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In vitro anti-inflammatory activity of *Elephantopus scaber* leaf tea through human red blood cell membrane stabilization

Aktivitas Anti Inflamasi Teh Daun Tapak Liman (Elephantopus scaber L.) Metode Stabilisasi Membran Sel Darah Merah

Wahdaniah Wahdaniah[1*], Gervacia Jenny Ratnawaty[1], Linda Triana[1], Bagus Muhammad Ihsan[1]

[1]Department of Medical Laboratory Technology, Poltekkes Kemenkes Pontianak, Pontianak, Indonesia

*Email: wahdasabolakna@gmail.com

ABSTRACT

Background: *Inflammation is a physiological response to tissue injury, infection, or irritation. The long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) may cause adverse effects, prompting the search for safer natural alternatives. Tapak liman (Elephantopus scaber L.) leaves contain flavonoids, saponins, tannins, and steroids with potential anti-inflammatory properties.*

Objective: *To evaluate the in vitro anti-inflammatory activity of E. scaber leaf tea using the red blood cell (RBC) membrane stabilization method.*

Methods: *A laboratory experimental study with a post-test-only control group design was conducted. Anti-inflammatory activity was assessed based on the inhibition of hypotonic solution-induced erythrocyte hemolysis. Tapak liman leaf tea was prepared by steeping dried leaf powder in hot distilled water. RBC suspensions at concentrations of 0.25%, 0.5%, and 1% were used. Sodium diclofenac (0.1%) served as the positive control and distilled water as the negative control. Hemolysis was measured spectrophotometrically at 560 nm.*

Results: *Tapak liman leaf tea inhibited erythrocyte hemolysis in a concentration-dependent manner. The highest membrane stabilization activity approached that of sodium diclofenac. All tested concentrations showed significantly greater membrane stabilization than the negative control ($p<0.05$).*

Conclusion: *Elephantopus scaber leaf tea prepared as a hot-water infusion exhibits significant in vitro anti-inflammatory activity through erythrocyte membrane stabilization and has potential for further development as a natural phytotherapeutic agent.*

Keywords: *anti-inflammatory activity, erythrocyte membrane, flavonoid, herbal tea, tapak liman*

ABSTRAK

Latar Belakang: Inflamasi merupakan respons fisiologis terhadap cedera jaringan, infeksi, atau iritasi. Penggunaan jangka panjang obat antiinflamasi nonsteroid (OAINS) dapat menimbulkan berbagai efek samping sehingga diperlukan alternatif alami yang lebih aman. Daun tapak liman (Elephantopus scaber L.) mengandung flavonoid, saponin, tanin, dan steroid yang berpotensi memiliki aktivitas antiinflamasi.

Tujuan: Mengevaluasi aktivitas antiinflamasi in vitro teh daun tapak liman menggunakan metode stabilisasi membran sel darah merah (eritrosit).

Metode: Penelitian eksperimental laboratorium dengan desain post-test only control group dilakukan menggunakan metode penghambatan hemolisis eritrosit yang diinduksi larutan hipotonik. Teh daun tapak liman dibuat dengan menyeduh serbuk daun kering menggunakan air panas. Suspensi eritrosit dengan konsentrasi 0,25%, 0,5%, dan 1% digunakan dalam pengujian. Natrium diklofenak 0,1% digunakan sebagai kontrol positif

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dan akuades sebagai kontrol negatif. Hemolisis diukur secara spektrofotometri pada panjang gelombang 560 nm.
Hasil: Teh daun tapak liman mampu menghambat hemolisis eritrosit secara bergantung konsentrasi. Aktivitas stabilisasi membran tertinggi mendekati efek natrium diklofenak. Seluruh konsentrasi uji menunjukkan stabilisasi membran yang secara signifikan lebih tinggi dibandingkan kontrol negatif ($p<0,05$).
Kesimpulan: Teh daun tapak liman (Elephantopus scaber L.) yang disiapkan sebagai seduhan air panas memiliki aktivitas antiinflamasi in vitro yang signifikan melalui mekanisme stabilisasi membran eritrosit dan berpotensi dikembangkan sebagai agen fitoterapi alami.

