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Phytochemicals and anti-hemorrhoidal activities of Tapak Liman (*Elephantopus Scaber*) leaves

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ABSTRACT

Hemorrhoids occur due to inflammation of the rectoanal venous plexus, which causes inflammation, pain, and can cause lumps. This inflammation may cause difficulty in defecation and may even cause heavy bleeding. Empirically, Tapak Liman (*Elephantopus scaber* L.) leaves have been used to treat hemorrhoids and have been known to contain flavonoids and terpenoid lactones which are active as anti-inflammatory agents. To determine the anti-hemorrhoidal activity of *E. scaber* leaves in reducing inflammation in hemorrhoids in Wistar rats, the research begins with the extraction of *E. scaber* leaves with 70% ethanol as solvent. Extracts were analyzed for total flavonoid content with quercetin standard. Anti-hemorrhoidal activity was measured based on the degree of edema formed based on the weight of the rectum through the surgical process. Hemorrhoid was induced with croton oil for 3 days on the rectoanal area in rats. The extracts were treated at concentration of 50, 100, and 200 mg/kgW for 7 days. The drug control group used oral diclofenac sodium for 7 days. *E. scaber* leaf ethanol extract contains alkaloids, flavonoids, saponins, and polyphenols. The flavonoid level was 3.433 mgQE/g extract. This study showed that *E. scaber* leaf extract had the best anti-hemorrhoidal activity at a dose of 100 mg/kgW of extract with a significance value of $p<0.05$. Also, the extract of *E. scaber* leaves containing flavonoids provides a fairly good anti-hemorrhoid activity compared to the control drug diclofenac sodium. It is suggested that this plant extract might be used as a new herbal anti-hemorrhoid drug.

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INTRODUCTION

Hemorrhoids are disorders that occur in the anorectal area. The pathophysiology of hemorrhoids is still unknown, possibly due to multiple factors such as; displacement of the anus pads, hemorrhoidal plexus hyperfusion, vascular abnormalities, tissue inflammation, and prolapse (lumps) on the internal or external rectal [1]. Hemorrhoids occur due to inflammation and widening of the hemorrhoidal venous plexus, and if the dilation is wider, bleeding occurs. Treatments often given to people with hemorrhoids are astringent, anti-inflammatory, and bioflavonoid supplements [2].

According to data from the Ministry of Health in 2018, it was estimated that 5.7% of the Indonesian population had hemorrhoids, but only 1.5% were diagnosed [3]. Empirically, the Indonesian people use the leaves of the *Elephantopus scaber* plant with the local name "Tapak Liman" as an alternative treatment to treat hemorrhoids and bleeding. In Indonesia, this plant is also used to cure anemia, sore throat, menstrual pain, cough, vaginal discharge, dysentery, chicken pox, and inflammation of the kidneys [1]. Recent research has shown that *E. scaber* leaves contain 4 sesquiterpene lactones and 5 flavones, which have been shown to have anti-inflammatory and hepatoprotective activity [4–6]. However, the efficacy of the *E. scaber*, especially for treating hemorrhoids, still must be

scientifically proven. Thus, this study aimed to determine the anti-hemorrhoid activity effects of *E. scaber* leaves during hemorrhoids and identifying the levels of flavonoid compounds contained as anti-hemorrhoid agents.

MATERIALS AND METHODS

Materials

The instruments used in this study were animal handling equipment, surgical equipment, rotary evaporator (Buchi® Switzerland), and ultraviolet-visible spectrophotometer (Genesys 105®). The materials used in this study consisted of aluminum chloride (Merck®), acetic acid glacial (Merck®), hydrochloric acid (Merck®), sulfuric acid (Merck® Germany), acetone (Merck®), croton oil (Sigma Aldrich®), ethanol p.a (Merck®), ferric chloride (Merck®), 10% formaldehyde, ketamine HCl (KTM-100®), quercetin (Sigma Aldrich® Germany), NaCMC (Sigma Aldrich®), diclofenac sodium 5mg/KgW (Sigma Aldrich®), Dragendrof reagent, Meyer's reagent, Wagner's reagent, pyridine (Merck®), $AlCl_3$ (Merck®), potassium acetate (Merck®), and magnesium (Merck®).

