

## 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 have been used for 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

21 control was used allopurinol 36 mg/200 g b.w, test preparation were used 3 doses, i.e,  
22 175 mg/200 g b.w, 350 mg/200 g b.w and 700 mg/200 b.w which suspended in Na-  
23 CMC 0.5%

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

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

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

## 34 1. INTRODUCTION

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

41 Normal uric acid levels in humans around 4 mg / dl. Uric acid levels in the blood can  
42 increase beyond normal levels and is called hyperuricemia, due to increased production  
43 or decreased excretion of uric acid. Increased blood uric acid levels can cause build up  
44 of uric acid crystals that form like needles, especially in joints. As a result will cause  
45 pain in the joints (1, 2).

46 Gout can be treated with one or more of the following drug, namely:

- 47 a. Nonsteroidal anti-inflammatory drugs, such as ibuprofen, naproxen and
- 48 celecoxib etc.
- 49 b. Colchicine
- 50 c. Probenecid
- 51 d. Allopurinol
- 52 e. Salicylic acid etc.

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

61 scarlet coloured seeds are described.

## 62 **2. MATERIAL AND METHODS**

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

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

### 73 **2.1. Compound and phytochemical screening of *E.scaber* extract**

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

#### 77 **2.1.1. Determination of the Alkaloid Group**

78 A total of 0.5 grams of *E. Scaber* extract was dissolved in 1% hydrochloric acid and  
79 filtered. The filtrate was divided into two parts, one part was dropped with Mayer's  
80 reagent and the other was dropped with Dragendorff reagent. Positive results were

81 indicated by the formation of white precipitation with Mayer reagent and red  
82 precipitation with Dragendorf reagent

### 83 **2.1.2. Determination of the Flavonoid Group**

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

### 87 **2.1.3. Determination of the Saponin Group**

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

### 92 **2.1.4. Determination of Terpenoid Groups and Steroids**

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

### 99 **2.1.5. Determination of the Tannin and Polyphenol Groups**

100 As much as 0.5 gram of E scaber extract was dissolved in 5 mL aquadest then drops of  
 101 10% iron (III) chloride solution, if it was formed in blue or blackish green color showed  
 102 the presence of tannins

## 103 **2.2. Treatment of test preparations in experimental animals**

104 The male white rats, strain of Sprague-Dawley with 3-4 months old (weight 190-250 g)  
 105 were acclimatized for two weeks and maintained on 12hours light, 12hours dark cycle  
 106 on temperature 25°C. Procedure maintenance of rats and conducting experiments on  
 107 animals were done based on norms of Committee for the Purpose of Control and  
 108 Supervision on Experiments on Animals /CPCSEA, 2003(9). The rats qualified for the  
 109 experiment were divided into 6 groups. The number of rats per group was calculated  
 110 based on Federer's formula (10), where for 6 group were greater than 15, therefore the  
 111 number of rats per group was obtained:

$$112 \quad (n-1).(t-1) = (6-1).(4-1) > 15. \text{ Or each group consists of 4 rats.}$$

113 **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%

120 3 Positive control was given caffeine 27 mg/200 g BW and allopurinol 36 mg/200  
121 g BW in solution of Na-CMC 0.5%

122 4 Low dose was given caffeine 27 mg/200 g BW in solution of Na-CMC 0.5%  
123 and 175 mg/200 g BW extract *E. Scaber* in solution of Na-CMC 0.5%

124 5 Middle dose was given caffeine 27 mg/200 g BW in solution of Na-CMC 0.5%  
125 and 350 mg/200 g BW extract *E. Scaber* in solution of Na-CMC 0.5%

126 6 High dose was given caffeine 27 mg/200 g BW in solution of Na-CMC 0.5%  
127 and 700 mg/200 g BW extract *E. Scaber* in solution of Na-CMC 0.5%

128

129 In this experiment were calculated, the dose of allopurinol as a positive control used for  
130 humans was 200 mg / day. The conversion factor from human to rat was 0.018 and the  
131 pharmacokinetics factor used is 10 Therefore dose for rat was  $200 \text{ mg} \times 0,018 \times 10 =$   
132  $36 \text{ mg} / 200 \text{ g b.w.}$  The dose of caffeine that used for humans is 150 mg / day. The  
133 conversion factor from human to rat was 0.018 and the pharmacokinetics factor used is  
134 10. (b). Therefore dose for rat is  $150 \text{ mg} \times 0,018 \times 10 = 27 \text{ mg} / 200 \text{ g b.w.}$  As shown in  
135 table 1.

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

140 On the seventh day, the treatment was given based on each group every day. Caffeine  
141 was also administered to all groups except the normal group. Measurement of blood  
142 uric acid levels continued on the day, 9th, 12th and 15th.