Kata kunci: aktivitas antiinflamasi; stabilisasi membran eritrosit; Elephantopus scaber; tapak liman; teh herbal

INTRODUCTION

Inflammation is a complex biological response of body tissues to harmful stimuli such as pathogens, damaged cells, or irritants. It acts as a protective mechanism aimed at eliminating the initial cause of cell injury and initiating tissue repair. However, uncontrolled or prolonged inflammation may result in tissue damage and chronic diseases such as arthritis, cardiovascular disorders, and cancer[1], [2], [3]. Synthetic anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) are widely used but their long-term administration is often associated with adverse effects, including gastrointestinal irritation, renal impairment, and cardiovascular complications[4]. Therefore, natural products with anti-inflammatory potential have become an attractive alternative due to their safety, affordability, and biocompatibility.

Elephantopus scaber L., commonly known as tapak liman, is a medicinal herb traditionally used in Indonesia and other Asian countries for treating fever, wounds, and inflammation. Phytochemical studies have shown that E. scaber L. contains bioactive compounds such as flavonoids, tannins, saponins, phenolic acids, and triterpenoids that contribute to its pharmacological activities, including antioxidant, antimicrobial, and anti-inflammatory effects [5], [6]. Recent studies have demonstrated that polysaccharides and phenolic compounds isolated from E. scaber can inhibit the production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and suppress nitric oxide formation in lipopolysaccharide (LPS)-stimulated macrophage cells[7], [8]. The flavonoid and triterpenoid constituents of E. scaber are thought to contribute to membrane stabilization and protection against oxidative damage in erythrocytes, which may be associated with the observed anti-inflammatory activity[9], [10].

The red blood cell (RBC) membrane stabilization assay is a well-established in vitro method for screening the anti-inflammatory activity of natural compounds. This assay evaluates the ability of a test sample to prevent erythrocyte hemolysis induced by hypotonic or heat stress. The RBC membrane is considered analogous to the lysosomal membrane; therefore, its stabilization is used as a surrogate model for lysosomal membrane stabilization during inflammation. Stabilization of the lysosomal membrane may inhibit the release of proteases, hydrolases, and other inflammatory mediators, thereby contributing to the attenuation of the inflammatory response[11], [12]. Stabilization of the lysosomal membrane prevents the release of inflammatory mediators such as proteases and hydrolases, thereby reducing the inflammatory response[13], [14].

Given the growing demand for safe, natural, and affordable anti-inflammatory agents, herbal teas prepared from E. scaber L. offer promising potential as functional beverages

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for health maintenance. However, only limited research has investigated the anti-inflammatory mechanisms of tapak liman in tea formulation. Therefore, this study aims to evaluate the anti-inflammatory activity of tapak liman leaf tea (Elephantopus scaber L.) using the red blood cell membrane stabilization method. The findings are expected to provide scientific evidence supporting the use of E. scaber L. tea as a natural anti-inflammatory agent and contribute to the development of herbal-based therapeutic alternatives.

METHODS

Study design

This study employed an experimental laboratory design using a post-test only control group approach. The anti-inflammatory activity of Elephantopus scaber L. leaf tea was evaluated using the red blood cell (RBC) membrane stabilization assay, which serves as an in vitro surrogate model of lysosomal membrane stabilization during inflammation[15].

The main experimental variable in this study was the infusion time of the tea preparation, specifically 5 minutes and 10 minutes, to assess its effect on erythrocyte membrane stabilization activity.

Data source and sampling prosedures

Blood samples were obtained from three (n = 3) healthy adult volunteers who met the inclusion criteria and provided written informed consent prior to participation. Approximately 5 mL of venous blood was collected from each donor, resulting in a total volume of 15 mL for the preparation of erythrocyte suspensions. Venous blood was collected into Alsever's solution (1:1, v/v) and processed to obtain erythrocyte suspensions. For each experimental condition, assays were performed in triplicate as technical replicates using erythrocyte suspensions prepared from the pooled blood samples of all donors to ensure data reliability and reproducibility. All laboratory procedures were conducted in the Hematology and Phytochemistry Laboratories of Poltekkes Kemenkes Pontianak from March to May 2025.

