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Original Research Article

The effect of ethanol extract of *Elephantopus scaber* Linn in decreasing blood uric acid levels of hyperuricemic male rats

Abstract

Background: Gout causes attacks of pain and swelling in one or more joints and control of serum uric acid level has been used as one of the therapeutic methods for gout. Inhibition method of xanthine oxidase (XO) activity which can oxidize hypoxanthine to uric acid has been commonly used to decrease serum uric acid level. On the other hand, *Elephantopus scaber* Linn leaf has been used for the treatment of rheumatoid arthritis as folk medicine by some people in Indonesia

Aims: This study was to determine the hypouricemic effect of ethanol extract of *Elephantopus scaber* leaf by in vivo study in caffeine (PO)-induced hyperuricemic male rats.

Methodology: The *E. scaber* leaf was obtained from Research Institute for Spices and Medicinal Plants, Bagor, Indonesia. Preparation of *E. scaber* leaf extract was done by cold maceration extraction technique using ethanol 70%. Male rats (Sprague-Dawley) were induced by using caffeine with dose 27 mg/200 g b.w until the levels in the blood of male rats become hyperuricemic. Rats were divided into 6 groups, as a positive

control was used allopurinol 36 mg/200 g b.w, test preparation were used 3 doses, i.e, 175 mg/200 g b.w, 350 mg/200 g b.w and 700 mg/200 b.w which suspended in Na-CMC 0.5%

Results: The result of a decrease in blood uric acid levels in rats, on the 15th days showed that a dose of 350 mg / 200 g b.w gave decrease in the highest percentage ie 43%. Statistical analysis on the 15th day showed that all of the test preparation groups of *E. scaber* had effects decrease uric acid in blood serum of rats and significantly different from negative controls ($p \leq 0.05$). The dose of 350 mg/200 g b.w of *E. scaber* had the same effect with a dose of 36 mg/200 g b.w allopurinol in reducing uric acid in experimental rats.

Conclusions: *E. scaber* is a plant that is quite potential to be used in the treatment of gout

Keywords: *Elephantopus scaber,* gout, join pain, rat, uric acid.

1. INTRODUCTION

Uric acid disease or gout is a type of joint disease that occurs due to too high levels of uric acid in the blood. Under normal conditions, uric acid dissolves in the blood and exits through urine. But under certain conditions, the body can produce excessive amounts of uric acid or experience disruption in removing excess uric acid, so that uric acid builds up in the body. Uric acid is the end product of the metabolism of the destruction of purine compounds, a nucleotide that has many roles in the functioning of

cells. Normal uric acid levels in humans around 4 mg/dl. Uric acid levels in the blood can increase beyond normal levels and is called hyperuricemia, due to increased production or decreased excretion of uric acid. Increased blood uric acid levels can cause build up of uric acid crystals that form like needles, especially in joints. As a result will cause pain in the joints (**1, 2**). Gout can be treated with one or more of the following drug, namely: Nonsteroidal anti-inflammatory drugs, such as ibuprofen, naproxen and celecoxib etc., Colchicine, Probenecid, Allopurinol, Salicylic acid etc.

Side effect of these gout drugs, generally is drowsiness, headache, diarrhea, vomiting, stomach discomfort, nausea, cramping (**3, 4**). *E scaber* is highly potential for treating gout, because *E scaber* contains chemical compounds that have structure-activity relationship (SAR) with allopurinol, salicylate and synthetic drugs for other gout drugs, namely the group of sesquiterpenes of lactone, phenolic acids and flavonoids as compounds chemistry of *E. scaber* (**5, 6,**), as shown in Fig. 1. Based on this reason, we conducted the research by giving ethanol 70% extract of *E.scaber* orally to rats and determined the effect of the efficacy of *E scaber* leaf extract in rat blood.

2. MATERIAL AND METHODS

E. scaber leaf was obtained from Research Institute for Spices and Medicinal Plants (BALITRO) Bogor, Indonesia and to determine plant authentication was carried out in Biology research center, Indonesian Institute of Sciences, Bogor, Indonesia.

A total of 500 mg leaf powder of *E. scaber* was extracted by repeated maceration method using 70% ethanol solvent and performed occasional shaking. The process was carried out for 3 weeks, were once in two days the solvent was replaced and filtered to obtain a liquid extract, then the liquid extract was evaporated with a vacuum rotary evaporator to obtain a viscous extract and dried using a freeze dryer.

