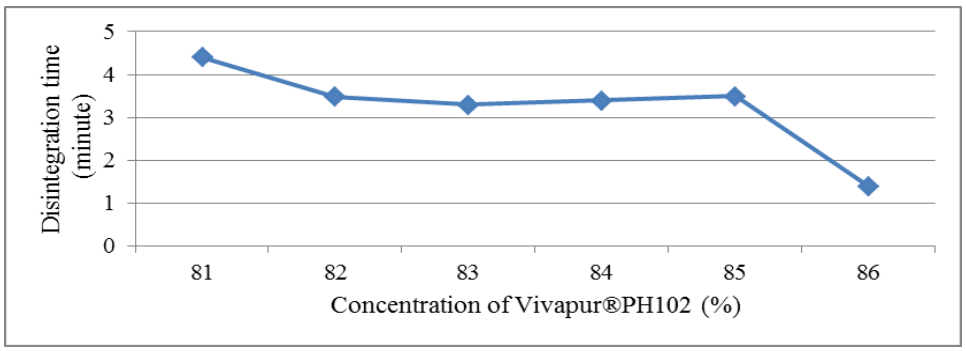

Fig.1 Correlation between Vivapur®PH102 concentration and disintegration time

This result was compared to the work of Lahdenpää et al (1997) who concluded that the increasing of Avicel® PH-102 granular and especially Avicel® PH-200 in the mixture of tablet, resulted a lower crushing strength, shorter disintegration time, and smaller weight variation [19].

The result of i*n vitro* study could be seen in Fig.2 showed the inhibition percentage of uric acid formation by *M. pudica* tablet (blue), *M. pudica* extract (red), and allopurinol (green). Trendline and data labels were provided for the tablet, extract, and drug control in different colours. The data showed that IC_{50} of *M. pudica* tablet is 68.04 ppm, IC_{50} of the extract is 32.75 ppm, whilst IC_{50} of allopurinol is 18.73 ppm.

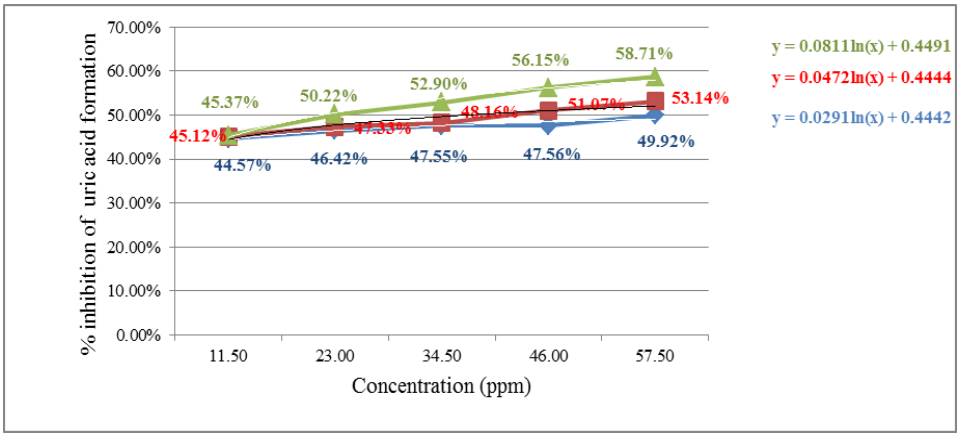

Fig.2 Inhibition percentage of uric acid formation by *M. pudica* tablet (blue), *M. pudica* extract (red), and allopurinol (green)

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This data proved that direct tableting of *M. pudica* extract decreases the activity to 48.13% . Although previous study of Marczyiński concluded that direct tableting, omitting granulation, prevented biological activity loss of active substances [20].

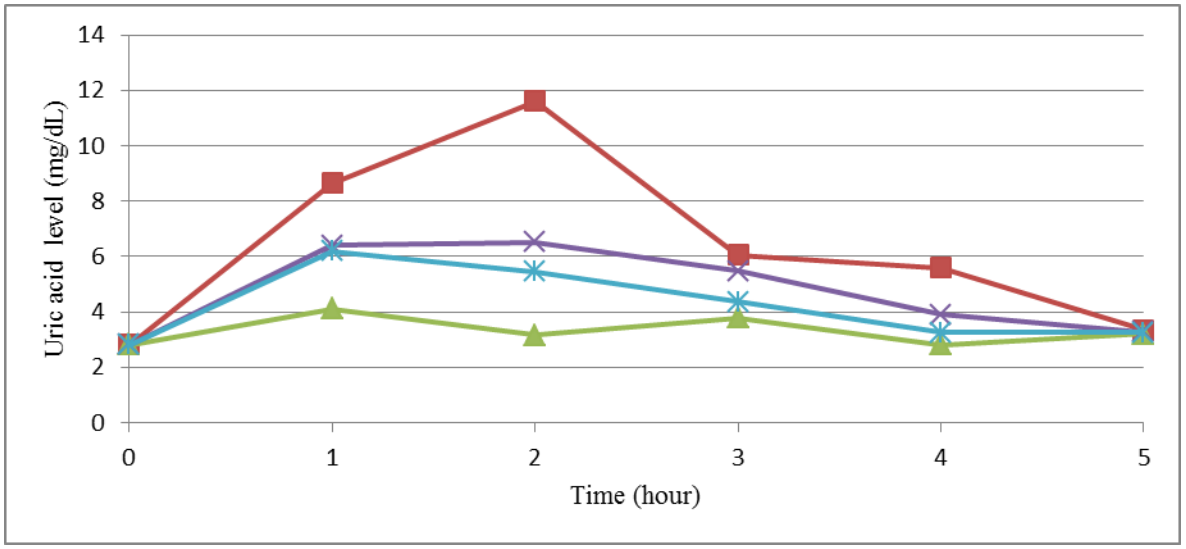

Fig.3 Inhibitory activity on uric acid formation by *Mimosa pudica* tablet (purple), extract (blue) compared to negative control (red) and allopurinol (green)

The result of *ex vivo* study could be seen in Fig.3. Fig.3 showed the inhibitory activity on uric acid formation by *Mimosa pudica* tablet and extract. The result showed that *Mimosa pudica* L. tablet 125 mg/kg of body weight and extract reduced 36% and 43%, repectively, of uric acid level in hyper-urisemic mice (α=0.05).

Results

Tabel 1 Average concentrations of uric acid for Five Minutes

Treatment	Uric acid concentration (units / mL)
Alopurinol	57,287
Mimosa pudica extract Tablet	59,259
Mimosa pudica extract	53,126
Negative Control	112,247
Alopurinol	57,287

CONCLUSIONS

Mimosa pudica L. herb extract tablet 125 mg/kg of body weight inhibited uric acid formation in hyper-uricemic mice, therefore this pharmaceutical dosage form could be proposed as anti-hyperurisemic drug.

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