Variable of the study

The independent variables were the infusion time of *Elephantopus scaber* L. leaf tea (5 and 10 minutes) and the erythrocyte suspension concentration used in the membrane stabilization assay (0.25%, 0.5%, and 1%). The dependent variable was the percentage of erythrocyte membrane stabilization, expressed as inhibition of hemolysis. Control variables included the infusion temperature (90°C), incubation temperature (37°C), incubation time (30 minutes), hypotonic saline composition, and assay conditions. Sodium diclofenac (0.1%) was used as the positive control, while distilled water served as the negative control.

Data collection

Preparation of E. scaber Leaf Tea

Tea infusion was prepared separately from the RBC preparation. Dried leaves of E. scaber L. were oven-dried at 40°C, ground into fine powder, and used for infusion. One gram of dried leaf powder was infused in 200 mL of distilled water at 90°C for 5 and 10 minutes, filtered, and allowed to cool to room temperature. The resulting infusion was used directly as the test solution without further dilution.

Preparation of Erythrocyte Suspension

Venous blood was collected into Alsever's solution (1:1, v/v) and centrifuged at 3,000 rpm for 10 minutes. The packed erythrocytes were washed three times with isotonic saline to remove plasma and leukocytes. A 10% (v/v) erythrocyte stock suspension was prepared and further diluted with isotonic saline to obtain working

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RBC suspensions at concentrations of 0.25%, 0.5%, and 1% (v/v), which were used in the membrane stabilization assay.

RBC Membrane Stabilization Assay

For each test, 0.5 mL of RBC suspension (0.25%, 0.5%, or 1%) was mixed with 0.5 mL of E. scaber leaf tea infusion and 2.5 mL of hypotonic saline solution. Sodium diclofenac (0.1%) was used as the positive control, while distilled water served as the negative control. The mixtures were incubated at 37°C for 30 minutes and subsequently centrifuged at 3,000 rpm for 10 minutes. The absorbance of the supernatant was measured at 560 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800) to determine hemoglobin release due to hemolysis.

The percentage of membrane stabilization was calculated using the following formula:

$$\text{Percent inhibition of hemolysis} = \left(1 - \frac{A_1}{A_0}\right) \times 100$$

where A_1 represents the absorbance of the test sample and A_0 represents the absorbance of the negative control.

Measurement and instrument

Phytochemical Screening

Preliminary qualitative phytochemical screening of the tea infusion was performed to identify the presence of major secondary metabolites, including flavonoids, tannins, saponins, phenolics, and triterpenoids, using specific qualitative tests with standard reagents.

Flavonoids were detected using the Shinoda test (magnesium powder and concentrated hydrochloric acid), indicated by the formation of a red to pink coloration. Tannins and phenolic compounds were identified using the ferric chloride ($FeCl_3$) test, indicated by the appearance of a dark blue or greenish-black coloration. Saponins were detected using the frothing test, indicated by the formation of stable persistent foam after vigorous shaking. Triterpenoids and steroids were detected using the Liebermann–Burchard test (acetic anhydride and concentrated sulfuric acid), indicated by the formation of a green or reddish-brown coloration[16].

Ethical considerations

The study protocol was approved by the Health Research Ethics Committee of Poltekkes Kemenkes Pontianak (Ethical Clearance No: 301/KEPK-PK.PKP/V/2025). All blood donors provided written informed consent prior to participation, and all procedures were conducted in accordance with the Declaration of Helsinki and institutional biosafety guidelines.

Data analysis

Data from triplicate measurements were expressed as mean ± standard deviation (SD). Prior to inferential analysis, the assumptions of normality and homogeneity of variance were evaluated using the Shapiro–Wilk test and Levene's test, respectively. Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to assess differences among groups. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 26.0 (IBM Corp., USA).

RESULTS

The anti-inflammatory activity of Elephantopus scaber L. tea was evaluated using the red blood cell (RBC) membrane stabilization method. The absorbance of each sample was measured at a wavelength of 560 nm, and the percentage of membrane stabilization

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was calculated by comparing the absorbance of the test groups to that of the control. The results are summarized in Table 1.