2.1. Compound and phytochemical screening of *E.scaber extract*

Compound and phytochemical screening of *E.scaber* extract was done based on Harbone and Farnsworth methods, namely for the groups of alkaloid, flavonoid, saponin, steroid, triterpenoid, tannin, quinone and essential oil (**7, 8**), . as follows:

2.1.1. Determination of the Alkaloid Group

A total of 0.5 grams of *E. Scaber* extract was dissolved in 1% hydrochloric acid and filtered. The filtrate was divided into two parts, one part was dropped with Mayer's reagent and the other was dropped with Dragendorf reagent. Positive results were indicated by the formation of white precipitation with Mayer reagent and red precipitation with Dragendorf reagent

2.1.2. Determination of the Flavonoid Group

A total of 0.5 grams of *E. Scaber* extract was dissolved with 2 mL of 70% ethanol and 3 drops of NaOH solution were added. The change in the intensity of the yellow color becomes colorless on the addition of sulfuric acid indicating the presence of flavonoids

2.1.3. Determination of the Saponin Group

As much as 0.5 gram of extract *E. Scaber* was put into the test tube, 10 ml of hot water was added, cooled and then shaken vertically for 10 seconds. The formation of foam as high as 1–10 cm which was stable for not less than 10 minutes showed the presence of saponins. At the addition of 1 drop of 2N HCl, the foam was not lost

2.1.4. Determination of Terpenoid Groups and Steroids

The contents of the secondary metabolites of the terpenoid group and the steroid extract of *E. scaber* were determined by using Liebermann-Burchard reagent. A total of 0.5 gram of extract was added 5 mL of chloroform, then acetic anhydride was added and a few drops of concentrated sulfuric acid. The test results were positive for terpenoids when dark green was formed. Positive test results for steroids if pink or red were formed

2.1.5. Determination of the Tannin and Polyphenol Groups

As much as 0.5 gram of E scaber extract was dissolved in 5 mL aquadest then drops of 10% iron (III) chloride solution, if it was formed in blue or blackish green color showed the presence of tannins

2.2. Treatment of test preparations in experimental animals

The male white rats, strain of Sprague-Dawley with 3-4 months old (weight 190-250 g) were acclimatized for two weeks and maintained on 12hours light, 12hours dark cycle on temperature 25°C. Procedure maintenance of rats and conducting experiments on

animals were done based on norms of Committee for the Purpose of Control and Supervision on Experiments on Animals /CPCSEA, 2003(**9**). The rats qualified for the experiment were divided into 6 groups. The number of rats per group was calculated based on Federer's formula (**10**), where for 6 group were greater than 15, therefore the number of rats per group was obtained:

(n-1).(t-1) = (6-1).(4-1) > 15. Or each group consists of 4 rats.

Table 1. Groups of test animals

No	Groups
1	Normal control was given only solution Na-CMC 0.5%
2	Negative control was given caffeine 27 mg/200 g BW in solution of Na-CMC 0.5%
3	Positive control was given caffeine 27 mg/200 g BW and alopurinol 36 mg/200 g BW in solution of Na-CMC 0.5%
4	Low dose was given caffeine 27 mg/200 g BW in solution of Na-CMC 0.5% and 175 mg/200 g BW extract *E. Scaber* in solution of Na-CMC 0.5%
5	Middle dose was given caffeine 27 mg/200 g BW in solution of Na-CMC 0.5% and 350 mg/200 g BW extract *E. Scaber* in solution of Na-CMC 0.5%

6 High dose was given caffeine 27 mg/200 g BW in solution of Na-CMC 0.5% and 700 mg/200 g BW extract *E. Scaber* in solution of Na-CMC 0.5%

In this experiment were calculated, the dose of allopurinol as a positive control used for humans was 200 mg / day. The conversion factor from human to rat was 0.018 and the pharmacokinetics factor used is 10 Therefore dose for rat was 200 mg x 0,018 x 10 = 36 mg / 200 g b.w. The dose of caffeine that used for humans is 150 mg / day. The conversion factor from human to rat was 0.018 and the pharmacokinetics factor used is 10. (b). Therefore dose for rat is 150 mg x 0,018 x 10 = 27 mg/ 200 g b.w. As shown in table 1.

In this case before the experiment was done, the rats were fasted for 12 hours. To increase uric acid levels in rats blood, the rats were induced with caffeine 27 mg/200 g b.w. Based on experiment that was done by Azizahwati et al (2005) uric acid levels in rats blood were already high on the sixth day (11).

