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IN VITRO AND *IN VIVO* EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF *GYNURA PROCUMBENS* (LOUR.) MERR. LEAF EXTRACT

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Summary

This study evaluated the anti-inflammatory and analgesic effects of a 95% ethanol extract of *Gynura procumbens* leaves. These effects were assessed using both *in vitro* and *in vivo* models. *In vitro* anti-inflammatory activity was assessed using the egg white albumin denaturation assay, while *in vivo* efficacy was evaluated *via* xylene-induced ear edema and hot plate models in mice at doses of 37.5, 50, and 75 mg/mL. Chemical analysis revealed 22.3 mg essential oil/kg of extract and 22.44 mg quercetin equivalents/g of extract. It inhibited albumin denaturation with an IC_{50} of 0.34 mg/mL, significantly lower than the IC_{50} of diclofenac. In the xylene-induced ear edema model, all doses reduced ear thickness, with the 75 mg/mL dose exhibiting an effect comparable to diclofenac. In the hot plate test, the 50 and 75 mg/mL doses significantly increased response latency. These findings demonstrate that the ethanol extract of *G. procumbens* leaves possesses significant anti-inflammatory and analgesic properties, supporting its potential as a natural therapeutic candidate for inflammation and pain management.

Keywords: *Gynura procumbens*; *Anti-inflammatory; Analgesic.*

1. Introduction

Inflammation and pain are common symptoms in pathologies such as arthritis and rheumatoid arthritis (RA), often being chronic and significantly impacting the quality of life. Current treatments primarily rely on glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs). Although effective, these drugs are associated with various side effects, including gastric ulceration, immunosuppression, hypertension, and cardiovascular diseases. To mitigate systemic side effects, topical formulations are increasingly favored. However, even these can cause local adverse reactions such as dermatitis and skin atrophy [1],[2]. In light of this situation, there is a compelling necessity for the development of safe and effective natural-origin products. *Gynura procumbens* (Lour.) Merr. (Asteraceae), commonly known as Longevity spinach (or Bầu đất in Vietnamese), is a well-known medicinal plant in Southeast Asia. It is traditionally used in cuisine and folk medicine for various therapeutic applications, including hypoglycemic, hypolipidemic, hepatoprotective, and antihypertensive effects. Furthermore, the leaves are traditionally applied topically to reduce swelling and inflammation and to treat insect bites, owing to their recognized folk anti-inflammatory action [3]. The essential oil of *G. procumbens* (GPEO), whose main components include alpha-pinene, 3-carene, and limonene, has demonstrated anti-inflammatory and analgesic effects by inhibiting the activity of the COX-2 enzyme [4]. Iskander et al. (2002) reported that the ethyl acetate fraction derived from the total ethanol extract of *G. procumbens* exhibited significant anti-inflammatory activity in a mouse ear edema model induced by croton oil, achieving up to 65.2% inhibition of the inflammatory response at a concentration of 37.5 mg/mL [5]. In addition to the essential oil, *G. procumbens* is rich in antioxidant compounds, with flavonoids being a major class responsible for this activity [6]. The primary flavonoid components identified in *G. procumbens* are quercetin, rutin, kaempferol, and astragalin. Among these, quercetin is a crucial component in reducing carrageenan-induced inflammation, while rutin is effective in chronic inflammatory processes, particularly as an adjuvant in arthritis treatment [7],[8]. Despite this existing knowledge, scientific research within Vietnam regarding the active compounds and pharmacological effects of *G. procumbens* remains limited. Therefore, this study aims to

evaluate the *in vitro* and *in vivo* anti-inflammatory and analgesic effects of the ethanol extract from *G. procumbens* leaves.

2. Materials and methods

2.1. Plant materials

Leaves of *G. procumbens* were collected in Xuan Son commune, Do Luong district, Nghe An province, Vietnam. The leaves were harvested during the spring season, specifically in the early morning on sunny days, ensuring the selection of mature leaves (neither too old nor too young). The plant specimen was authenticated, with sample code JI038223, by the Laboratory of Nam Khoa Services and Trading Co., Ltd. (Ho Chi Minh City).

Following thorough washing, the leaves were dried in the shade at a temperature of less than 40°C and stored in a cool, well-ventilated, and light-protected place. Prior to extraction, the dried material was ground and sieved through a 1.4 mm mesh, to obtain the leaf powder.