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

145 The test results data were analyzed using data processing software SPSS-19 and  
146 presented in the mean and standard deviation of each group. The data were processed  
147 using statistical analysis with normality test, homogeneity test, One Way ANOVA and  
148 Kruskal-Wallis Test.

### 149 **3. RESULTS AND DISCUSSION**

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

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

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

158 The result of Phytochemical Analysis for the identification of the chemical constituents  
159 present in the 70% ethanol extract based on Harborne and Farnsworth methods were



160 obtained chemical compound groups as shown in table 2. In this study we used 70%  
 161 ethanol solvent, because 70% ethanol solvent was a more powerful solvent in isolating  
 162 chemical compounds in natural products compared to other organic solvents (7).

163 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	-

164 Note:

165 (+) contain chemical compounds

166 (-) does not contain chemical compounds

167

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

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

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

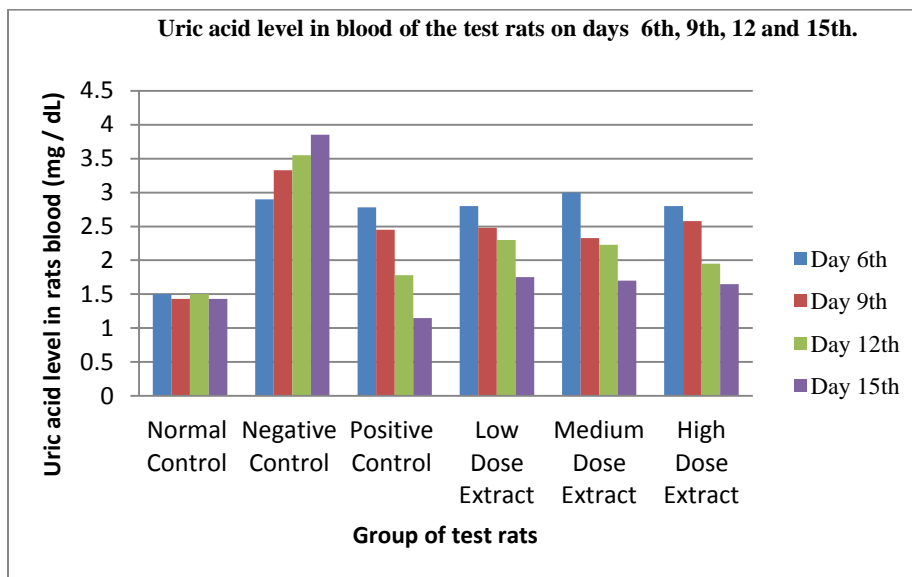
179 Table 3. The mean measurements of uric acid blood levels of the test animals during the  
 180 experiment (mg / dL)

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

181

182 Statistical test of one-way ANOVA and LSD on day 12th, the levels of blood uric acid  
 183 on high dose and positive control were not significantly different ( $p \geq 0.05$ ) with the  
 184 normal control group. This was because the work of caffeine to increase uric acid was  
 185 almost same with the work of test preparation to decrease uric acid levels in these  
 186 groups of rats

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



192  
193

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

196 While uric acid level in the blood of negative control was still high, because on this  
197 group caffeine still increase uric acid level and there was no drug or chemical  
198 compound that work to increase uric acid level in the blood. Whereas on positive  
199 control, low dose, middle dose and high dose, the work of test preparation can increase  
200 of uric acid reduce uric acid which was enhanced by caffeine or in other words, the gout  
201 on rats and human can be treated by *E. scaber* extract. As shown in Table 3 and Figure  
202 1. Percentage (%) of increase and decrease of uric acid levels after was induced with  
203 caffeine and administration of test preparations on the 9th, 12th and 15th days,  
204 compared to 6th day, ie before the administration of the test preparation, can be shown  
205 in Table 4 & Figure 2.