On the seventh day, the treatment was given based on each group every day. Caffeine was also administered to all groups except the normal group. Measurement of blood uric acid levels continued on the day, 6th, 12th and 15th.

The measurement level of uric acid in the blood was done by taking the blood on the rats tail and measured with equipment (Easy Touch) by using uric acid strip.

The test results data were analyzed using data processing software SPSS-19 and presented in the mean and standard deviation of each group. The data were processed

using statistical analysis with normality test, homogeneity test, One Way ANOVA and Kruskal-Wallis Test.

3. RESULTS AND DISCUSSION

The results of determination of plant taxonomy was done by Herbarium Bogoriense, Biological Research Center, Indonesian Institute of Sciences, indicating that the plant used was *E.scaber*.

The result extraction of 500 mg simplicia powder (*E. scaber*) was obtained 92.6 g extract or the yield of simplicia was 92.6 g divided by 500 g was 18.52%.

The results of phytochemical screening of *E.scaber* was done based on the Harbone and Farnswoth methods, *E. scaber* contains groups of chemical compounds, as shown in table 2.

The result of Phytochemical Analysis for the identification of the chemical constituents present in the 70% ethanol extract based on Harborne and Farnswoth methods were obtained chemical compound groups as shown in table 2. In this study we used 70% ethanol solvent, because 70% ethanol solvent was a more powerful solvent in isolating chemical compounds in natural products compared to other organic solvents (7).

Table 2. The results of phytochemical screening of *E scaber* 70% ethanol extract.

Group of chemical compounds	Screening results
a. Alkaloid	+
b. Flavonoid	+
c. Saponin	+
d. Steroid/triterpenoid	+
e. Tannin	+

f. Quinone	-
g. Essential oil	+
h. Qoumarin	-

Note:
(+) contain chemical compounds
(-) does not contain chemical compounds

The result of chemical compounds in this research was obtained the group of alkaloid, flavonoid, saponin, steroid/triterpenoid, tannin, essential oil, as shown in Table 2.

As shown in Table 3 and Figure 1, the results of rats were induced with caffeine cause increase uric acid level in the blood on day sixth on all groups of rats and differed significantly from normal rats, namely rats group that were not induced with caffeine ($p \leq 0.05$).

Statistical test of one-way ANOVA and Least Significance Different (LSD) on day 9th, the levels of blood uric acid on all groups were still significantly different ($p \leq 0.05$) with the normal control group. This was because the work of caffeine to increase uric acid was stronger than the work of test preparation to decrease uric acid levels in all groups of rats.

Table 3. The mean measurements of uric acid blood levels of the test animals during the experiment (mg / dL)

Days	Normal Control	Negative Control	Positive Control	Low Dose	Medium Dose	High Dose
0	1.65	1.48	1.30	1.60	1.25	1.53
6	1.50	2.90	2.78	2.80	3.00	2.80
9	1.43	3.33	2.45	2.48	2.33	2.58
12	1.50	3.55	1.78	2.30	2.23	1.95
15	1.43	3.85	1.15	1.75	1.70	1.65

Statistical test of one-way ANOVA and LSD on day 12th, the levels of blood uric acid

on high dose and positive control were not significantly different ($p \geq 0.05$) with the normal control group. This was because the work of caffeine to increase uric acid was almost same with the work of test preparation to decrease uric acid levels in these groups of rats

Statistical test of one-way ANOVA and LSD on day 15th, the levels of blood uric acid on positive control, low dose, middle dose and high dose were not significantly different ($p \geq 0.05$) with the normal control group. This was because the work of caffeine to increase uric acid was almost same with the work of positive control, low dose, middle dose and high dose to decrease uric acid level in the blood of these groups.