Sample preparation

E. scaber leaf samples were obtained in Samarinda City, East Kalimantan, Indonesia. This plant has been determined at the Dendrology and Forest Ecology Laboratory, Faculty of Forestry, Mulawarman University (No.122/UN17.4.08/LL/2021). The fresh leaves were weighed, washed, sorted, and dried in the simplicia oven at 50°C for 3 h to become dry. Dried leaves were cut into small pieces until ready to be extracted.

Extraction

E. scaber leaves were weighed as much as 200 grams and extracted using the maceration method using 2 L, 70% ethanol (3×24 h immersion). The filtrate was filtered using filter paper and then concentrated using a rotary evaporator at a temperature of 50°C. The viscous extract was then evaporated in a desiccator to obtain a dry extract.

Phytochemical screening

5 grams of *E. scaber* extract was dissolved in 50 mL of ethanol. For alkaloids test, 2 mL of extract solution was added with 5 mL of 2N HCl and divided into 3 test tubes. Wagner's reagent was added to the first tube, then Dragendorff's reagent was added to the second tube, and Mayer's reagent was added to the third tube. For flavonoid test, 2 mL of extract solution was added with Mg powder, and then concentrated HCl was added. For the saponin test, 2 mL of extract solution is added to 2 mL of hot water, then shaken for 5 minutes until foam is formed. Stable foam will continue to be seen for 5 minutes and does not disappear at adding one drop of 2 N hydrochloric acids [7]. For polyphenol test, take 2 mL of extract solution, then added 1% ferric chloride solution [8]. Measurement of total flavonoid content was done using quercetin standard. Preparation of a standard solution of 25mg quercetin with 25 mL (1000 ppm). Then dilute 40, 60, 80, 100, and 120 ppm in 10 mL of ethanol p.a. Mix 1.0 mL of standard solution, 0.2 mL of 10% aluminum chloride, 0.2 mL, 1M acetate acid with 5.6 mL of distilled water and mixed properly. After being incubated for 30 minutes in a light-protected place, it was measured at 300-600 nm. At a concentration of 80 ppm standard solution, the maximum wavelength was determined in triples. The concentration series of the standard solution was measured for absorption

at the maximum wavelength so that the standard curve value of quarcetin was obtained. Analysis of the flavonoid content of the ethanol extract of *E. scaber* leaves began with the preparation of an extract solution of 1000 ppm in ethanol. Similarly, as described above, 1.0 mL of the ethanol extract solution was reacted with aluminum chloride and acetic acid. The flavonoid content in the extract was calculated as the equivalent quercetin content [9].

Animal experiments

Rats with male Wistar strain were selected for as many as 24 tails aged 10-12 weeks and weighing 200-300 grams. Rats were kept in a laboratory with a standard animal handling procedures. This research work has been ethically approved by the Health Research Ethics Committee of the Faculty of Pharmacy, Mulawarman University (No.83/KEPK-FFUNMUL/EC/EXE/12/2021). Rats were given food and water ad libitum. Rats were randomly divided into six groups: normal control group, negative control group (crotone oil induction only), positive control group, and 3 test groups (50, 100, and 200 mg/KgW) [10].

Hemorrhoid induction

Induction of hemorrhoids in rats using 6% Croton oil solution by mixing distilled water, pyridine, acetone, and Croton oil (1:4:5:10). Before being induced by Croton oil, rats fasted overnight. Induction was given into the rectum (rectoanal section, about 20 mm from the anal canal) as much as 100 µl. Croton oil induction was carried out for three consecutive days, then rectal inflammation was observed after 8 h [11].

Anti-hemorrhoid test

On day 4, each rat of the test group was given a dose orally for 7 days. On day 11, all animals were anesthetized using ketamine HCl 10 mg/KgW intramuscularly. The rats were weighed and the rectum (1 cm) was surgically collected. The rectal organs were weighed and analyzed for changes in weight in the control and test groups. The value of the rectoanal coefficient (RAC) of rats was calculated using the following equation as described previously [12,13]:

$$Rectoanal\ Coefficient\ (RAC) = \frac{Weight\ of\ rectoanal\ tissue\ (mg)}{Body\ weight\ (g)}$$

Statistical analysis

The research design used Post Test Randomized Controlled Group Design. Normality tests were analyzed using Shapiro-Wilk. Intergroup variability was analyzed using one-way ANOVA. Statistical analysis of hemorrhoidal activity using SPSS® version 23rd with a significance level of $p<0.05$. Advanced statistical analysis used Tukey's HSD test to compare the anti-hemorrhoidal activity with the control drug diclofenac sodium.