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

208

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

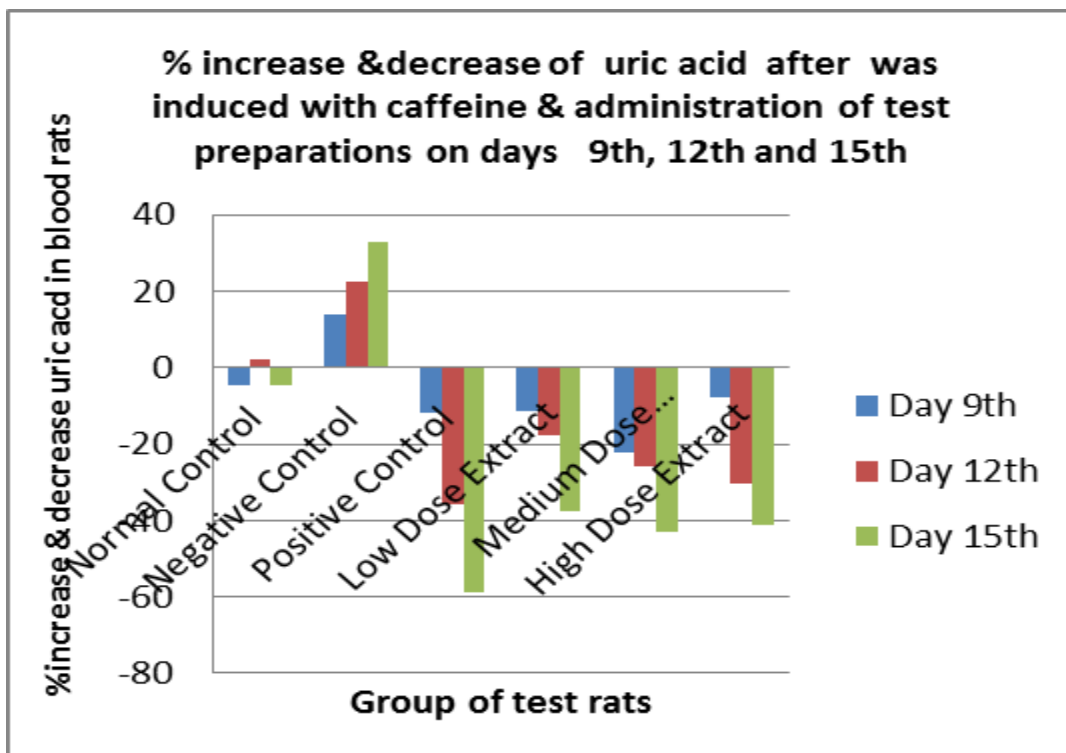
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210 In this case on normal control group because there was no administration caffeine and  
 211 test preparation, uric acid level just experience a slight fluctuation value, decrease on  
 212 day 9th about 4.66% and increase + 0,02 % on day 12th and increase again on day  
 213 15th. On negative control group because there was administration caffeine 27 mg/200  
 214 g b.w every day from first day until day 15th of experiment has caused uric aci level in  
 215 the blood increase and increase from day to day far beyond uric acid levels in the  
 216 normal control group, which reaches levels 3.85 mg/dL on the day 15th. While uric acid  
 217 level in the blood of normal control just about 1.50 mg/dL. Percentage (%) decrease  
 218 happened on day 15th with value for Positive Control, Low dose, Medium Dose, High  
 219 Dose (-)58.63%, (-)37.50%, (-)43.00% and (-)41.00% respectively.

220 As shown in Table 4 and Figure 2, Negative control. Group always increase from day  
 221 6th until day 15th, caused was induced with caffeine, while Positive Control, Low dose,  
 222 Medium Dose, High Dose always decrease from day 9th until day 15th, caused the  
 223 word of test preparation.

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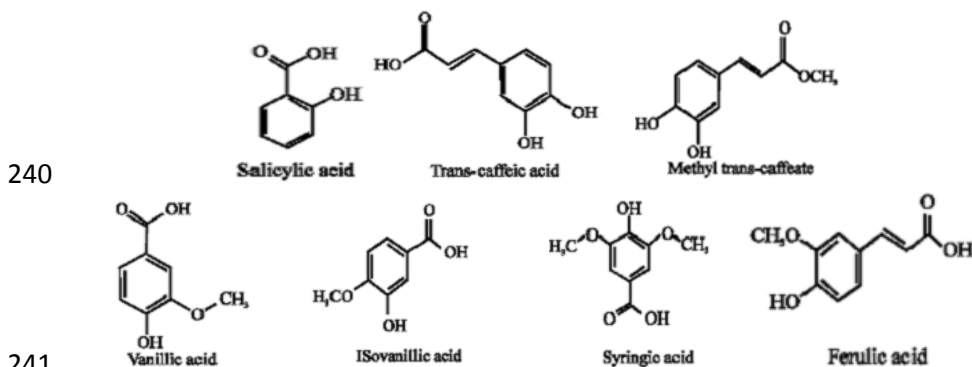
226  
227  
228  
229

**Figure 2.** % increase & decrease of uric acid after was induced with caffeine & administration of test preparations on days 9<sup>th</sup>, 12<sup>th</sup> & 15<sup>th</sup>. compared day 6<sup>th</sup>

230 Structure Activity Relationships (SAR) are relations between the molecular structure  
231 and biological or physicochemical activity of chemicals or in pharmacology, chemical  
232 compounds that have the same chemistry and differ in functional groups, will have the  
233 same properties but differ in potential efficacy (12, 13, 14, 15).