2.2. Preparation of the crude extract

The crude extract from *G. procumbens* leaves was prepared as follows: 200 g of leaf powder was macerated in 95% ethanol (in a ratio of 1:10 w/v) for 7 days at room temperature, protected from light. The extract was then filtered and concentrated in vacuo at 40°C until dryness, yielding a crude extract (extract yield: 3.76%, moisture content: 9.81%). The crude extract was stored in a refrigerator throughout the study.

2.3. Experimental animals

Male ICR mice, aged 5–6 weeks and weighing 18–22 g, were used in the study. The mice were confirmed healthy and were supplied by the Nha Trang Institute of Vaccines and Medical Biologicals (Vietnam). The animals were subjected to a two-week acclimatization period prior to the experiments. They were housed at 8 animals per cage (25 × 35 × 15 cm) under standard laboratory conditions, with *ad libitum* access to standard food pellets and water throughout the study.

2.4. Chemicals, solvents, and equipment

Chemicals and solvents used included: 95% ethanol, xylene, methanol, dimethyl sulfoxide (DMSO), propylene glycol, K_2HPO_4, KH_2PO_4, $AlCl_3$, and *n*-hexane (sourced from China, Germany, and Spain). Standard reference compounds were quercetin 97% and diclofenac sodium 99.7% (both obtained from the Ho Chi Minh City Institute of Drug Quality Control). Diclofenac 1% commercial gel (Voltaren Emulgel®) was used as a positive control.

Equipment utilized included a Heidolph rotary evaporator, a UV–Vis spectrophotometer SP8001 (Axiom), a Bio-Rad ELISA reader, a Sartorius analytical balance, a DF-15 grinder, an ISTEK pH meter, and a Memmert drying oven.

2.5. Quantification of essential oil in alcoholic extract by hydrodistillation

Three hundred grams of dried crude drug powder were extracted with 3 liters of 95% ethanol for 7 days to maximize the essential oil yield. The resulting extract was then subjected to hydrodistillation using a Clevenger-type apparatus for 3 hours. To minimize the loss of essential oil, 5 mL of *n*-hexane was precisely added [9]. Upon completion, the apparatus was allowed to cool completely, including the condenser and the essential oil receiver, before dismantling. The essential oil dissolved in the *n*-hexane layer was collected. The *n*-hexane was then removed by evaporation under reduced pressure until no *n*-hexane vapor was visible in the condenser tube, to yield the essential oil. The collected essential oil was dried over anhydrous sodium sulfate (Na_2SO_4), then transferred into a dark glass vial, tightly stoppered, and stored under refrigeration. The mass was subsequently determined by weighing.

2.6. Quantification of total flavonoids by the spectrophotometric method

Principle: Flavonoids belonging to the flavonol group (e.g., quercetin, rutin) readily react with Al^{3+} metal ions to form colored complexes that absorb light in the range of 410 - 430 nm [10].

Construction of the quercetin standard curve

Precisely 10 mg of standard quercetin was weighed and dissolved in methanol in a 10 mL volumetric flask to obtain a stock solution with a concentration of 1000 µg/mL. One milliliter of the 1000 µg/mL stock solution was diluted with methanol in a 10 mL volumetric flask to obtain a

quercetin working solution of 100 µg/mL.

A series of standard concentrations ranging from 1 to 50 µg/mL was then prepared from the working solution. The absorbance was measured using a 96-well microplate spectrophotometer. Based on the collected results, five concentrations were selected to construct the quercetin standard curve.

Quantification of total flavonoids in the extract sample

Precisely 0.5 mL of the crude extract sample was added to an Eppendorf tube containing 0.5 mL of 2% aluminum chloride ($AlCl_3$) solution prepared in methanol. The mixture was incubated for 10 minutes at room temperature, protected from light.

Absorbance was then measured at a wavelength of 415 nm using a 96-well microplate spectrophotometer. Each sample was measured in triplicate, and the average value was recorded.