RESULTS

Extraction and phytochemical screening of *E. scaber* leaves

E. scaber leaf extract obtained 24.08 grams (12.04% yield) against the weight of simplicia. The extracts were tested for phytochemicals by observing the reaction of the color change. Based on the results of phytochemical testing, it can be seen that *E. scaber* leaves contain alkaloids, flavonoids, saponins, and polyphenols. The results of the phytochemical screening test can be seen in Table 1.

Table 1. Results of phytochemical screening of *E. scaber* leaf extract.

No.	Method	Parameter	Result
1.	Alkaloid:		
	a) Wagner	Brown precipitate [7]	(+) Brown precipitate
	b) Dragendorf	Orange-red precipitate [7]	(+) Red precipitate
	c) Mayer	White to yellowish precipitate [7]	(+)Yellow precipitate
2.	Flavonoid	Red, yellow, or orange [7]	(+) Yellow
3.	Saponin	Stable foam [7]	(+)Stable foam
4.	Polifenol	Dark blue/green [8]	(+) Greenish blue
5.	Flavonoid content	Absorbance (+$AlCl_3$) [9]	3.433 mgQE/g±0.075

Description (+): Detected

The result of scanning the maximum wavelength of quercetin is 440 nm. The standard curve is very good, with a linearity value of R2 = 0.989. The graph of the standard curve for quercetin can be seen in Figure 1. The total flavonoid content of the ethanolic extract of *E. scaber* leaves was calculated based on the absorbance value using standard linear regression equations. The flavonoid content obtained was 3.433 mgQE/g (Table 1).

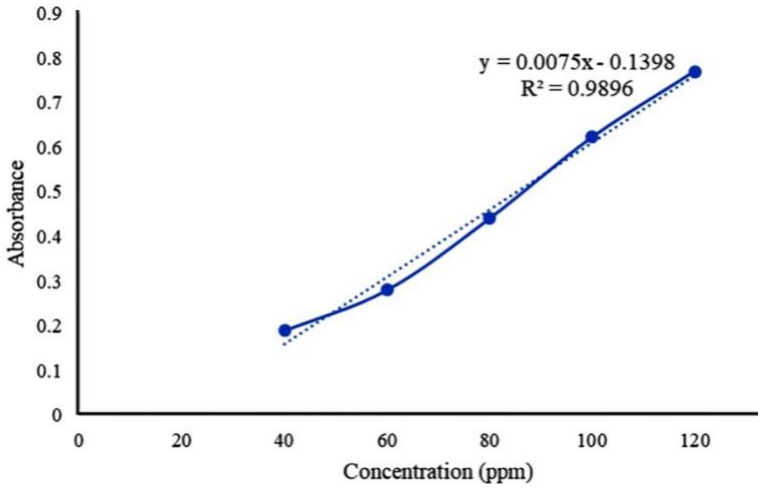

Figure 1. Standard curve for quercetin.

Anti-hemorrhoidal activity of *E. scaber* leaves ethanol extracts

Hemorrhoids in the rectum were successfully formed after the administration of croton oil (Figure 2). The mechanism of this inducing agent in causing inflammation is through the activation of protein kinase C. Observation of hemorrhoids shows the various shape of rectum in rats. Figure 3 showed that the whole area of the rectal tissue contained

edema. Consistently, common symptoms in hemorrhoids are tissue prolapse and mucus discharge accompanied by bleeding [14].

Figure 2. The rectum has hemorrhoids after induction with croton oil on day 1 to day 3.

