

In vitro anti-inflammatory activities of ethanolic extract *Elephantopus scaber* leaves

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ABSTRACT

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Background: Inflammation is a protective physiological response to tissue injury that can be caused by harmful stimuli. If the inflammatory process is prolonged and cannot restore to homeostatic conditions, this may lead to pathological effects that can damage cells and cause various diseases. *Elephantopus scaber* is a plant that can easily be found in Indonesia. *Elephantopus scaber* is a type of plant that is often used as a traditional medicines. Several studies have shown that the compound bioactive content contained in plants has enormous potential as alternative medicine.

Objective: This present study was to investigate the anti-inflammatory activity of ethanolic extract of *Elephantopus scaber* leaves.

Methods: The *Elephantopus scaber* leaves were extracted using ethanol solvent into different concentration (50 mg/mL, 100 mg/mL and 120 mg/mL). Diclofenac sodium was used as the standard. Anti-inflammatory assays were performed by the human red blood cell (HRBC) membrane stabilization method and heat-induced hemolysis method. Phytochemical screening that used in the present study was a conventional method.

Results: Phytochemical screening showed the presence of flavonoids, tannins and saponins. In the present study, ethanolic extract of *Elephantopus scaber* leaves has anti inflammatory activity by protecting the stability of red blood cell membrane. The highest protection capability possessed by the ethanolic extract of *Elephantopus scaber* leaves in both human red blood cell (HRBC) membrane stabilization method and heat-induced hemolysis method was at a concentration of 100 mg/mL.

Conclusion: The ethanolic extract of *Elephantopus scaber* has anti-inflammatory activities by in vitro assays.

Latar Belakang: Inflamasi merupakan respon protektif fisiologi terhadap cedera pada jaringan yang dapat disebabkan oleh stimulus berbahaya. Jika proses inflamasi berlangsung lama dan tidak dapat mengembalikan ke kondisi homeostasis, dapat menyebabkan efek patologis yang dapat menyebabkan kerusakan sel dan menimbulkan berbagai penyakit. *Elephantopus scaber* merupakan tumbuhan yang dapat dengan mudah dijumpai di Indonesia. *Elephantopus scaber* merupakan tumbuhan yang sering digunakan sebagai obat-obat tradisional. Berbagai penelitian menunjukkan bahwa senyawa bioaktif yang terkandung dalam tumbuh-tumbuhan memiliki potensi yang besar untuk dijadikan sebagai alternatif obat-obatan.

Tujuan Penelitian: Penelitian ini bertujuan untuk mencari aktifitas anti-inflamasi ekstrak etanol daun *Elephantopus scaber*.

Metode: Daun *Elephantopus scaber* diekstraksi dengan menggunakan etanol dan dibuat menjadi

konsentrasi yang berbeda-beda (50 mg/mL, 100 mg/mL, and 120 mg/mL). Natrium diklofenak merupakan obat standar. Anti-inflamasi diukur dengan menggunakan metode stabilisasi membran sel darah merah dan metode hemolisis yang diinduksi panas. Skrining fitokimia yang digunakan dalam penelitian ini adalah metode konvensional.

Hasil: Skrining fitokimia menunjukkan adanya flavonoid, tanin, dan saponin yang terkandung dalam ekstrak. Pada penelitian ini, ekstrak etanol daun *Elephantopus scaber* memiliki aktifitas anti-inflamasi dengan melindungi stabilitas membran sel darah merah. Kemampuan proteksi yang paling tinggi yang dimiliki oleh ekstrak etanol daun *Elephantopus scaber* baik metode stabilisasi membran sel darah merah maupun hemolisis yang diinduksi panas adalah pada konsentrasi 100 mg/mL.

Kesimpulan: Ekstrak etanol daun *Elephantopus scaber* memiliki aktifitas anti-inflamasi dengan pengujian in-vitro.

INTRODUCTION

Inflammation is a protective physiological response to tissue injury that can be caused by a physical trauma, destructive chemicals agent and microbiological agent infections. The healing processes in inflammation can occur with neutralizing harmful stimuli, repairing damaged tissue and fighting the microbial infection process by activating the immune response.¹⁻³ In inflammation, there is a release of vasoactive, chemoattractant and proliferative substances that cause transfer of plasma fluid and mobilization of phagocytic cells to the site of inflammation. Inflammation is characterized by symptoms such as redness, heat, pain, swelling, and loss of function. Although inflammation plays an important role in physiological processes, if the inflammatory process is prolonged and cannot restore to homeostatic conditions, this may lead to pathological effects that can damage cells and cause various diseases.^{1,3,4}

Non-steroidal anti-inflammatory drugs (NSAIDs) are often used to treat pain. NSAIDs act by inhibiting cyclooxygenase (COX) enzymes or protecting the lysosome membranes from breakdowns. COX is an enzyme that is responsible for the formation of prostaglandin. COX consists

of COX-1 and COX-2 isoforms that have different roles. COX-1 is constitutively expressed in almost all tissues such as bloodvessels, kidneys and stomach. It is expressed in a physiological state to maintain the functioning of tissue homeostasis. In the other hand, COX-2 is expressed in a pathological condition. COX-2 is induced by pro-inflammatory agents, hormones, and growth factors. Most of NSAIDs act by inhibiting COX-1 and COX-2, therefore, this mechanism often cause adverse effects on cardiovascular, kidney, and gastrointestinal tracts.^{2,5-7}