The total flavonoid content was calculated and expressed as milligrams of quercetin equivalent per gram of crude extract (mg QE/g extract), using the following formula:

$$X = \frac{A_t - b}{a.(100 - h).C} \; x\; 2\; x\; 100$$

Where:

a, b: coefficients of the linear regression equation from the quercetin standard curve

A_t: absorbance at 415 nm of the test sample

C: concentration of the crude extract (mg/mL)

h: moisture content of the crude extract (%)

2.7. In vitro anti-inflammatory activity assay by inhibition of egg white albumin denaturation

The *in vitro* anti-inflammatory activity was evaluated using the egg white albumin denaturation model. Absorbance was measured at 660 nm following heat treatment [11]. The percentage inhibition and the IC_{50} values for both diclofenac sodium and the crude extract were determined.

Sample preparation

Diclofenac sodium control solutions: Standard powdered diclofenac sodium was dissolved in DMSO to create a stock solution with a concentration of 5 mg/mL. This stock solution was then used to prepare diclofenac sodium solutions in DMSO for the assay at concentrations of 0.4, 0.8, 1.2, 1.6, and 2.0 mg/mL.

95% Ethanol extract solutions: Precisely 50 mg of the 95% ethanol crude extract was weighed and dissolved in DMSO to form a stock solution at a concentration of 5 mg/mL. This stock was used to prepare the test solutions of the 95% ethanol extract in DMSO at concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL.

These prepared diclofenac sodium and 95% ethanol extract solutions were used to investigate the inhibitory activity against egg white albumin denaturation.

The procedure for preparing the samples to determine the inhibitory activity against egg white albumin denaturation is presented in **Table 1**.

Table 1. Sample preparation for determining inhibitory activity against egg white albumin denaturation

Component	Control (µL)	Sample (µL)	Blank sample (µL)
Egg white albumin mixture	750	750	–
pH 6.4 buffer solution	–	–	750
DMSO	500	–	–
Test solution	–	500	500

All samples were incubated for 15 minutes at 37°C. Subsequently, they were heated at 65 ± 1°C for 5 minutes in a heat incubator. The samples were then cooled, thoroughly mixed, and the absorbance was measured at a wavelength of 660 nm. The inhibitory capacity of the 95% ethanol extract was compared with diclofenac sodium, a common NSAID anti-inflammatory drug. All samples were analyzed in triplicate.

The half-maximal inhibitory concentration (IC_{50}) of the test solutions was calculated based on a 4-parameter non-linear model (or 4-parameter logistic model). This model describes the relationship between a substance's concentration and its inhibitory effect using a non-linear curve defined by four parameters: maximal inhibition, minimal

inhibition, inflection point, and slope. The IC_{50} value is defined as the concentration that inhibits 50% of the protein denaturation.

The percentage inhibition of denaturation for each test solution concentration was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{[\text{ODcontrol} - (\text{ODsample} - \text{ODblank sample})]}{\text{ODcontrol}} \times 100$$

Where:

OD_control: Absorbance of the control sample

OD_sample: Absorbance of the test sample

OD_blank sample: Absorbance of the test blank sample

2.8. Anti-inflammatory effect on the mouse ear edema model induced by xylene

Three concentrations of the 95% ethanol extract were prepared by dissolving 375, 500, and 750 mg of the 95% ethanol extract in 10 mL of propylene glycol solvent to obtain three test extract solutions with concentrations of 37.5 mg/mL (0.75 mg/20 µL), 50 mg/mL (1 mg/20 µL), and 75 mg/mL (1.5 mg/20 µL), respectively.

The test mice were randomly divided into 5 groups (n = 8), and inflammation was induced using xylene. A volume of 20 µL of xylene solvent was evenly applied to the inner and outer surfaces of the mouse's right ear (10 µL on each surface).

After 15 minutes, 20 µL of the test solutions were topically applied, including: *G. procumbens* extract at concentrations of 37.5–75 mg/mL; propylene glycol (negative control); diclofenac sodium (Voltaren Emulgel®, positive control). The application method involved using a micropipette to measure the exact volume of the test sample, which was then evenly applied to the mouse ear using a plastic stick. Ear thickness was measured before treatment and at 4 hours after treatment. The percentage reduction in inflammation was calculated based on the average ear thickness among the groups [12].

2.9. Study of analgesic effect on the hot plate model

The experiment was performed as described by Huang and Hunskaar [4, 13]. Twenty-four hours prior to the experiment, mice were placed on a hot plate at a temperature of 55°C ± 0.5°C. The time taken from placing the mouse on the hot plate until it exhibited forepaw licking, paw withdrawal, or jumping response within 30 seconds was used as a criterion for selection to proceed with the study.

