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Effectiveness of gel formulation of mahogany (*Swietenia macrophylla*) bark extract and its potential as an anti-inflammatory in white male rats (*Rattus norvegicus*)

SHAFA GEULISTIA NURANI[1], NAFASYA NEGIY DELUNA[1], PUTRI NABILA[2], SYAMSUL FALAH[1,♥]

[1]Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl Tanjung, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia. Tel./fax.: +62-251-8423267, ♥email: syamsulfa@apps.ipb.ac.id

[2]Department of Forest Resources Conservation and Ecotourism, Faculty of Forestry and Environment, Institut Pertanian Bogor. Jl. Ulin Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia

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Abstract. *Nurani SG, Deluna NN, Nabila P, Falah S. 2022. Effectiveness of gel formulation of mahogany (*Swietenia macrophylla*) bark extract and its potential as an anti-inflammatory in white male rats (*Rattus norvegicus*). Nusantara Bioscience 14: 117-121.* Mahogany bark contains phytochemical compounds that have anti-inflammatory activity. This study aims to determine the anti-inflammatory activity of mahogany-bark extract gel formulation in-vivo. Gel formulations of 96% ethanol extract of mahogany bark at different concentrations were tested for anti-inflammatory activity on the paws of rats induced by 1% carrageenan. The data observed were the thickness of the foot edema of rats. Positive control was anti-inflammatory drugs (Desoximetasone), and negative control was the gel formulation without extract. Data on the thickness of rat paw edema were analyzed using the ANOVA test. The results of the anti-inflammatory test showed that gel formulation 2 with an extract concentration of 10% had a thickness reduction value of 41.83%, which was not significantly different from the positive control treatment of 44.38%. The results of the dispersion test showed that formulation 2 and formulation 3 had good dispersibility, as suggested in SNI, and all gel formulations had good adhesion. Based on these results, it was concluded that formulation 2 was the most effective in reducing rat paw edema.

Keywords: Anti-inflammatory, gel formulation, in vivo test, *Swietenia macrophylla*

INTRODUCTION

Indonesia is a country that has high biodiversity, one of which is plant diversity. About 30,000 plant species in Indonesia, and 9,600 are medicinal plants (Novaryatiin and Indah 2019). The use of medicinal plants in Indonesia has been known for a long time in some communities. For example, Indonesian people used to consume jamu as herbal medicine. Based on the traditional utilization, jamu is being developed into a therapeutic formulation using phytopharmaceuticals by herbal practitioners (Sangat and Larashati 2002; Woerdenbag and Kayser 2014; Fathir et al. 2021; Utaminingrum et al. 2022).

Mahogany (*Swietenia macrophylla* G.King, family Meliaceae) is a medicinally important plant indigenous to tropical and subtropical. It is commonly found in Indonesia. The plant parts commonly used as medicine are fruits, seeds, and leaves. Previous studies indicate various pharmacological activities of *S. macrophylla*, which exhibit antimicrobial, anti-inflammatory, antioxidant effects, antimutagenic, anticancer, antitumor, and antidiabetic activities (Telrandhe et al. 2022). In addition, various other activities include anti-nociceptive, hypolipidemic, antidiarrhoeal, and anti-infective, antiviral, antimalarial, acaricidal, antifeedant, and heavy metal phytoremediation activity (Moghadamtousi et al. 2013).

Wood processing activities produce bark as a waste that is rarely used. Bark as a waste or by-product has benefits as other plant parts. Mahogany bark extract could serve as a suitable adjuvant and promising antidiabetic phytomedicines (Falah et al. 2010). In addition, mahogany bark also has anti-inflammatory activity due to alkaloids, flavonoids, saponins, and terpenoid content (Yasotha et al. 2019).

Inflammation is the body's defense mechanism to eliminate harmful agents that damage and prepare the body for tissue recovery. Characteristics of inflammation in the body include swelling/edema, redness, heat, and pain (Naclerio et al. 2010). Inflammation that occurs in the long term and is persistent is called chronic inflammation and harms the body (Bondy et al. 2021). There are two types of anti-inflammatory drugs, namely steroid and non-steroidal anti-inflammatory drugs, which can be in the form of oral and topical drugs. Topical drugs are absorbed faster because they do not pass through the digestive tract. Topical drugs are widely used for healing inflammatory skin diseases. One of the most commonly used topical drugs is the gel form due to its good drug release and cooling effect on the skin (Garg et al. 2015).