Since ancient times, the knowledge and utilization of traditional medicinal plants have long been done. This often becomes the basis for the development and discovery of new alternative medicine. *Elephantopus scaber* is a plant that can easily be found in Indonesia including in the Southeast Celebes.⁸ *Elephantopus scaber* is a type of plant that is often used as a traditional medicine to treat headaches, fever, diarrhea, and hepatitis.⁹⁻¹¹ Several studies have shown that the compound bioactive content contained in plants has enormous potential as antioxidants, anti-inflammatory and anti-cancer.¹²⁻¹⁴ The present study aimed to investigate the anti-inflammatory activity of ethanolic extract of *Elephantopus scaber*.

METHOD

Drugs and chemicals

The diclofenac sodium salt was obtained from Tocris Biosciences, McKinley Place NE, Minneapolis, USA. Alsever's solution was obtained from Sigma Aldrich, Saint Louis, Missouri, USA. Sodium hydroxide, chloroform, sulfuric acid, acetic anhydride and other agents were obtained from the local supplier.

Preparation of extract

The leaves material *Elephantopus scaber*, was collected from South Konawe, Southeast Celebes, Indonesia. The extraction was performed using standard procedures. The plant leaves were thoroughly washed with tap water. Then the leaves were dried and made into the powder. Dry powder material was extracted by maceration

method using ethanol 70% solvent and then was evaporated by vacuum rotary evaporator.

Preliminary phytochemicals screening

Preliminary phytochemical screening was performed using conventional method.¹⁵

Alkaloids test

The powder samples of *Elephantopus scaber* (1 g) were taken in a conical flask and added ammonia solution (3 mL). It was allowed to stand for few minutes to evaluate free alkaloids. Chloroform (10 mL) was added to the conical flask shaken by hand gently and then filtered. The chloroform was evaporated from the crude extract by water bath and added Mayer's reagent (3 mL). The presence of alkaloid was characterized by the formation of a cream color precipitation.

Flavonoids test

The ethanolic extract of *Elephantopus scaber* (1 mL) was taken in a test tube and added few drops of dilute NaOH solution. The presence of flavonoids was characterized by a change of color from intense yellow to colorless when added a few drops of dilute acid.

Tannins test

The ethanolic extract of *Elephantopus scaber* (3 mL) was taken in the test tube and diluted with chloroform and an acetic anhydride (1 mL) was added. The presence of tannins was characterized by a green color that is formed when there is a sulfate (1 mL) addition that is carefully on the side of the test tube to the solution.

Steroids test

The crude plant extract *Elephantopus scaber* (1 mg) was taken in a test tube and dissolved with chloroform (10 mL), then added the same volume of concentrated sulfuric acid to the test tube by sides. The presence of steroids was characterized by a change in color to the red and the sulfuric acid layer appears yellow with green fluorescence.

Triterpenoids test

The dry crude plant extract *Elephantopus scaber* (5 mg) was dissolved in chloroform (2 mL) and then acetic anhydride (1 mL) was added to it. Concentrated sulphuric acid (1 mL) was added to the solution. The presence of triterpenoids was characterized by the formation of reddish violet color.

Saponins test

The ethanolic extract of *Elephantopus scaber* (1 mL) was taken in a test tube and diluted with 20 mL of distilled water. It was shaken by hand for 15 minutes. The presence of saponins was characterized by a layer of the foam obtained at the top of the test tube.

Anti Inflammatory Assays

The human red blood cell (HRBC) membrane stabilization method

The method was used for the present study following the methodology of Gupta et al. (2013) with some modification.¹⁶ The blood was collected from a healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and 10 % suspension was made. Different concentration of extracts was prepared (50, 100 and 120 mg/ml) using DMSO and to each concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37°C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated by spectrophotometric at 560 nm. Diclofenac sodium (100 mg/ml) was used as a standard drug and a control was prepared by omitting the extracts. The procedures were performed in duplo. Mean values of the duplo were considered.

Percentage Protection (%) = $100 - \left[\frac{\text{optical density (OD) of sample}}{\text{OD of control}} \times 100 \right]$

Heat-induced hemolysis method

The reaction mixture (2 ml) consisted of 1 ml of test sample solution and 1 ml of 10 % RBCs suspension, instead of test sample the only saline was added to the control test tube. Diclofenac sodium (100 mg/ml) was used as standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30 minutes. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 minutes and the absorbance

of the supernatants was taken at 560 nm. The experiment was performed in duplo for all the test samples. Mean values of the duplo were considered.

$$\text{Percentage of Protection (\%)} = 100 - \left[\frac{\text{OD of sample}}{\text{OD of control}} \times 100 \right]$$

RESULTS

Phytochemical Screening

The phytochemicals screening of *Elephantopus scaber* showed the presence of phytochemical various constituents (Table 1).