Thirty-two mice that responded well to the hot plate (55°C ± 0.5°C) were selected, divided into 4 groups (n = 8), and each mouse was topically applied on the outer surface of the soles of the feet, with 20 µL of the *G. procumbens* extract dissolved in propylene glycol on each limb at the following concentrations:

- Group 1: *G. procumbens* extract at a low dose of 37.5 mg/mL
- Group 2: *G. procumbens* extract at a medium dose of 50 mg/mL
- Group 3: *G. procumbens* extract at a high dose of 75 mg/mL
- Group 4 (Control): Propylene glycol

Evaluation Parameter: The thermal latency of the mice was recorded before and 60 minutes after drug application (in seconds). During the 60-minute interval after drug application, the mice were closely monitored to prevent paw-licking behavior.

Thermal latency is defined as the time from the moment the mouse is placed on the hot plate until it exhibits behaviors such as forepaw licking, paw withdrawal, or jumping response.

2.10. Analysis of results and statistical data processing

Results are presented as the mean ± standard error of the mean (SEM). Statistical analysis was performed using Microsoft Excel and GraphPad Prism software. The One-way ANOVA test was used to examine differences between groups, followed by a post-hoc test (Tukey HSD) to specify the differences, if any. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Quantification of essential oil in ethanol extract by the hydrodistillation method

The alcoholic extract, obtained from soaking 300 g of dried raw material in 95% ethanol, was

subjected to hydrodistillation (steam distillation). After hydrodistillation, a mixture of essential oil dissolved in *n*-hexane was collected. Removal of *n*-hexane by evaporation yielded 6.69 mg of essential oil. Thus, the 300 g of dried raw material contained 6.69 mg of total essential oil, corresponding to an essential oil content in the dry crude drug of 22.3 mg/kg.

3.2. Quantification of total flavonoids

The standard curve for absorbance versus quercetin concentration is presented in Fig. 1.

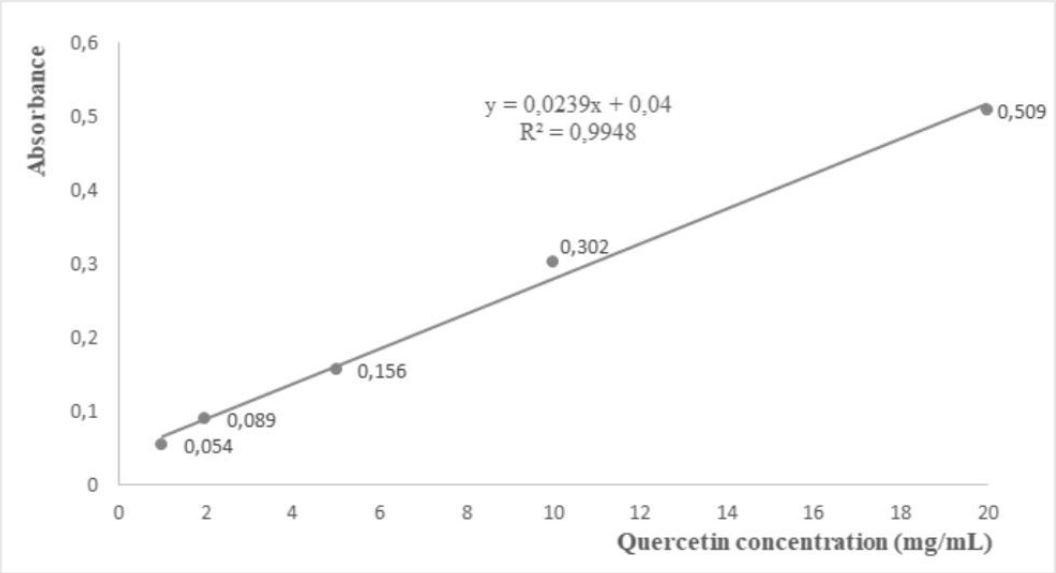

Fig. 1. Linear relationship showing the correlation between absorbance and quercetin concentration

In the concentration range of quercetin from 1 to 20 µg/mL, there was a correspondence between absorbance and quercetin concentration, with the regression equation y = 0.0239x - 0.04 and a correlation coefficient $R^2 = 0.9948$.