Mahogany bark extract was formulated as a topical anti-inflammatory drug in gel formulations. Therefore, this research is expected to be a source of innovation. Therefore, this research aims to evaluate the anti-inflammatory effects of mahogany bark extract gel on white male rats induced by carrageenan.

MATERIALS AND METHODS

Sample preparation

The mahogany bark was obtained from a 38-year-old mahogany tree planted in the research forest of the Research Institute for Agroforestry Technology, Ministry of Environment and Forestry in Ciamis, West Java, Indonesia. First, mahogany bark is cut into small pieces of 2 cm x 2 cm. The small pieces are then put into the Hammer Mill to make powder and filtered to get a powder size of 40-80 mesh.

Sample extraction

In this study, using the maceration method, two hundred g of mahogany bark powder with a moisture content of 7.53% was extracted with 96% ethanol solvent in a ratio of 1:10 (%w/v). Maceration was carried out for one day in a closed container and repeated three times. The filtrate was filtered using filter paper and then evaporated using a vacuum evaporator to produce concentrated ethanol extract.

Bark extract gel formulation

Three gel formulations with various extract concentrations (F1, F2, F3) were used in this study. In addition, gel formulation without extract was used as negative control (F4) (Table 1) and formulated as the previous methods (Sugihartini and Wiradhika 2017). The gel was made in two phases, namely phase A and phase B. Phase A was composed of a gel base, namely Carbopol (6 g), dissolved in 100 mL of distilled water at 70°C. Phase B consists of methylparaben (0.18 g) dissolved in a small amount of water and added with glycerin and propylene glycol. The two phases were mixed, and 20 g of distilled water was added and stirred until homogeneous.

Anti-inflammatory activity test

The in-vivo anti-inflammatory activity was performed using carrageenan-induced male white rats (*Rattus norvegicus* Berkenhout, 1769). There were five treatment groups, namely the negative control group (F4), three gel formula treatment groups (F1, F2, F3), and the positive control (F0). The positive control used corticosteroid anti-inflammatory drugs, namely Desoximetasone®. A 1% carrageenan was made to induce rat paws to cause edema. Carrageenan (0.1 g) was put into a container and dissolved in 10 mL of 0.9% NaCl.

Table 1. Gel formulation of mahogany bark extract

Materials	F1 (g)	F2 (g)	F3 (g)	F4 (g)
Bark extract	6	12	18	0
Carbopol	6	6	6	6
Glycerin	12	12	12	12
Propylene glycol	6	6	6	6
Methyl Paraben	0.18	0.18	0.18	0.18
Aquades	120	120	120	120

Rats were injected with 0.1 mL of 1% carrageenan solution intraplantar on the sole of the rat's right foot. One hour after an injection of carrageenan solution, the thickness of the rat paws was measured using a digital caliper and recorded as initial thickness (T0). Then, each group was treated topically on the soles of the feet according to the treatment group. The thickness of the rat's right paw after administration of the gel was measured every 60 minutes for 360 minutes and at 24 and 48 hours after carrageenan induction.

Gel formulation test

The gel preparation was tested by organoleptic, pH, dispersibility, and adhesion tests. Moreover, the organoleptic test was conducted by observing the texture, color, and smell. The pH test was conducted by smearing the gel preparation on a universal pH indicator. The dispersion test was carried out by placing 0.5 g of gel on a glass lined with millimeter block paper and covered with another glass that had been weighed, then adding a load of 50, 100, and 150 g and allowed to stand for one minute. The distribution diameter was recorded and repeated three times. The adhesion test was carried out by placing 0.5 g of gel on a slide and covering it with another slide which was then given a load of 1 kg for 5 minutes. Then the load weighing 100 g is released, and the time when the two slides are released is recorded and repeated three times.

