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# Nano Spray from Sambong (Blumea balsamifera) and Caricature (Graptophyllum pictum (L.) Griff.) Leaves Extract for Antiseptic Burn Treatment and Blood Clotting Agent

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**Abstract**

***Background**: Burn wound care remains a significant medical challenge, involving pain, bleeding, scarring, and potential microbial infections. The research explores a nano spray formulated from extracts of Blumea balsamifera and Graptophyllum pictum to address these issues in wound healing. The formulation's burn healing, blood clotting, and antiseptic properties were tested and analyzed. **Methods**: The leaves of B. balsamifera and G. pictum were dried and macerated. A nano spray was prepared with a 75:25 concentration of the extracts. The nano spray was tested for burn healing on animal models, blood clotting, and antibacterial effects. **Result:** The results of the phytochemical test showed that the two ethanol extracts of the leaf contain flavonoids, tannins, and phenols. In the antibacterial test,combination of Blumea balsamifera (sambong) and Graptophyllum pictum (purple) leaf extracts showed an inhibition zone of 16.03±0.28 mm, categorized as strong inhibition. This inhibition zone was larger than that produced by the positive control, which had an inhibition zone of 12.04±0.34 mm. Meanwhile, the inhibition zone produced by nano spray was smaller, at 4.06±0.12 mm, classified as weak inhibition. The blood clotting test results showed that the use of nano spray resulted in a clotting time of 664±166 seconds. In burn healing test, By the 14th day, a clear difference in wound healing was observed between the treatment groups. **Conclusion:** The effectiveness of the nano spray combining these extracts as an antiseptic was proven by antibacterial tests. The nano spray also accelerated blood clotting, as shown by the blood clotting test results.*

**Keywords:** *nano spray, Blumea balsamifera, Graptophyllum pictum*

## INTRODUCTION

The incidence of wounds, both in Indonesia and globally, is very high. A wound is a form of tissue damage on the skin caused by trauma. Burns are the most common type of wound, requiring quick treatment. Based on the healing process, burns go through three phases: the acute phase, the subacute phase, and the late phase. In open wounds, the healing process also involves three stages: the coagulation and inflammation phase, the proliferation or reconstruction phase, and the remodeling or maturation phase.[1]

Given this, there is a need for a medicinal product that can act as an antiseptic, speed up blood clotting, and accelerate skin regeneration. Existing medications do not offer all these benefits in a single formulation, making their use somewhat inefficient, as multiple medications are required for antiseptic purposes, blood clotting acceleration, and burn wound regeneration.[2]

Sembung leaves (*Blumea balsamifera* (L) DC), from the genus *Blumea* and family Asteraceae (Compositae), are often used in traditional medicine, either as an infusion or through extraction techniques, due to their content of alkaloids and flavonoids, which have potential as antioxidants. Additionally, *Blumea balsamifera* leaves have antifungal, antimicrobial, and anti-inflammatory properties due to their high tannin, phenolic, ether, and flavonoid content.[3]

Purple leaves (*Graptophyllum pictum* L. Griff) are a hairless shrub that grows to about 1.5-3 meters tall. The purple leaves contain flavonoids, tannins, saponins, alkaloids, and glycosides, which give them benefits as antioxidants, anti-inflammatory agents, analgesics, and antibacterials (Indradi and Sartika, 2021).[2]

One of the most commonly used medical formulations is nano spray. The advantage of nano spray is that it can penetrate body tissues more easily compared to conventional sprays, which have larger particles. The smaller particle size of nano spray allows the active compounds to exert their effects on the tissues more efficiently.[2] The combination of an effective delivery system and the bioactive compounds from *Blumea balsamifera* and *Graptophyllum pictum* extracts has the potential to become a medical innovation for addressing various health problems while promoting the diversity of Indonesian medicinal plants.