Table 1. Effect of Tapak Liman (Elephantopus scaber L.) Tea Infusion on RBC Membrane Stabilization

Treatment Group	Mean Absorbance (560 nm) ± SD	% Membrane Stabilization ± SD
Negative control (distilled water)	0.652 ± 0.015	—
Positive control (sodium diclofenac 0.1%)	0.317 ± 0.012	51.38 ± 1.82
Tea infusion 5 min	0.441 ± 0.020	32.35 ± 2.14
Tea infusion 10 min	0.392 ± 0.018	39.87 ± 1.95

Values are presented as mean ± standard deviation (SD) (n = 3 donors, each measured in triplicate).

Table 1 shows that membrane stabilization increased with longer infusion time, from 32.35 ± 2.14% at 5 minutes to 39.87 ± 1.95% at 10 minutes, suggesting enhanced extraction of anti-inflammatory compounds. One-way ANOVA demonstrated a significant difference among groups ($p < 0.05$). Post hoc analysis showed that both infusion groups had significantly higher membrane stabilization than the negative control ($p < 0.05$). The 10-minute infusion was not significantly different from sodium diclofenac ($p > 0.05$), whereas the 5-minute infusion showed significantly lower stabilization than diclofenac ($p < 0.05$). In addition, the 10-minute infusion had significantly greater membrane stabilization than the 5-minute infusion ($p < 0.05$). This effect may be related to the higher extraction of water-soluble bioactive compounds. Supporting this finding, phytochemical screening of the infusion (Table 2) confirmed the presence of flavonoids, tannins, saponins, and phenolics, which are known to contribute to anti-inflammatory activity.

Table 2. Phytochemical Constituents of Tapak Liman (Elephantopus scaber L.) Tea Infusion

Phytochemical Component	Test Result
Flavonoids	+ (present)
Tannins	+ (present)
Saponins	+ (present)
Phenolic compounds	+ (present)
Steroids/Triterpenoids	+ (present)

The presence of these bioactive compounds may be associated with the membrane-stabilizing and anti-inflammatory activities observed in this study. Flavonoids and phenolic compounds have been reported to protect erythrocyte lipid bilayers from oxidative damage and may contribute to the stabilization of lysosomal membranes, thereby potentially limiting the release of inflammatory mediators..

DISCUSSION

The present study demonstrated that Elephantopus scaber L. (tapak liman) leaf tea exhibits significant anti-inflammatory activity, as evidenced by its ability to stabilize the red blood cell (RBC) membrane under hypotonic conditions. An increase in membrane stabilization was observed with longer infusion time, indicating more efficient extraction of water-soluble bioactive compounds that contribute to membrane protection against hemolysis. This finding suggests a positive relationship between infusion duration and the availability of active constituents in the tea preparation.

The RBC membrane stabilization assay is a well-established in vitro model for preliminary screening of anti-inflammatory activity. Because the erythrocyte membrane is structurally analogous to the lysosomal membrane, agents that inhibit hypotonic-

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induced RBC lysis are considered capable of stabilizing lysosomal membranes during inflammatory processes. Such stabilization may reduce the release of inflammatory enzymes, including proteases and phospholipases, thereby attenuating tissue damage and amplification of inflammation[11], [17]. In this study, the 10-minute infusion showed membrane-stabilizing activity that was not significantly different from sodium diclofenac, although its mean percentage of stabilization was lower. This finding suggests meaningful biological activity without implying equivalence to the standard anti-inflammatory drug.

The observed anti-inflammatory effects of E. scaber L. tea are likely associated with its phytochemical constituents, particularly flavonoids, tannins, phenolic compounds, and saponins. These secondary metabolites have been widely reported to exhibit antioxidant and anti-inflammatory properties by scavenging reactive oxygen species, reducing lipid peroxidation, and modulating the production of pro-inflammatory mediators such as prostaglandins, nitric oxide, TNF-α, and IL-6 [10], [18], [19]. Flavonoids and phenolic compounds are thought to contribute to membrane stabilization and protection against oxidative damage in erythrocytes, which may support the anti-inflammatory effects observed in this study. Additionally, saponins may enhance membrane resistance to lytic stress through interactions with membrane components[20], [21].