Data analysis

The data were analyzed using the Analysis of Variance (ANOVA) with a 95% confidence level to determine the significant differences in the treatments. In addition, the differences between treatments were analyzed with the Least Significance Different (LSD) test.

RESULTS AND DISCUSSION

Anti-inflammatory activity

Anti-inflammatory activity was observed based on the ability of the gel to increase or decrease the edema size on the soles of the rats' paws. That observation is made every hour. However, the paws of rats experienced inflammation after being induced by carrageenan, which was characterized by swelling due to increased blood flow and edema (tumor), as well as redness (rubor) (Figure 1).

The thickness of the rat paw edema in carrageenan-induced rats was presented in Table 2. Table 2 shows that formulation two (F2) is the closest formulation to the treatment using anti-inflammatory drugs as the positive control treatment.

The results showed that formulation 2 had a percentage reduction in edema thickness of 41.93%, that not significantly different from the percentage reduction of positive control (44.38%) (Table 3). On the other hand, formulations 1,3, and 4 had a lower percentage reduction of edema thickness which was 21.21%. 30.50%. 13.78%, respectively.

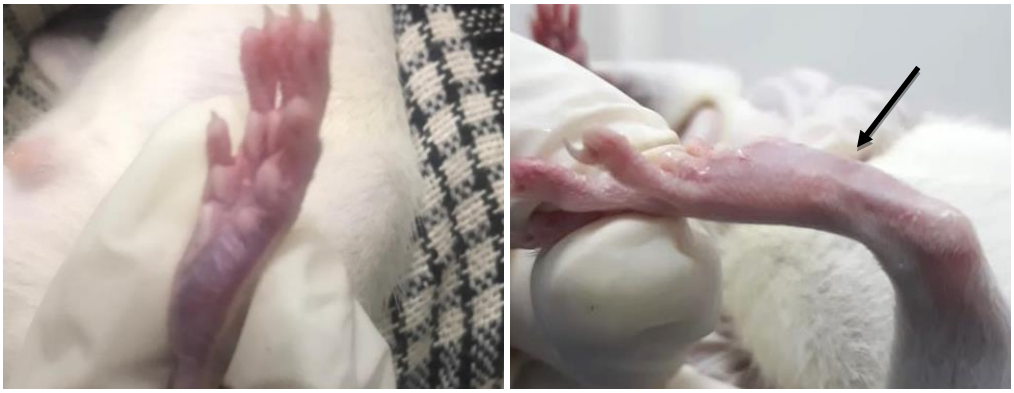

Figure 1. A. The paws of rats before carrageenan induction, B. The soles of rats' feet after carrageenan induction. Arrows indicate swelling

Table 2. The average thickness of the rat paw edema (%) in carrageenan-induced rats for two days of observation

Groups	The average thickness of edema (mm) per hour (x ± SD)								
	0	1	2	3	4	5	6	24	48
Positive control	3.28 ± 0.55	2.85 ± 0.49	2.25 ± 0.54	2.58 ± 0.39	2.78 ± 0,46	2.22 ± 0.44	2.45 ± 0.36	2.65 ± 0.64	1.80 ± 0.26
F1	2.73 ± 0.59	3.02 ± 0.66	2.68 ± 0.32	2.75 ± 0.19	3.35 ± 0.73	2.70 ± 0.51	2.45 ± 0.33	2.53 ± 0.21	2.07 ± 0.29
F2	3.40 ± 0.71	2.52 ± 0.20	2.63 ± 0.16	2.73 ± 0.42	2.13 ± 0.57	2.32 ± 0.32	2.07 ± 0.46	2.28 ± 0.23	1.85 ± 0.29
F3	2.83 ± 0.31	2.25 ± 0.24	2.58 ± 0.48	2.57 ± 0.37	2.28 ± 0.35	2.77 ± 0.37	2.27 ± 0.42	2.33 ± 0.26	1.93 ± 0.23
F4	2.73 ± 0.18	2.45 ± 0.36	2.41 ± 0.10	2.73 ± 0.87	2.82 ± 0.31	2.73 ± 0.23	2.75 ± 0.73	2.90 ± 0.29	2.35 ± 0.41

Note: Corticosteroid anti-inflammatory drug, Desoximetasone® was used as a positive control