Test samples were prepared, and the flavonoid content of the extracts from *G. procumbens* leaves was quantified. The results are shown in Table 2.

Table 2. Results of total flavonoid quantification in 95% ethanol extract

Parameter	Test 1	Test 2	Test 3
Weight weighed (mg)	20.48	20.12	20.16
Dilution factor	100	100	100
Extract concentration (mg/mL)	0.20	0.20	0.20
OD sample	0.670	0.645	0.648
OD blank sample	0.579	0.559	0.560
OD sample − OD blank sample	0.091	0.086	0.088
Flavonoid content in 1 g of crude extract (mg QE/g)	23.01	22.21	22.09
Average (mg QE/g)	22.44		
Standard deviation	0.50		

The total flavonoid content of the 95% ethanol extract was 22.44 mg QE/g.

3.3. In vitro anti-inflammatory activity

The percentage inhibition of albumin denaturation by diclofenac sodium solutions at concentrations of 0.4, 0.8, 1.2, 1.6, and 2 mg/mL was 13.52%, 23.66%, 45.03%, 67.20%, and 81.76%, respectively. Similarly, the percentage

inhibition of albumin denaturation by the 95% ethanol extract solutions at concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL was 22.04%, 27.80%, 39.82%, 58.56%, and 65.58%, respectively. The IC_{50} concentrations of diclofenac sodium and the 95% ethanol extract were calculated based on the four-parameter nonlinear model, yielding results of 1.41 mg/mL and 0.34 mg/mL, respectively.

3.4. In vivo anti-inflammatory effect

Table 3. Results of the *in vivo* anti-inflammatory effect study

Group	Ear thickness before xylene (mm)	Ear thickness 4 h after treatment (mm)	Increase in ear thickness (mm)
Vehicle control	0.12 ± 0.01	0.25 ± 0.01	0.13 ± 0.01
Voltaren Emulgel®	0.13 ± 0.01	0.17 ± 0.01	0.04 ± 0.01*
G. procumbens extract 37.5 mg/mL	0.12 ± 0.01	0.20 ± 0.01	0.08 ± 0.01*
G. procumbens extract 50 mg/mL	0.12 ± 0.01	0.18 ± 0.01	0.06 ± 0.01*
G. procumbens extract 75 mg/mL	0.11 ± 0.01	0.15 ± 0.01	0.04 ± 0.01*

*$p < 0.001$ compared to the vehicle control group.

The ear thickness of mice in all groups increased after xylene application. In the vehicle control group, where mice received only propylene glycol, the increase in ear thickness was the highest. The test groups treated with the *G. procumbens* extract and the positive control Voltaren Emulgel® all showed an anti-inflammatory effect, with a significantly lower increase in ear thickness compared to the vehicle control group ($p < 0.001$). A comparison among the *G. procumbens* extract groups showed a clear dose-dependent trend ($p < 0.05$), with the 75 mg/mL extract yielding the strongest anti-inflammatory effect, followed by the 50 mg/mL and 37.5 mg/mL concentrations. Significantly, the 75 mg/mL *G. procumbens* extract produced an equivalent effect to the positive control Voltaren Emulgel®, with no statistically significant difference ($p > 0.05$).

3.5. In vivo analgesic effect

The results of the analgesic effect on the hot plate model are presented in Fig. 2.