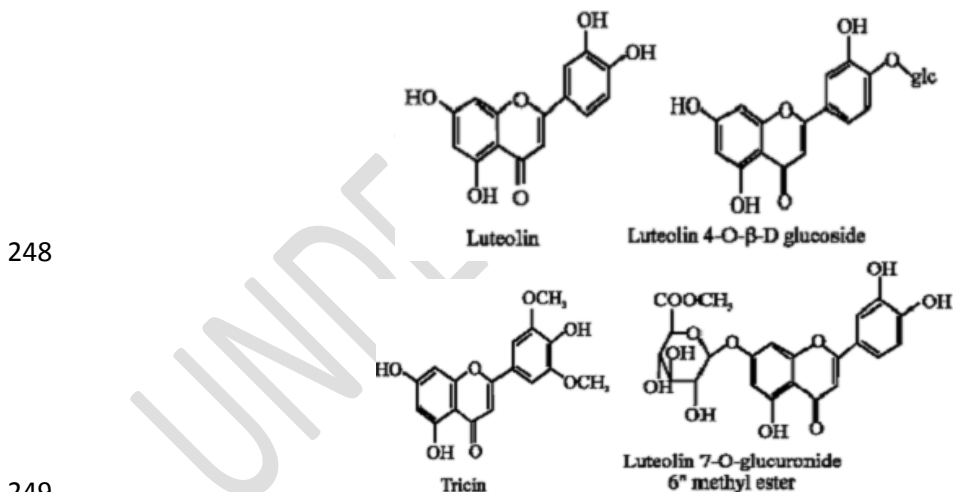
234 Chemical compound that work in decrease uric acid level in blood of rats, probably  
235 derived from Some phenolic acid and flavonoid compound. According to Kabeer and  
236 Prathapan (2014), Chang et al (2011) were obtained some chemical compound of  
237 phenolic acid and flavonoid compound in *E. scaber* as shown in Figure 3. and Figure 4,  
238 (5, 6). These chemical compounds have the same basic structure with chemical

239 compounds that are currently widely used to treat gout, as shown in Figure 5.

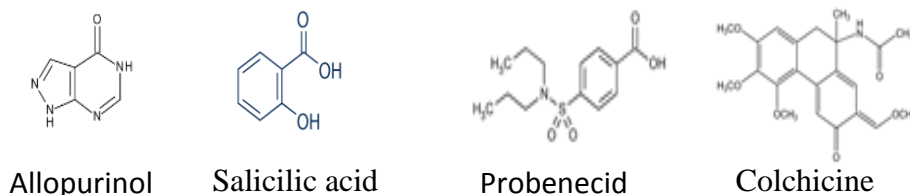


**Figure 3**, there are 7 chemical compounds in *E. scaber* that have basic structure similar with salicylic acid, namely salicylic acid, trans-caffeic acid, methyl trans cafeate, vanillic acid, iso-vanillic acid, syringic acid, ferrulic acid

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



Allopurinol

Salicylic acid

Probenecid

Colchicine

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

255

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

259 The results of this study are similar to the results of research by Jennie et al (2016),  
 260 where they used *E. scaber* powdered leaves extracted using methanol and distilled  
 261 water to obtain test extracts. The administration of the test preparation was carried out  
 262 by using a single subcutaneous injection of 0.1 ml Complete Freund Adjuvant emulsion  
 263 (CFA). Their experiments also found that *E. scaber* worked significantly in reducing  
 264 uric acid in gout (16, 17)

#### 265 4. CONCLUSIONS

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

269 There are about 7 of the salicylic acid derivatives and there are about 4 of the phenolic  
 270 acids and flavonoid derivatives in *E. scaber*, these chemical compounds are strongly  
 271 suspected have properties for decrease uric acid in the blood, because their basic

272 structure is very similar with drugs to decrease uric acid. Further research is needed, to  
273 find out what chemicals are better for treating gout in *E. Scaber*.

#### 274 **CONFLICTS OF INTEREST**

275 The authors declare no conflicts of interest.

276

#### 277 **COMPETING INTERESTS DISCLAIMER:**

278

279 Authors have declared that no competing interests exist. The products used for this  
280 research are commonly and predominantly use products in our area of research and  
281 country. There is absolutely no conflict of interest between the authors and producers  
282 of the products because we do not intend to use these products as an avenue for any  
283 litigation but for the advancement of knowledge. Also, the research was not funded by  
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285

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