Table 3. The percentage of the thickness of rat paw edema (%) compared to the thickness at 0-hour observation

Observation time (hour)	Groups				
	Positive control	F1	F2	F3	F4
0	100.0	100.00	100.00	100.00	100.00
1	88.90	116.30	77.46	80.36	90.14
2	70.03	100.50	81.15	92.58	88.59
3	80.65	104.44	82.25	92.61	99.68
4	86.33	127.50	62.62	81.38	103.06
5	68.99	102.34	71.89	98.72	100.01
6	77.09	91.57	62.19	81.14	100.32
24	81.24	95.57	71.64	83.48	106.07
48	55.62	78.78	58.07	69.50	86.22
∆t (0-48)	44.38^{c}	21.21^{a}	41.93^{c}	30.50^{b}	13.78^{a}

Note: Different letters indicate significant differences based on the LSD test

Table 4. The organoleptic test of the gel formulation of mahogany bark extract

Parameters	F1	F2	F3	F4
Texture	Gel	Gel	Gel	Gel
Color	Brown	Brown	Brown	Clear
Smell	Extract smell	Extract smell	Extract smell	Gel smell
pH	4	4	4	3

Gel formulation

The gel preparations were tested organoleptically by observing the texture, color, odor, and pH (Table 4). The results of the organoleptic test showed that there were differences in each gel formulation. It is due to the differences in extract concentration in gel formulations. Furthermore, increasing mahogany bark extract concentration in the gel formulation increased the gel density, darker color, a sharp odor typical of mahogany bark extract, and a higher pH. However, the pH value of the gel is still quite acidic. It is due to the acidic base of the carbopol gel. Therefore, adding other ingredients, such as triethanolamine (TEA), is necessary to increase the gel's pH.

The dispersion test resulted in the spreadability of a gel formulation of mahogany extract after loading 150 g (Figure 2). In addition, spreadability was carried out to determine the ability of the gel to spread when applied to the skin. The results of the gel dispersion in the standard range were gel formulations 2 and 3 of 6.95 cm2 and 6.60 cm2, respectively. Figure 3 shows the ability of each gel to stick in seconds in the adhesion test

Discussion

Carrageenan was chosen as an inducer of inflammation because carrageenan induction in rat paws has been widely used as a model of acute inflammation (Morris 2003). The mechanism of carrageenan-induced inflammation was as follows: the first (early) phase is about 1 hour after injection, which is associated with the release of inflammatory mediators such as histamine, bradykinin, and serotonin by mast cells in damaged tissues.

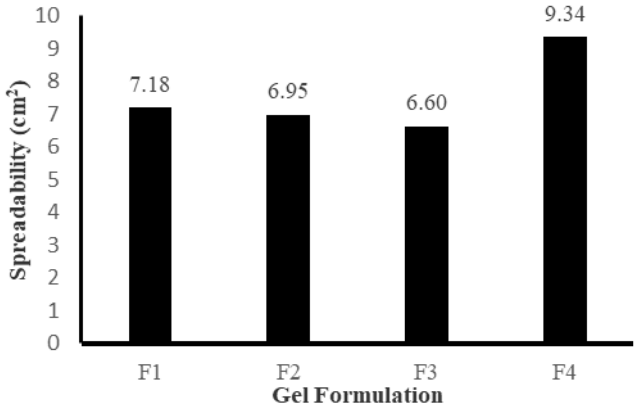

Figure 2. The spreadability of mahogany bark extract gel when added with a load of 150 g in the dispersion test