## METHODS

The process began with the preparation of extracts from *Blumea balsamifera* (sambong) and *Graptophyllum pictum* (purple) leaves. The leaves were cleaned and then dried in an oven at 50°C for 24 hours. After drying, they were ground and sieved through a 20-mesh sieve to obtain a fine powder. The powdered samples were then extracted using the maceration method by weighing the leaf powder and adding 1.5 L of 90% ethanol. The mixture was left in a sealed container at room temperature for 3x24 hours, with stirring every 24 hours, followed by filtration. The extract was then re-macerated with 500 mL for 24 hours. Afterward, evaporation was performed at 60°C at 100 rpm to obtain a thick extract. This extract was then analyzed for its phytochemical content, specifically flavonoids, tannins, and phenols.

The preparation of the nano spray began with the creation of an acetic acid solution and a NaTPP solution, which were homogenized with chitosan and distilled water (aquades). These two solutions were then mixed with the extract, using the best concentration ratio.

Phytochemical tests were conducted to determine the levels of flavonoids, tannins, and phenols in the combination of *Blumea balsamifera* and *Graptophyllum pictum* extracts. The diameter of the clear zones (indicating bacterial inhibition) was recorded and compared.

The antibacterial activity test was performed using the Kirby-Bauer method. A total of 200 µL of *Streptococcus aureus* was suspended in Mueller Hinton Agar (MHA) medium and spread using a sterile cotton swab. The samples were incubated at 37°C for 24 hours. Each plate contained six sterile discs, 6 mm in diameter, each treated with 20 µL of the test substance. The antibacterial test results were interpreted and classified into four categories based on the inhibition zone diameter: weak (0-5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (> 20 mm).

The blood clotting test involved collecting 6 mL of human venous blood, spraying it with the nano spray, and vortexing it. Blood clotting time was measured every 30 seconds. The treatment group was compared to the control group, which was measured using the same method.[4]

The burn healing test was conducted on 12 male rats, aged 2-3 months, weighing 150-200 grams, divided into 3 groups. Each group contained 4 rats, which were acclimatized for 1-2 weeks. The burn wounds were created using a 1x1 cm metal heated over a spirit lamp for 1 minute, then applied to the rats for 30 seconds, causing a first-degree burn. Treatment was carried out by spraying the

nano spray with the best formulation once a day on each rat until the wound healed. The effectiveness of healing was observed based on the speed of wound healing for each extract combination compared to the untreated group.

## RESULTS

**Phytochemical Test Results**

| Extract | B. balsamifera | G. pictum |
|---|---|---|
| **Flavonoid (mg/100g)** | 5315.67 ± 52.25 | 1293.68 ± 23.75 |
| **Tanin (mg/100g)** | 5299.07 ± 15.6 | 1986.61 ± 12.46 |
| **Phenol (mg/100g)** | 4637.04 ± 92.9 | 2191.94 ± 52.43 |

Based on Table 1, *Blumea balsamifera* (sambong leaves) contains 5315.67 ± 52.25 mg/100g of flavonoids, 5299.07 ± 15.66 mg/100g of tannins, and 4637.04 ± 92.94 mg/100g of phenols. Meanwhile, *Graptophyllum pictum* (purple leaves) contains 1293.68 ± 23.75 mg/100g of flavonoids, 1986.61 ± 12.46 mg/100g of tannins, and 2191.94 ± 52.43 mg/100g of phenols.

**Antibacterial Test (Kirby-Bauer)**

| Sample | Inhibition Zone Diameter (mm) | Inhibition Zone |
|---|---|---|
| **Extract Combination** | 16.03 ± 0.28 | Strong |
| **Positive Control** | 12.04 ± 0.34 | Strong |
| **Negative Control** | 0.00 ± 0.00 | Weak |
| **PENTADINE** | 4.06 ± 0.12 | Weak |

The results from the combination of *Blumea balsamifera* (sambong) and *Graptophyllum pictum* (purple) leaf extracts showed an inhibition zone of 16.03±0.28 mm, categorized as strong inhibition. This inhibition zone was larger than that produced by the positive control, which had an inhibition zone of 12.04±0.34 mm, also categorized as strong inhibition. Meanwhile, the inhibition zone produced by PENTADINE nano spray was smaller, at 4.06±0.12 mm, classified as weak inhibition.