The findings of this study are consistent with previous reports demonstrating the anti-inflammatory potential of E. scaber L. Wong and Natesan (2019) reported strong antioxidant and anti-inflammatory activities of E. scaber extracts attributed to their high phenolic and flavonoid content[22]. Similarly, Wang et al. (2024) Similarly, Wang et al. (2024) showed that polysaccharides derived from E. scaber significantly reduced systemic inflammation in animal models by modulating cytokine production and enhancing antioxidant defenses[23]. These studies support the present results and suggest that water-soluble constituents extracted during tea preparation play an important role in the observed membrane-stabilizing activity.

In addition, the membrane stabilization approach provides a safe and cost-effective preliminary model for evaluating the anti-inflammatory potential of herbal preparations. Unlike conventional NSAIDs, which can cause gastric irritation and renal impairment due to cyclooxygenase inhibition, natural plant-based agents like *E. scaber* L. achieve inflammation reduction through physicochemical stabilization and antioxidant pathways[24]. This characteristic highlights the potential of tapak liman tea as a functional herbal beverage with fewer side effects compared to synthetic anti-inflammatory drugs.

One of the strengths of this study lies in the application of the RBC membrane stabilization assay as a simple, reproducible, and cost-effective in vitro screening method with translational relevance. The use of E. scaber L. in tea formulation further enhances the practical significance of this study, as it reflects a culturally accepted and accessible mode of herbal consumption. Together with phytochemical screening, this approach provides supportive evidence linking the presence of bioactive compounds to membrane stabilization and anti-inflammatory activity.

One of the main strengths of this study is the application of the red blood cell (RBC) membrane stabilization assay as a simple, reproducible, and cost-effective in vitro method for preliminary screening of anti-inflammatory activity. This assay is widely accepted because the erythrocyte membrane closely resembles the lysosomal membrane, and stabilization of RBCs under hypotonic conditions reflects the ability of a substance to prevent lysosomal membrane disruption during inflammation[11], [25]. In

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addition, the use of Elephantopus scaber L. in tea formulation enhances the translational relevance of this study, as it represents a practical and culturally accepted mode of herbal consumption. The inclusion of phytochemical screening further strengthens the findings by supporting the mechanistic role of flavonoids, tannins, saponins, and phenolic compounds in membrane stabilization and anti-inflammatory activity [10], [18].

Nevertheless, several limitations should be acknowledged. The anti-inflammatory activity observed was evaluated exclusively using an in vitro model, which cannot fully represent the complexity of inflammatory responses in vivo. Inflammatory processes involve multiple cell types, signaling pathways, and immune mediators that extend beyond erythrocyte membrane stabilization alone[26], [27]. Furthermore, this study did not quantify individual bioactive compounds or measure specific inflammatory biomarkers, and the use of limited laboratory-scale replication may affect the generalizability of the findings. Despite these limitations, the results provide scientific support for the traditional use of E. scaber L. as an anti-inflammatory herbal remedy. The demonstrated membrane-stabilizing activity suggests that tapak liman tea may serve as a safe, affordable, and natural complementary agent for inflammation management. Future studies are warranted to perform quantitative phytochemical analyses, evaluate dose–response relationships, and confirm efficacy through in vivo models and clinical trials to strengthen its development as an evidence-based functional herbal beverage[30], [31].

CONCLUSION

The present study demonstrated in vitro that Elephantopus scaber L. (tapak liman) leaf tea exhibits significant anti-inflammatory activity through stabilization of red blood cell membranes under hypotonic conditions. The 10-minute infusion showed the highest membrane-stabilizing effect and did not differ significantly from sodium diclofenac, although the mean stabilization percentage remained lower, indicating that the observed activity is associated with longer infusion time rather than differences in erythrocyte concentration. Phytochemical screening confirmed the presence of flavonoids, tannins, saponins, and phenolic compounds, which are thought to contribute to membrane stabilization and protection against inflammatory damage. These findings suggest that E. scaber L. leaf tea has the potential to be developed as a natural and affordable anti-inflammatory herbal product with a favorable safety profile to be further evaluated. However, further studies involving quantitative phytochemical analysis, in vivo models, toxicity assessment, and clinical trials are necessary to confirm its efficacy and safety for human use.

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