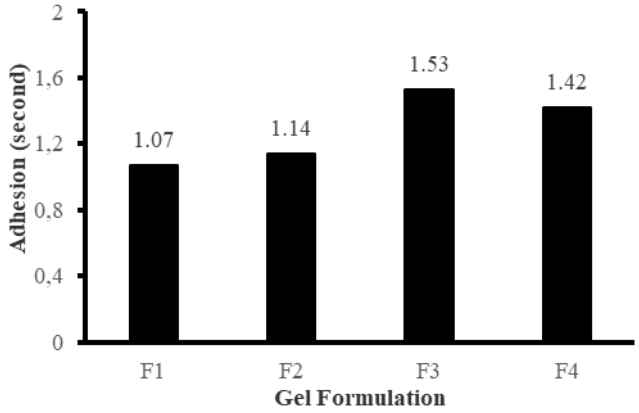

Figure 3. The adhesive properties of mahogany bark extract gel

The second phase is mainly attributed to the stage of prostaglandin production by the cyclooxygenase (COX) enzyme. This phase took about 3-6 hours after the carrageenan injection. Therefore, anti-inflammatory activity was evaluated by inhibiting the formation of these mediators, especially the product of the COX-2 enzyme. The COX-2 enzyme is an enzyme that plays a vital role in the synthesis of prostaglandin mediators as a vasodilator for edema formation (Karim et al. 2019).

Cyclooxygenase (COX) is an enzyme that regulates prostaglandins' formation from arachidonic acid, known as prostaglandin-endoperoxide synthase (PTGS). Prostaglandins are one of the mediators in the body's inflammatory response. The COX enzyme has two isoforms, namely COX-1 and COX-2. COX-1 plays a role in maintaining tissue homeostasis, synthesized essentially in various cells, and COX-2 is synthesized during inflammation induction. Therefore, COX-2 becomes the primary target inhibiting anti-inflammatory processes (Sharma et al. 2019).

COX-2 regulates the formation of prostaglandins initiated by converting arachidonic acid to prostaglandin G2 (PGG2). Followed by the peroxidase process to form prostaglandin H2 (PGH2). PGH2 is converted into several isoforms with specific functions by various specific synthase enzymes, such as TXA2, PGF2α, PGE2, PGD2, and PGI2 (Korbecki et al. 2014). Thromboxane A2 (TXA2) activates platelet aggregation and smooth muscle contraction, mainly produced from COX-1 platelets. PGI2 is synthesized by COX-1 to produce gastric mucosa and plays a role in maintaining tissue homeostasis, especially in the digestive tract. On the other hand, COX-2 is more dominant in synthesizing PGE2, leading to inflammation characterized by redness, swelling, and pain due to vasodilation of blood vessels and edema (FitzGerald and Ricciotti 2011).

Formulation 4 had the highest dispersion due to the highest extract concentration. The spreadability test showed gel formulations had a good spreadability range from 5-7 cm2. That is in the Indonesian National Standard (SNI) range number 06-2588 (Ningsih et al. 2019). Formulation 1 contains less extract than other formulations; it contains fewer phytochemical compounds that affect its anti-inflammatory activity. Formulation 3 contains more extract, but the amount of extract also affects the spreadability related to the gel's ability to deliver the drug to the skin. The increase in the extract amount decreased gel dispersion and the active substance diffusion rate through the membrane. Gels with high spreadability result in good drug delivery (Patil et al. 2019). Stickiness is also a determinant factor in gel properties (Ruis et al. 2007). The stickiness of all gel formulations is considered good because it has an average of three repetitions in one second. The requirement for good adhesion is more than one second. All gel formulations have good adhesion to the skin because they meet these requirements. Formulation 2 has good anti-inflammatory activity, pH, spreadability, and adhesion. Therefore, formulation 2 is the best gel formulation.

The anti-inflammatory activity of mahogany has been reported in seed due to the sweetening content that can downregulate the COX-2 enzyme in vitro (Mak et al. 2021). In addition, a study by Chen et al. (2015) showed that other limonoid compounds extracted from mahogany seeds inhibit nitric oxide (NO), in which the limonoid is one of the inflammatory mediators. Therefore, it can be used as an anti-inflammatory agent. Furthermore, an anti-inflammatory compound in mahogany can also be found in fruit which inhibits the superoxide anion on neutrophils (Chen et al. 2010).

Mahogany bark contains phytochemical compounds that have anti-inflammatory activity. The gel of mahogany stem bark extract at a concentration of 10% had an anti-inflammatory activity that closely resembled corticosteroid anti-inflammatory drugs. So, it can be concluded that the best gel formulation was formulation 2, with an extract concentration of 10%. Therefore, gel formulation 2 also has the potential as an anti-inflammatory herbal therapeutic drug.

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