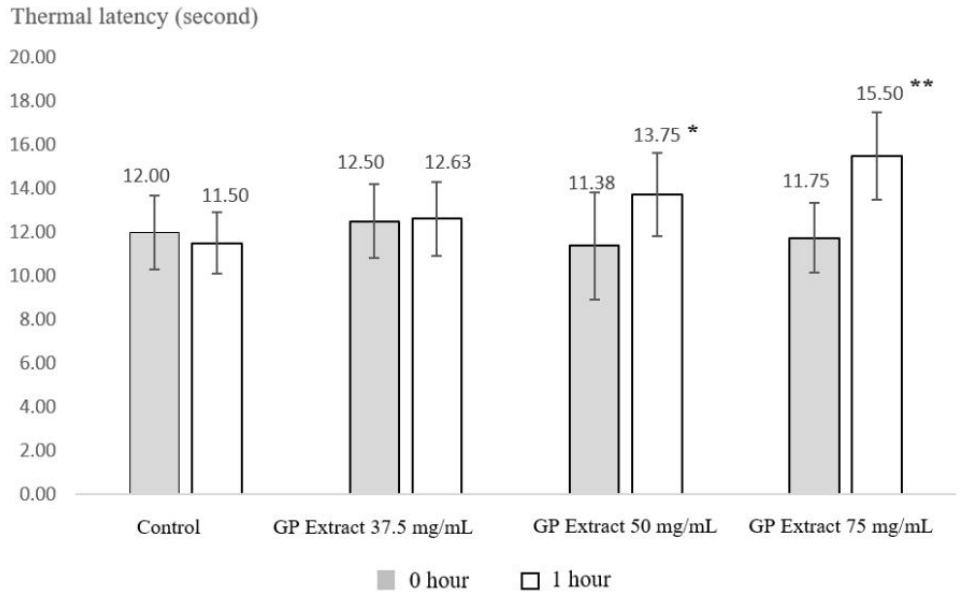

Fig. 2. Analgesic effect of the tested extracts on the hot plate model

(Fig. 2 caption): *$p < 0.05$; **$p < 0.01$ compared to before drug application

In the groups treated with the *G. procumbens* extract, the thermal latency (pain tolerance time) increased compared to before topical application, with statistically significant differences observed in the groups treated with 50 mg/mL and 75 mg/mL *G. procumbens* extract ($p < 0.05$). In contrast, the control group (propylene glycol) exhibited a decrease in thermal latency. These findings demonstrate the ability of the *G. procumbens* extract to enhance thermal tolerance in mice.

4. Discussion

4.1. Chemical composition of G. procumbens leaves and potential biological activity selected for study

The analgesic and anti-inflammatory effects of *G. procumbens* leaves have long been applied in folk medicine: in Vietnam, *G. procumbens* leaves are used to treat eye pain, while in Thailand, the plant is used to relieve symptoms of inflammation, rheumatism, and diseases caused by viruses [3],[7].

According to Tan et al., *G. procumbens* leaves are rich in phenolic compounds and flavonoids, as well as essential oils. The main flavonoid components in *G. procumbens* are quercetin, rutin, kaempferol, and astragalin. These compounds are known to possess various biological activities such as anti-inflammation and analgesia. Specifically, quercetin and rutin have been proven to be important components with anti-inflammatory effects against carrageenan-induced inflammation and are effective in chronic inflammatory processes, supporting the treatment of arthritis. Meanwhile, the essential oil components reported in *G. procumbens* leaves include α-pinene, 3-carene, and limonene, which have mostly been shown to exhibit anti-inflammatory and analgesic effects in experimental models [8],[14],[15].

Therefore, we proceeded to select the anti-inflammatory and analgesic activities of *G. procumbens* leaves for initial assessment using *in vitro* and *in vivo* models.

4.2. Selection of extraction solvent, extraction yield, and total essential oil and total flavonoid

The main biologically active compounds of *G. procumbens* leaves (*α*-pinene, quercetin) are highly soluble in organic solvents such as ethanol, DMSO, or dimethylformamide. Therefore, 95% ethanol was chosen as the extraction solvent to maximize the extraction of essential oils and most flavonoids.

In the study by Algariri et al., which evaluated the biological activity of *G. procumbens* leaves, 95% ethanol and the maceration method were also used. In that study, the extraction yield was lower than ours, 0.42% versus 3.76%. This difference may be due to the variation in extraction time between the two studies, 3 days versus 7 days [16].

For the total essential oil content, the recorded amount calculated based on the dried raw material was 22.3 mg/kg. Compared to data from Huang, this essential oil content is higher than that extracted in Hubei province (10.56 mg/kg) but lower than that in Hunan province (175.03 mg/kg) in China. This discrepancy is clearly due to differences in geographical location and varying soil conditions [4].

The total flavonoid content was also determined to ensure that the extract contained biologically active chemical components along with essential oils. The quantification results showed a total flavonoid content of 22.44 ± 0.50 mg QE/g of crude extract, comparable to the result in Algariri's study (24.52 mg QE/g), despite differences in extraction time and temperature, where our extraction method did not involve heating but had a longer duration [16].

