Proceeding of the International Conference on Health Sciences and Nursing
Volume. 1, Number. 1, Year 2024
Page 93-99
Available online at: https://prosiding.arikesi.or.id/index.php/ICHSN

Effectiveness of Styrax Paralleloneurus Extract as Anti-Inflammatory Agent on Incision Wounds in Male White Mice *(Mus Musculus)*

Meriani Herlina[1], Noradina Noradina[2], Bernita Silalahi[3], Ratna Dewi[4], Syahrul Handoko Nainggolan[5], Ali Asman[6], Ririn Mariska Sitanggang[7]
[1,2] Universitas Sumatera Utara, Indonesia
[3-7] Universitas Imelda Medan, Indonesia

Abstract: *Incision wounds are characterized by tissue damage or loss caused by sharp objects. Inflammation is the body's protective response to infection, irritation, or injury, marked by redness, heat, swelling, pain, and tissue dysfunction. Styrax paralleloneurus resin has potential wound-healing properties. This study aimed to investigate the anti-inflammatory effects of Styrax paralleloneurus extract on incision wounds in male white mice. Twenty-five mice were divided into five groups: positive control (Betadin), negative control (Vaseline), and three groups receiving 5%, 10%, and 15% Styrax paralleloneurus extract concentrations. Incision wounds (1cm x 0.2cm) were created on the dorsal area. Wound length and closure were observed daily using calipers. ANOVA analysis revealed significant differences (p=0.138 > 0.05) among the extract concentrations, indicating a concentration-dependent anti-inflammatory effect. The 15% concentration demonstrated the fastest wound healing. Bioactive compounds, such as flavonoids, saponins, and terpenoids, likely contribute to the anti-inflammatory effects. This study supports previous research on Styrax paralleloneurus' wound-healing properties. The results suggest Styrax paralleloneurus extract as a potential phytotherapeutic agent for wound healing. Further clinical trials and stability testing are recommended to develop a phytopharmaceutical product. Future studies should investigate the extract's mechanism of action and potential interactions with other medications. Conclusion The 15% concentration of Styrax paralleloneurus extract exhibited the fastest anti-inflammatory effects on incision wounds in male white mice.Recommendations: Conduct clinical trials to assess the stability, efficacy, and safety of Styrax paralleloneurus extract for phytopharmaceutical product development.*

Keywords: *Styrax Resin Extract (Styrax paralleloneurus), Incised Wounds, Inflammation, Male White Mice, Phytotherapy.*

1. INTRODUCTION

Wounds are characterized by damage to anatomical structures (Tamuntuan et al., 2021). Wounds often cause pain, bleeding, and disability, and impact clinical practice. The wound-healing process consists of interconnected stages: hemostasis, inflammation, proliferation, and remodeling (Aisyah et al., 2022). Incision wounds can lead to bleeding, involving hemostatic mechanisms and subsequent inflammation (Arum et al., 2022). According to the World Health Organization (WHO), wound incidence rates have increased annually, affecting acute and chronic wounds (Huda et al., 2022). A recent American study reported a prevalence of 3.50 wounds per 1,000 population. The majority of global wounds result from surgical procedures or trauma (48%), foot ulcers (28%), and pressure ulcers (21%) (Harun et al., 2023). Prevalence and Etiology A 2022 American Wound Association study reported: 11.03 million surgical wounds, 160 million trauma cases, 2.04 million abrasions, 10 million burns, 850 million pressure ulcers, 1.25 million venous ulcers, 3.50 million diabetic ulcers, 20 million amputations, 60 million skin cancers, and 10 million melanoma cases annually. In Indonesia, 70.9% of wounds are abrasions, followed by 23% lacerations. Falls (40.9%) and motorcycle

Received: October30, 2024; Revised: November 30, 2024; Accepted: December 18,2024; Published: December 30, 2024

accidents (40.6%) are the primary causes. Other causes include sharp/ blunt objects (7.3%), transportation (7.1%), and falls (2.5%) (Wintoko & Yadika, 2020).

Inflammation is the body's protective response to infection, irritation, or injury, characterized by redness, heat, swelling, and pain. Primary causes include blunt trauma, foreign objects, vibrations, and chronic low-intensity pressure (Emelda et al., 2023). Inflammation mechanisms involve stimulus-induced cell damage, releasing phospholipids, including arachidonic acid. Prostaglandins and leukotrienes contribute to inflammatory symptoms (Fitriyanti et al., 2020). Inflammatory responses manifest as rubor, kalor, dolor, tumor, and functional impairment (Sugihartini et al., 2020).