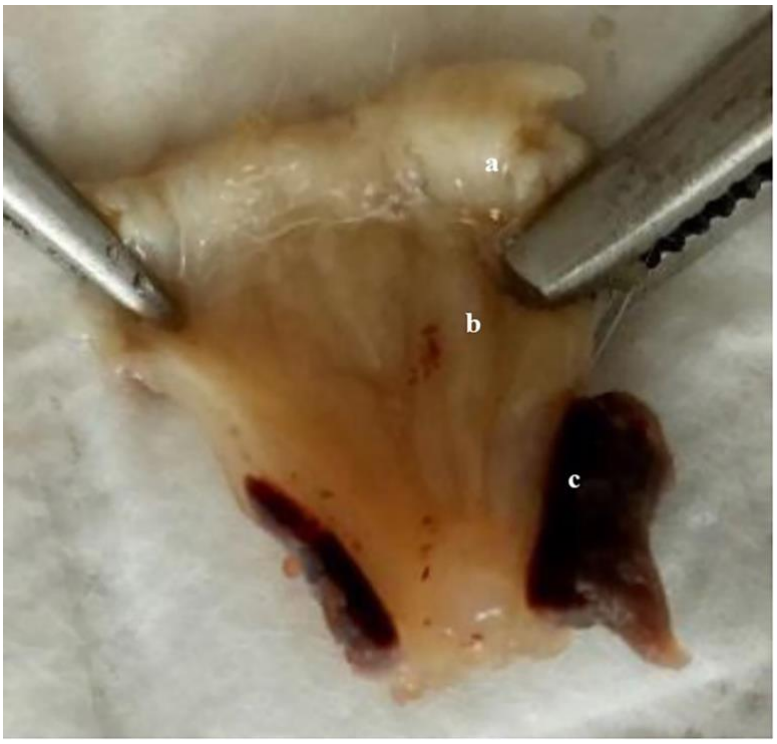

Figure 3. Rectum with hemorrhoids: (a) External hemorrhoidal tissue; (b) Anal columns of morgagni; and (c) Internal hemorrhoidal tissue.

Figure 4 is showing the rectal weight of the experimental groups. Based on the rectal weight, croton oil (CO) treatment increased edema in rats. Interestingly, it was found that *E. scaber* leaf extract reduced the degree of edema in CO-treated rats.

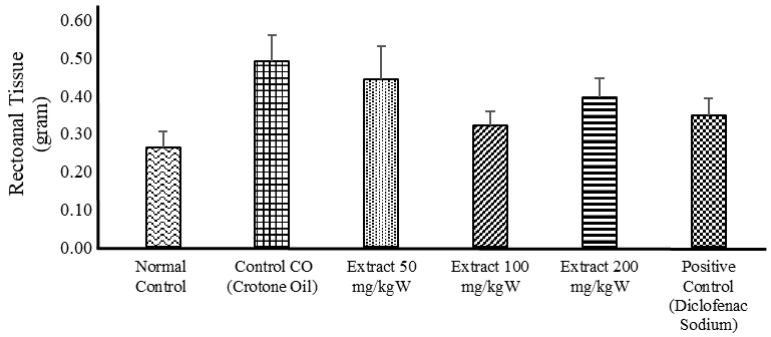

Figure 4. Rectal weight after treating the *E. scaber* leaf extracts in rats for reducing edema.

The rectoanal coefficient (RAC) was used to determine the activity of anti-hemorrhoids on the rectoanal tissue organs based on the body weight of the rats. Figure 5 shows the potency of *E. scaber* extract where the extract concentration of 100 mg/KgW had the best RAC value in reducing the degree of edema. This result was better than the positive control group of 5mg/KgW diclofenac sodium which is almost close to the value of the normal control group (Figure 5).

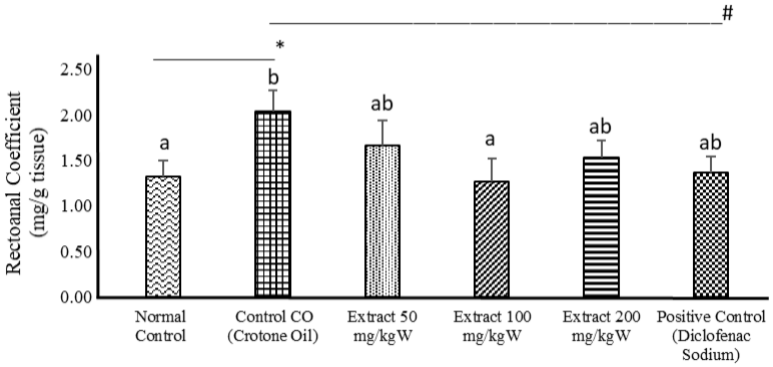

Figure 5. Rectoanal coefficient (RAC) in rats. * Indicates significance between control Croton oil (CO) and normal control; and # indicates that 100 mg/kgW *E. scaber* leaf extract has significantly anti-hemorrhoidal potential compared to 50, 100 mg/kgW extract and 5mg/KgW diclofenac sodium control.

DISCUSSION

Based on the phytochemical screening, *E. scaber* extract contains flavonoid compounds that play an essential role as anti-inflammatory agents. Flavonoids inhibit the COX-2 enzyme, which plays a role in the metabolism of inflammatory prostaglandins [15]. The level of flavonoid compounds is essential to predict its activity as an anti-inflammatory agent. Quantitative determination of total flavonoid content was carried out using UV-Visible spectrophotometry. In this study, aluminum chloride was used as a stable complex with flavonoid compounds to produce a yellow color. The color formed is due to a shift in wavelength [9,16].