Table 1. Phytochemical screening of *Elephantopus scaber*

Phytochemical Test	Result
Alkaloids	-
Flavonoids	+
Tannins	+
Steroids	-
Triterpenoids	-
Saponins	+

The human red blood cell (HRBC) membrane stabilization method

In the present study, it can be seen that each concentration of ethanol extract could provide protection against stabilization of human red blood cell membrane (Table 2). Based on the

present study, ethanolic extract of *Elephantopus scaber* leaves has the potential as the anti-inflammatory. In the present study, we found that the highest protection capability possessed by ethanol extract *Elephantopus scaber* was at concentration 100 mg / mL.

Table 2. Effect of ethanolic extract of *Elephantopus scaber* on HRBC membrane stabilization method

Concentration	% Protection
50 mg/mL	98.40
100 mg/mL	99.04
120 mg/mL	98.28
Standard	98.58

Heat-induced hemolysis method

In the present study, it can be seen that each concentration of ethanol extract also could provide protection against heat-induced

hemolysis (Table 3). In the present study, we also found that the highest protection capability possessed by ethanolic extract of *Elephantopus scaber* leaves was at concentration 100 mg / mL.

Table 3. Effect of ethanolic extract of *Elephantopus scaber* on heat induced hemolysis method

Concentration	% Protection
50 mg/mL	52,0
100 mg/mL	94.7
120 mg/mL	70.7
Standard	97,0

DISCUSSION

Several studies have shown that the bioactive content possessed by plants has great potential to be a source of anti-inflammatory drug discovery.^{17,18} In the present study, we found anti-inflammatory activities of extract ethanolic of *Elephantopus scaber*. Based on the phytochemical screening, we found that the phytochemicals constituent content possessed by extract ethanolic. In the present study were flavonoids, saponins, and tannins. The content of flavonoids, tannins, and saponins in ethanolic extract of *Elephantopus scaber* may be responsible for the anti-inflammatory activity.

In the present study, the extract of *Elephantopus scaber* could inhibit lysis of the red blood cells membrane. The red blood cells membrane is analogous to the lysosome membrane. Several studies have shown that the content of flavonoids and saponins in plants could inhibit the phospholipase A2 (PLA2s).^{12,19,20} Phospholipase A2 is an enzyme that acts to break down the Sn-2 fatty acid of membrane phospholipid that encloses arachidonic acid (AA) which plays an important role in the inflammatory process.²¹ The arachidonic acid is subsequently converted to prostaglandin by COX-1 and COX-2 enzymes. COX-2 is an enzyme responsible for the inflammatory process and its activity is induced by inflammatory, hormonal, and growth factors.⁶ Flavonoids and saponins are also known to have an activity that can inhibit COX-2 which is a non-steroidal anti-inflammatory drug target.²⁰ In the present study also could be seen that the extract ethanolic of *Elephantopus scaber* could provide protection against heat-induced hemolysis red blood cells. Ethanolic extract of *Elephantopus scaber* could protect the

lysis of red blood cell membranes by exposure to harmful stimuli such as heat. Harmful stimuli could induce expression of TNF- α by activating immune response. TNF- α plays an important role in inflammation. TNF- α causes changes in vascular endothelium. In physiological conditions, leukocytes move freely along the vascular endothelium. TNF- α causes the vascular endothelium to become pro-inflammatory, subsequently increasing the adhesion of leukocytes to the vascular endothelium, trans-endothelial leukocyte migration, vascular leakage and increased thrombosis.^{22,23} Saponins contained in plants can inhibit TNF- α .²³ In addition, the anti-inflammatory mechanisms in this study may be due to the ability possessed by ethanolic extract of *Elephantopus scaber* in inhibiting prostaglandin E2 (PGE₂). PGE₂ plays an important role in the inflammatory process by increasing the permeability of blood vessels and causing pain. Flavonoids, saponins, and tannins present in plants have the activity to inhibit inducible Nitric Oxide Synthase (iNOS). iNOS is an NOS isoform that is highly expressed by macrophage cells during inflammation. iNOS can lead to increased production of PGE₂. Therefore, a substance that can inhibit iNOS may act as an anti-inflammatory agent.²⁴⁻²⁸ Further studies are necessary to investigate and to isolate the bioactive compounds contained in the ethanolic extract of *Elephantopus scaber* that responsible for this anti-inflammatory action.

CONCLUSION

In the present study showed that the ethanolic extract of *Elephantopus scaber* has anti-inflammatory activities by in vitro assays. The presence of flavonoid, saponin and tannin

may be responsible for the anti-inflammatory activities. The present study suggests that the ethanolic extract of *Elephantopus scaber* has a promising bioactive compound that can be used as an anti-inflammatory. Further investigations are needed to isolate the bioactive compound of the extract *Elephantopus scaber* and to confirm the mechanism that responsible for anti-inflammatory activities.

CONFLICT OF INTEREST

We declare there is no conflict of interest

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