Indonesia boasts an immense wealth of plant species, with approximately 30,000 varieties in its tropical forests. Of these, 9,600 species are known to possess medicinal properties, while 200 species are crucial for the traditional pharmaceutical industry due to their use as raw materials (Fadhilah et al., 2022). The *Styrax paralleloneurus* tree is predominantly cultivated in Tapanuli Utara, accounting for nearly 67% of Indonesia's *Styrax* plantations (Sipayung & Eddyanto, 2022). *Styrax* exhibits anti-inflammatory properties by mimicking nonsteroidal anti-inflammatory drugs (NSAIDs) (Nurwahyuni et al., 2022). Toba *Styrax* contains high levels of cinnamic acid, boasting superior resin quality compared to other varieties. Cinnamic acid, a primary compound in *Styrax paralleloneurus* sap, demonstrates biological activity as an anti-inflammatory agent (Sipayung & Eddyanto, 2022). Research conducted by Harahap and Marpaung (2018) revealed a 26.43% cinnamic acid content in Tapanuli Utara *Styrax paralleloneurus* resin using gas chromatography (Dari et al., 2018). Studies by Erlia Anggrainy Sianipar (2023) and Kamila Alawiyah et al. (2023) indicate that *Styrax paralleloneurus* resin contains active compounds, primarily cinnamic acid, which exhibits pharmacological effects, including anti-inflammatory and antibacterial properties. These findings support traditional uses of *Styrax paralleloneurus* for various ailments, such as dental infections, tumors, dysentery, nausea, and fever.

2. RESEARCH METHOD

This study employed an experimental method to investigate the anti-inflammatory effects of *Styrax paralleloneurus* resin extract on male white mice with incision wounds. The research objectives were to determine whether *Styrax paralleloneurus* resin extract formulations exhibit anti-inflammatory effects. The study consisted of sample preparation, *Styrax paralleloneurus* resin extraction, formulation preparation, and efficacy testing.

The research was conducted from April to August 2024 at the Imelda University Pharmacy Laboratory and the Sumatera Utara University Plant Systematics Laboratory.

Equipments and Materials

1. Equipment: porcelain bowls (Glassco), hotplate (Ika), analytical balance (Fujitsu), blender (Turbo), funnel (Glassco), gram scale (Local), milligram scale (Local), glass jars (TPS), filter paper, 80-mesh sieve, stirring rod, test tubes, test tube rack, mouse cages, aluminum foil, lab coats, masks, gloves, head caps, hair clippers, scissors, and calipers.
2. Materials: 96% ethanol, distilled water, *Styrax paralleloneurus* resin, magnesium powder, hydrochloric acid, alcohol, 2N hydrochloric acid, Mayer's reagent, Bouchardat's reagent, Dragendorff's reagent, N-hexane, Liebermann-Burchard reagent, 1% iron(III) chloride, Betadin, Veet, male white mice, mouse food, and drinking water.

Formulation Materials

The formulation materials used to prepare *Styrax paralleloneurus* resin extract formulations are listed in Table 1:Table 1. Ingredients of Frankincense Resin Extract Formulation

Material	**Amount Material**		
Frankincense Resin Extract	5 g	10 g	15 g
Vaseline	95 g	90 g	95 g
mf ointment	100 g	100 g	100 g

Research Procedure

a. Sample Collection and Simplisia Preparation

1 kg of *Styrax paralleloneurus* resin samples were purchased from farmers in Lobusingkam Village, Sipoholon District, Tapanuli Utara Regency, North Sumatra, Indonesia. Samples were air-dried on cardboard in a shaded area until dry and brittle. The dried samples were then ground into powder.

b. *Styrax paralleloneurus* Resin Extract Preparation

The extraction method used was maceration. 500 g of *Styrax paralleloneurus* powder was extracted with 96% ethanol for 3 x 24 hours. The resulting liquid extract was filtered and evaporated over water to obtain a thick extract.

c. Anti-Inflammatory Test

i. Animal Preparation

Male white mice were acclimatized for 1 week before experimentation with access to a pellet diet and water. Healthy animals were characterized by a symmetrical body shape, glossy fur, and agile movement.

ii. Incision Wound Preparation

Mouse fur was shaved and cleaned with 70% alcohol. Mice were anesthetized with chloroform. A 1 cm long, 0.2 cm deep incision wound was made on the dorsal area using a sterile scalpel (Muhajri Agusfina, 2022).

iii. Extract Administration

Mice were divided into groups: positive control (Group 1), negative control (Group 2), and treatment groups with 5% (Group 3), 10% (Group 4), and 15% (Group 5) extract concentrations. Extract administration was performed twice daily, at 9:00 AM and 3:00 PM, until wound healing was complete. Changes in wound length, redness, and swelling were observed daily using calipers until complete wound closure.