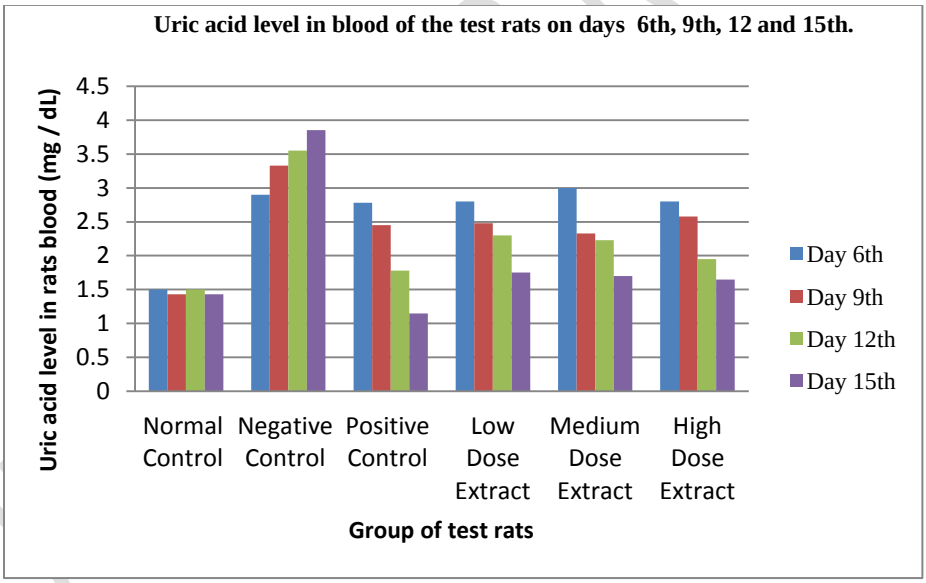

Figure 1. Uric acid level in blood of the test rats on days 6th, 9th, 12th and 15th after administration test preparation.

While uric acid level in the blood of negative control was still high, because on this group caffeine still increase uric acid level and there was no drug or chemical

compound that work to increase uric acid level in the blood. Whereas on positive control, low dose, middle dose and high dose, the work of test preparation can increase of uric acid reduce uric acid which was enhanced by caffeine or in other words, the gout on rats and human can be treated by *E. scaber* extract. As shown in Table 3 and Figure 1. Percentage (%) of increase and decrease of uric acid levels after was induced with caffeine and administration of test preparations on the 9th, 12th and 15th days, compared to 6th day, ie before the administration of the test preparation, can be shown in Table 4 & Figure 2.

Table 4. Percentage (%) of increase and decrease of uric acid levels after was induced with caffeine and administration of test preparations on the 9th, 12th and 15th days, compared day 6th

Days	Normal Control	Negative Control	Positive Control	Low Dose	Medium Dose	High Dose
9	(-)4.66	13.79	-11.87	-11.43	-22.33	-7.86
12	0.02	22.41	-35.97	-17.86	-25.67	-30.36
15	(-)4.66	32.75	-58.63	-37.50	-43.00	-41.00

In this case on normal controle group because there was no administration caffeine and test preparation, uric acid level just experience a slight fluctuation value, decrease on day 9th about 4.66% and increase + 0,02 % on day 12th and increase again on day 15th. On negative controle group because there was administration caffeine 27 mg/200 g b.w every day from first day until day 15th of experiment has caused uric aci level in the blood increase and increase from day to day far beyond uric acid levels in the normal control group, which reaches levels 3.85 mg/dL on the day 15th. While uric acid level in the blood of normal control just about 1.50 mg/dL. Percentage (%) decrease happened on day 15th with value for Positive Control, Low dose, Medium Dose, High

Dose (-)58.63%, (-)37.50%, (-)43.00% and (-)41.00% respectively.

As shown in Table 4 and Figure 2, Negative control. Group always increase from day 6th until day 15th, caused was induced with caffeine, while Positive Control, Low dose, Medium Dose, High Dose always decrease from day 9th until day 15th, caused the word of test preparation.