**Blood Clotting Time (Lee and White)**

| Research Subject | Results (s) |
|---|---|
| The testing time using PENTADINE | 664 ± 166 |
| The testing time without using PENTADINE (Negative Control) | 816 ± 261 |

The blood clotting test results showed that the use of PENTADINE nano spray resulted in a clotting time of 664±166 seconds. This was faster compared to the negative control, which had a clotting time of 816±261 seconds.

**Burn Wound Healing Test on Male Rats**

The burn wound healing test on male rats was conducted with 3 sample trials, and observations were made daily over 24 days. By the 14th day, a clear difference in wound healing was observed between the treatment groups.

## DISCUSSION

**Phytochemical Test of *B. balsamifera* and *G. pictum* Leaves Extract**

The results showed that *Blumea balsamifera* leaves contained 5315.67 ± 52.25 mg/100g of flavonoids, 5299.07 ± 15.66 mg/100g of tannins, and 4637.04 ± 92.94 mg/100g of phenols. Meanwhile, *Graptophyllum pictum* leaves contained

1293.68 ± 23.75 mg/100g of flavonoids, 1986.61 ± 12.46 mg/100g of tannins, and 2191.94 ± 52.43 mg/100g of phenols. *Blumea balsamifera* leaves have higher levels of flavonoids, tannins, and phenols compared to *Graptophyllum pictum* leaves. *Blumea balsamifera* grows in areas with sufficient sunlight, which tends to increase its flavonoid content.[5] Flavonoids can act as antibacterial, anti-inflammatory, antioxidant, and antidiabetic agents. In wound healing, flavonoids enhance collagen deposition, accelerate epithelialization, and aid in collagen tissue formation.[6] Additionally, the tannin content in *Graptophyllum pictum* can function as an astringent, helping tighten skin tissues and speeding up the cessation of bleeding.[2]

**Antibacterial Test (Kirby Bauer)**

The antibacterial diffusion test using gram-positive *Staphylococcus aureus* showed an inhibition zone, indicating the presence of antibacterial substances. This is consistent with a study by Sofianti et al (2023), which demonstrated that kombucha fermentation with butterfly pea flowers contained secondary metabolites such as alkaloids, flavonoids, and saponins. These secondary metabolites have the potential to inhibit the growth of test bacteria.[7] Based on the antibacterial test, the combination of *Blumea balsamifera* and *Graptophyllum pictum* extracts showed significant inhibition of *S. aureus*. The more *Blumea balsamifera* and *Graptophyllum pictum* were added to the nano spray, the stronger the antibacterial activity. The antibacterial effect in the inhibition zone was due to the active compounds in the extracts, such as phenols, flavonoids, and tannins.

**Blood Clotting Test (Lee and White)**

The blood clotting time was measured using the Lee-White method.[8] This method is based on the principle that when blood contacts glass, clotting factor XII and platelets are activated through the intrinsic pathway, leading to fibrin formation. Normal blood clots within 3 to 18 minutes at 37°C, which is considered the optimal temperature for reactions similar to those in the human body. The test showed that the nano spray with PENTADINE had a shorter or faster blood clotting time compared to without the nano spray. The in vitro blood clotting time can be affected by several factors. Prolonged clotting time may occur due to agitation of the test tube during the examination, which can cause blood lysis.[9]

**Burn Wound Healing Test on Male Rats**

On the 14th day of the burn wound healing test, a clear difference was observed between the treatment groups. The wound began to dry due to secondary metabolites such as flavonoids, saponins, and tannins, which play a role in the inflammatory phase with antimicrobial properties, helping to prevent and treat infections.[9] These antimicrobial properties work by directly destroying pathogens and reducing local inflammation and tissue damage.[10]

## CONCLUSION

The extracts of *Graptophyllum pictum* and *Blumea balsamifera* contain bioactive compounds, including flavonoids, tannins, and phenols. The effectiveness of the nano spray combining these extracts as an antiseptic was proven by antibacterial tests. The nano spray also accelerated blood clotting, as shown by the blood clotting test results. Furthermore, the nano spray accelerated burn wound regeneration, as observed in the wound healing test.

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