3. RESULTS AND ANALYSIS

Styrax paralleloneurus Resin Identification

Plant identification was conducted at the Medanese Herbarium Laboratory (MEDA), Universitas Sumatra Utara (USU). Based on identification number 2561/MEDA/224, the sample used in this study is *Styrax* resin (*Styrax paralleloneurus*) from the Styracaceae family.

Styrax paralleloneurus Resin Extraction Results

Extraction was performed using the cold maceration method. Before extraction, simplisia preparation was conducted. The purpose of simplisia is to extend the shelf life of raw materials without compromising quality, ensuring consistent production. One kilogram of sorted *Styrax paralleloneurus* resin was dried and then ground into powder using a mortar and stamper, yielding 700 grams. Five hundred grams of the powdered simplisia were weighed and extracted using the maserasi method with 96% ethanol solvent, fully immersing the sample in a glass container for 3 x 24 hours with occasional stirring. The resulting *Styrax paralleloneurus* resin extract weighed 264 grams, characterized by its thick and sticky texture, brown color, and distinct *Styrax paralleloneurus* aroma. According to Wardaningrum (2020), the extraction yield is considered good if it exceeds 10%. Calculation of the yield of white mustard leaf extract (Brassica rapa (L.)) as follows:Calculation of the yield of white mustard leaf extract (Brassica rapa (L.)) as follows:

$$Rendemen = \frac{\text{Weight of extract obtained (gr)}}{\text{Weight of the simple substance before extraction (gr)}} \times 100\%$$

$$Rendemen = \frac{264 \text{ grams}}{500 \text{ grams}} \times 100\%$$

Rendemen = 52 %

3). Phytochemical Screening of Resin *Styrax paralleloneurus* Extract

A phytochemical screening was performed to detect bioactive compounds in the *Styrax paralleloneurus* resin extract. The extract demonstrated the presence of alkaloids, flavonoids, saponins, tannins, and terpenoids. The results of the phytochemical screening are as follows:

Table 3. Phytochemical Screening Test Results

No	Parameter	Reagent	Results	Information
1	Flavonoid	FeCl3 5%	Black solution greenish	+
2	Alkaloid	Dragendorph	Brick red sediment	+
		Mayer	White sediment	+
3	Tannin	FeCl3 1%	Blackish green solution	+
4	Terpenoid	Liebermann- Burchard	Brownish red solution	+
5	Saponins	Aquadest	Foamy	+
6	Tannin	Fecl3 1%	Dark chocolate	-

Information:

Positive (+) = Presence of secondary metabolites Negative (-) = No secondary metabolites

4) Anti-inflammatory Test Results

The anti-inflammatory efficacy of *Styrax paralleloneurus* resin extract on incision wounds in male white mice was investigated. Results indicated that wounds treated twice daily (09:00 and 15:00) for 10 days demonstrated significant closure at a 15% concentration. This therapeutic effect is attributed to the increased concentration of bioactive compounds, particularly flavonoids, saponins, tannins, and glycosides, which facilitate wound healing by minimizing bacterial contamination (Prasongko, 2020). Average Anti-Inflammatory Test Results. Table 4: *Styrax paralleloneurus* Resin Preparation of Anti-Inflammatory Test Results:

Table 4. Average observation of incision wound length (mm)

Group	3	4	5	6	7	8	9	10
Betadine	8.54	8.28	7.72	6.86	5.46	4.24	2.82	0.94
Negative	9.44	8.70	8.32	7.96	7.38	6.9	6.36	5.46
5%	9.46	8.94	8.44	7.86	7.2	6.6	6.16	5.38
10%	8.83	7.14	6.52	5.9	5.24	4.42	3.12	2
15%	8.78	7.4	6.8	5.3	4.68	4.02	2.9	1.62

The statistical analysis using One-Way ANOVA revealed normally distributed data with homogeneous variance. The p-value (0.138 > 0.05) indicates significant differences among *Styrax paralleloneurus* resin extract preparations (5%, 10%, and 15%). The 15% concentration demonstrated accelerated wound healing in male white mice, with wound length reduction proportional to extract concentration. These findings align with Ginting's (2022) research on 15% frankincense extract preparations' efficacy in wound healing.

5. CONCLUSION

Based on the research conducted on *Styrax paralleloneurus* resin extract preparations, the following conclusions can be drawn: *Styrax paralleloneurus* resin extract exhibits anti-inflammatory effects on incision wounds in male white mice. The 15% concentration of *Styrax paralleloneurus* resin extract demonstrates the fastest anti-inflammatory effect on incision wounds in male white mice.

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