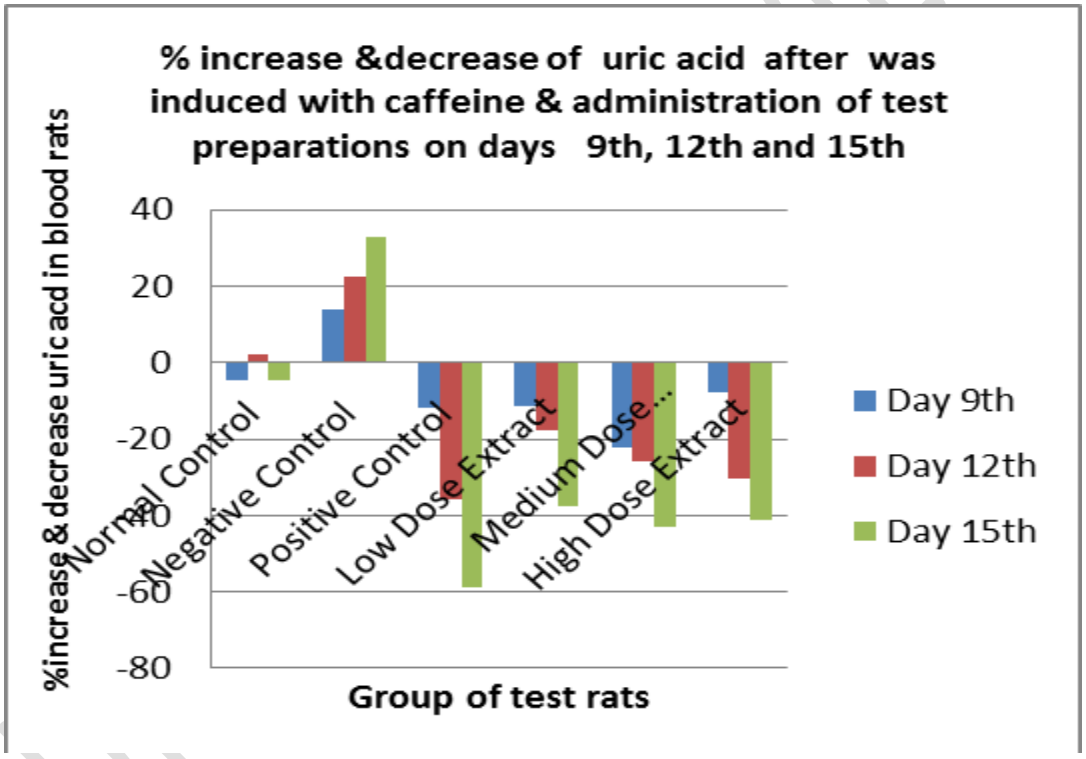

Figure 2. % increase & decrease of uric acid after was induced with caffeine & administration of test preparations on days 9^{th}, 12^{th} &15^{th}. compared day 6th

Structure Activity Relationships (SAR) are relations between the molecular structure and biological or physicochemical activity of chemicals or in pharmacology, chemical compounds that have the same chemistry and differ in functional groups, will have the

same properties but differ in potential efficacy (**12, 13, 14, 15**).

Chemical compound that work in decrease uric acid level in blood of rats, probably derived from Some phenolic acid and flavonoid compound. According to Kabeer and Prathapan (2014), Chang *et al* (2011) were obtained some chemical compound of phenolic acid and flavonoid compound in *E. scaber* as shown in Figure 3. and Figure 4, (**5, 6**). These chemical compounds have the same basic structure with chemical compounds that are currently widely used to treat gout, as shown in Figure 5.