The mechanism of this inducing agent in causing inflammation is through the activation of protein kinase C. This process affects the activity of the phospholipase A2 enzyme. Common symptoms in hemorrhoids are tissue prolapse and mucus discharge accompanied by bleeding [14]. This enzyme secretes the inflammatory mediator arachidonic acid (prostaglandins, leukotrienes, TNF, nitric acid, and bradykinin). In some test rat, the level of hemorrhoids causes heavy bleeding when passing feces. The

surgical process also confirms the process of observing hemorrhoids. Before rectal observation, the organs were given 10% formaldehyde to keep cells and tissue components in a "life-like state." This situation will prevent the degenerative process caused by the loss of blood supply to the tissue [12,17]. Based on ANOVA statistical analysis, the value of rectal weight between all test groups and the control group was significantly different ($p<0.05$). The difference in values between the groups could mean that the use of *E. scaber* leaf extract had an effect on rectal weight. The statistical advance test using the Tukey's HSD test (Figure 5), RAC obtained in the test group, *E. scaber* leaf extract with a concentration of 100 mg/kgW had the significant ($p<0.05$) best value in reducing the degree of edema. When compared with the positive control diclofenac sodium 5mg/KgW, the hemorrhoidal activity was not significant. This proves that the power can match the drug control, and the edema disappears as in the control crotone oil.

Some treatments against hemorrhoid use bioflavonoids as anti-inflammatory agents. These bioflavonoid compounds can also inhibit the release of prostaglandins as inflammatory mediators so that the edema formed will be reduced. Meta-analysis studies on flavonoids have been shown to prevent hemorrhoid symptoms. In another study, *E. scaber* ethanol extract was shown to have anti-inflammatory activity in stabilizing human red blood cell (HRBC) membranes by heat-induced hemolysis [15,18,19].

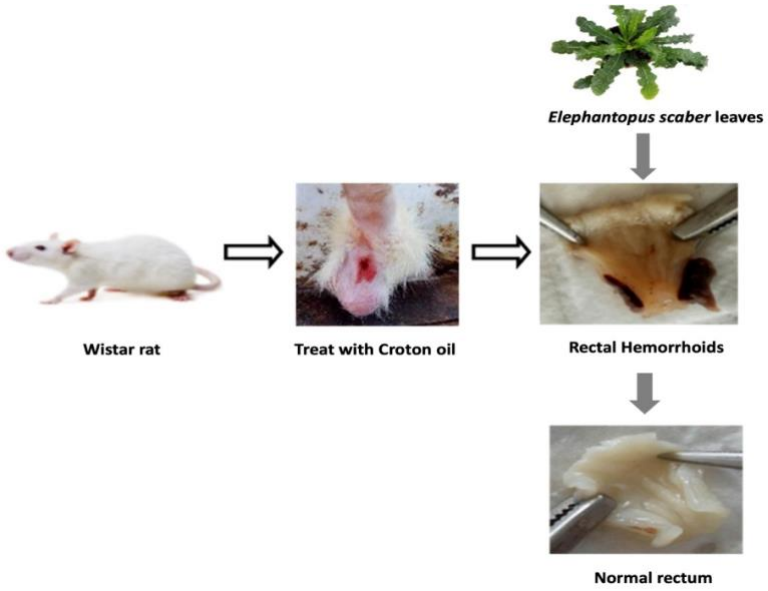

Figure 6. Anti-hemorrhoidal activities of Tapak liman leaves. This study showed that *E. scaber* leaf extract had the best anti-hemorrhoidal activity at a dose of 100 mg/kgW.

CONCLUSION

Phytochemical test results of ethanol extract of Tapak Liman *(E. scaber)* leaves contain flavonoid metabolites as anti-inflammatory agents with a total level of 3.433mgQE/g. The results of the anti-hemorrhoidal test showed that *E. scaber* leaf extract had better anti-hemorrhoidal activity at a concentration at 100 mg/kgW which was equivalent to activity of 5 mg/KgW diclofenac sodium (Figure 6). Considering all above, this plant extract needs to be clinically tested to support the opportunity to find new herbal anti-hemorrhoid drugs.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit it to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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