Since the objective was an initial investigation of the analgesic and anti-inflammatory effects, this study only quantified the total essential oil and total flavonoid content to ensure the test extract contained biologically active components. Based on the results obtained, future research will focus on specifically identifying the main essential oil and flavonoid components as a basis for standardizing the *G. procumbens* raw material

source in Nghe An, Vietnam.

4.3. In vitro and in vivo assay

The *in vitro* test results showed that the *G. procumbens* extract had an approximately 4-fold higher capacity to inhibit albumin denaturation compared to Voltaren Emulgel® (IC_{50} was 0.34 mg/mL versus 1.41 mg/mL), demonstrating the anti-inflammatory potential of the *G. procumbens* leaf extract. Consistent with other *in vitro* anti-inflammatory assay methods (human red blood cell membrane stabilization, bovine serum albumin denaturation), strict control over temperature, time, and sample dispersion was applied during the egg albumin denaturation method to ensure the accuracy of the results obtained.

The *in vivo* test results on the xylene-induced mouse ear edema model showed that the ear thickness in the vehicle control group doubled compared to baseline. Voltaren Emulgel® significantly reduced ear thickness compared to the vehicle control group, demonstrating that xylene-induced ear edema can be treated with medication. This model is suitable for evaluating topical anti-inflammatory effects, as successfully used in the studies by Huang and by Xiao et al. [4],[14]. In this model, the *G. procumbens* extract (notably at the high dose of 75 mg/mL) showed an effect equivalent to Voltaren Emulgel®, indicating the promising anti-inflammatory potential of the *G. procumbens* leaf extract.

The mechanism of xylene-induced inflammation involves increasing the expression of the COX-2 enzyme and the activity of MPO (myeloperoxidase) in the ear. Additionally, xylene significantly increases the concentration of NF-κB p65, TNF-α, IL-1β, and MDA in the ear and causes oxidative stress [17]. Flavonoids are known as a class of active compounds capable of activating antioxidant pathways that provide anti-inflammatory effects. They inhibit the activity of enzymes such as lysozyme and β-glucuronidase, and inhibit the formation of inflammatory prostaglandins, helping to reduce inflammatory reactions. The flavonoids found in *G. procumbens* leaves, such as quercetin and kaempferol, are known to regulate the expression and activation of IL-1β, TNF-α, IL-6, and IL-8; regulate the gene expression of various pro-inflammatory factors like NF-κB, AP-1, ICAM, and VCAM; and inhibit pro-inflammatory enzymes such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2, and lipoxygenase [18]. Furthermore, the COX-2 inhibitory activity of the essential oil in *G. procumbens* leaves has also been proven [4], contributing to clarifying the anti-inflammatory potential of *G. procumbens* leaves due to the presence of both flavonoid and essential oil components.

These results are consistent with the anti-inflammatory effect reported by Iskander et al., according to which the ethyl acetate fraction partitioned from the alcoholic extract showed an anti-inflammatory effect at a dose of 37.5 mg/mL in the croton oil-induced inflammation model [5], and by Huang et al. in 2019, which also demonstrated the anti-inflammatory effect, via COX-2 enzyme inhibition, of the total essential oil extracted from *G. procumbens* leaves [4].

In the hot plate model, the medium and high doses of the *G. procumbens* extract significantly increased the thermal latency of the mice ($p < 0.05$), showing a distinct analgesic effect, particularly at the high dose (75 mg/mL). This result is partially consistent with the study by Huang et al., which showed that the total essential oil mixture of *G. procumbens* leaves, at a high dose (1.73 mg/mL), increased the thermal tolerance threshold of the test mice [4]. This consistency suggests a central analgesic effect of the *G. procumbens* leaf extract, as it showed efficacy in the experimental model where pain is induced by thermal stimulation. When using this model, we conducted pre-screening to eliminate overly heat-sensitive individuals, which improved the reliability of the results. This model is simple, fast, and widely applied in research [4],[13]. These findings demonstrate the analgesic potential of *G.*

procumbens, providing a basis for further in-depth research on the analgesic effects of this plant on other models.

5. Conclusion

The 95% ethanol extract from *G. procumbens* leaves demonstrated both *in vitro* and *in vivo* anti-inflammatory and analgesic activities. These experimental results contribute data on the biological activity of *G. procumbens* leaves, providing a further basis for researching the application of *G. procumbens* leaves as a raw material for preparing external anti-inflammatory and analgesic formulations.

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