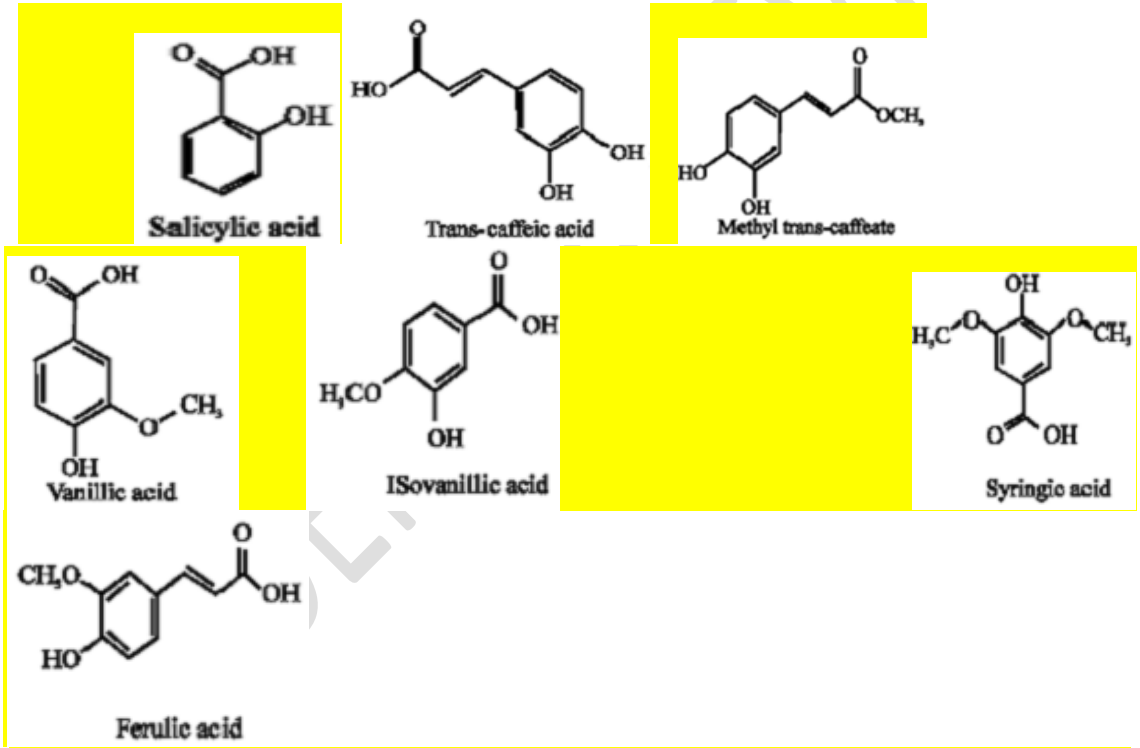

Figure 3, there are 7 chemical compounds in *E. scaber* that have basic structure similar with salicylic acid, namely salicylic acid, trans-caffeic acid, methyl trans caffeate, vanillic acid, iso-vanillic acid, syringic acid, ferrulic acid

Then, there are 4 chemical compounds in *E. scaber* that have basic structure similar with allopurinol, namely, luteolin, luteolin 4-o-β-D glucoside, luteolin 7-o-glucuronide, tricine, as shown in Figure 6.

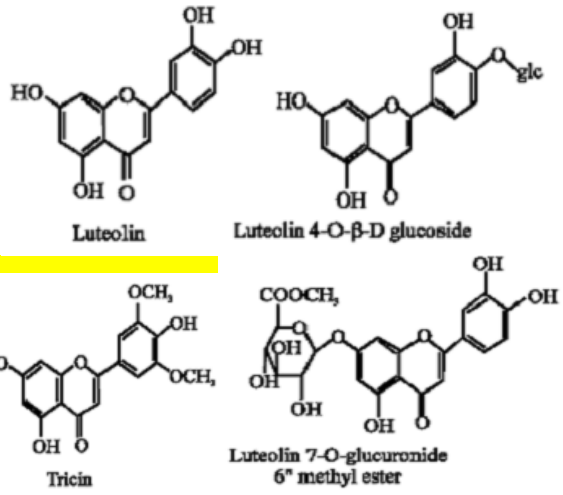

Figure 4. there are 4 chemical compounds in *E. scaber* that have basic structure similar with allopurinol, namely, luteolin, luteolin 4-o-β-D glucoside, luteolin 7-o-glucuronide, tricine.

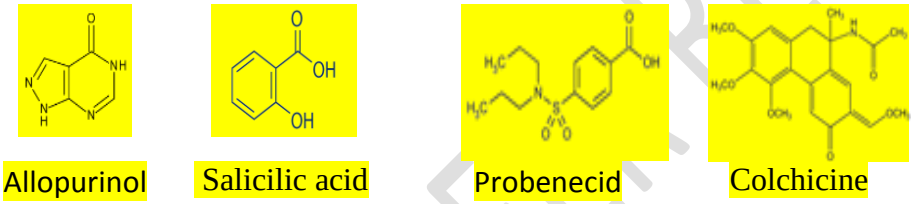

Figure 5. Chemical compounds that are often used to decrease uric acid in gout

There is also a strong possibility that the chemical compound in *E. scaber* in Figure 3 and Figure 4 above have the work of decrease uric acid better than the drugs for gout that exist today.

The results of this study are similar to the results of research by Jennie et al (2016), where they used *E. scaber* powdered leaves extracted using methanol and distilled water to obtain test extracts. The administration of the test preparation was carried out by using a single subcutaneous injection of 0.1 ml Complete Freund Adjuvant emulsion

(CFA). Their experiments also found that *E. scaber* worked significantly in reducing uric acid in gout (16, 17)

4. CONCLUSIONS

E.scaber has the effect decrease uric acid on gout. Effect of ethanol extract 70% of *E. scaber* with dose 175 mg / 200 g b.w on rat have the same effect with dose allopurinol of 36 mg / 200 g b.w rat.

There are about 7 of the salicylic acid derivatives and there are about 4 of the phenolic acids and flavonoid derivatives in *E. scaber*, these chemical compounds are strongly suspected have properties for decrease uric acid in the blood, because their basic structure is very similar with drugs to decrease uric acid. Further research is needed, to find out what chemicals are better for treating gout in *E. Scaber*.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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