Indonesian Journal of Pharmaceutical Science and Technology
Journal Homepage : http://jurnal.unpad.ac.id/ijpst/
*Research Article*

# Antioxidant and Antibacterial Activity of Extract Combination from *Sonneratia alba* and *Heterotrigona itama* Propolis

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Submitted 01 April 2024; Revised 16 October 2024; Accepted 20 October 2024; Published 26 December 2024

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**Abstract**

*Sonneratia alba* or rambai leaves are a plant from East Kalimantan that the community has used for generations as a cold powder. Antioxidants are needed to ward off free radicals, while bacteria are one of the causes of acne. Propolis also shows potential as an antioxidant and antibacterial agent. This research evaluated whether the combination of *Sonneratia alba* leaves extract and *Heterotrigona itama* propolis can increase antioxidant and antibacterial activity and whether this combination has synergistic potential. The *S. alba* leaves and *H. itama* propolis were macerated using ethanol as the solvent. The antioxidant activity test using the DPPH method with three comparisons of 1:1, 1:2, and 2:1 produced $IC_{50}$ values of 12.669 µg/ml, 32.756 µg/ml, and 19.694 µg/ml, respectively. The $IC_{50}$ value for ascorbic acid is 12.293 µg/ml. The results show that the combination of *S. alba* leaves and *H. itama* propolis ethanol extract has very strong antioxidant activity. The antibacterial activity test against *Staphylococcus aureus* bacteria with three concentrations of 60%, 80%, and 100% showed inhibition zones of 10.5 mm (medium category), 16 mm, and 17.5 mm (strong category), respectively.

**Keywords:** Antibacterial, Antioxidant, *Heterotrigona itama*, Propolis, *Sonneratia alba*.

# Aktivitas Antioksidan dan Antibakteri Kombinasi Ekstrak *Sonneratia alba* dan *Heterotrigona itama* Propolis

**Abstrak**

Daun rambai atau *Sonneratia alba* adalah tanaman asal Kalimantan Timur yang secara turun temurun dimanfaatkan masyarakat sebagai bedak dingin. Di sisi lain propolis juga menunjukkan potensi sebagai antioksidan dan antibakteri. Antioksidan diperlukan dalam menangkal radikal bebas, sedangkan bakteri merupakan salah satu penyebab terjadinya jerawat. Penelitian ini dilakukan untuk mengevaluasi apakah kombinasi ekstrak daun *Sonneratia alba* dan propolis *Heterotrigona itama* dapat meningkatkan aktivitas antioksidan dan antibakteri serta apakah kedua kombinasi tersebut mempunyai potensi sinergis. Daun *S. alba* dan propolis *H. itama* dilakukan ekstraksi menggunakan pelarut etanol. Uji aktivitas antioksidan menggunakan metode DPPH dengan tiga perbandingan 1:1, 1:2, dan 2:1 dengan menghasilkan nilai $IC_{50}$ masing-masing sebesar 12,669 µg/ml, 32,756 µg/ml, dan 19,694 µg/ml. Nilai $IC_{50}$ asam askorbat adalah 12,293 µg/ml. Hasil penelitian menunjukkan bahwa kombinasi ekstrak daun *S. alba* dan ekstrak etanol propolis *H. itama* mempunyai aktivitas antioksidan yang sangat kuat. Uji aktivitas antibakteri terhadap bakteri Staphylococcus aureus dengan tiga perbandingan konsentrasi yaitu 60%, 80%, dan 100% menunjukkan zona hambat masing-masing sebesar 10,5 mm (kategor sedang), 16 mm dan 17,5 mm (kategori kuat).

**Kata Kunci:** Antibakteri, Antioksidan, *Heterotrigona itama,* Propolis, *Sonneratia alba*.

## 1. Introduction

Recently, issues related to free radicals and bacterial infections have become an important focus of research in improving people's quality of life. Environmental pollution and unhealthy eating patterns have caused an increase in free radicals in the human body.[1] Free radicals are a natural product of the body's metabolism and play a role in the immune system. However, problems occur when too many free radicals are formed, causing the body to be unable to deal with the situation. Free radicals themselves can damage cells and body tissue in various diseases. Therefore, our bodies need substances that can help protect the body from free radical attacks. One of the natural or man-made substances that can prevent free radicals is antioxidants. The human body also produces antioxidants, namely the enzyme glutathione peroxidase and superoxide dismutase, proteins such as ferritin and ceruloplasmin, vitamin C, and vitamin E.[2] However, the antioxidant compounds produced by the body are not yet entirely sufficient to ward off free radicals that occur. Therefore, it is necessary to discover and develop effective sources of natural antioxidants, mainly to prevent and treat these diseases. In addition to antioxidants in maintaining healthy skin and for cosmetic use, antibacterials are needed to avoid acne on the skin.[3]

Faced with this trend of skin health problems, research on natural compounds with antioxidant and antibacterial activity is increasingly of interest. Combining two natural ingredients can increase bioactivity if the appropriate formulation is carried out. *Sonneratia alba* leaves and *Heterotrigona itama* propolis has been known to contain bioactive compounds with antioxidant and antimicrobial properties. *Sonneratia alba* or rambai leaves contain compounds such as terpenoids, saponins, and tannins. These compounds have various potential bioactivities.[4]

Meanwhile, *Trigona sp* propolis is now known as a natural product that is very beneficial for the world of health. *H. itama* is a species of stingless bee that lives in nests in tree holes, rock cavities, people's houses, and forests. The composition of propolis is influenced by species, age, climate, and the time at which propolis can be obtained. The H. itama bee species produces more abundant propolis than other propolis-producing bees.[5] Propolis contains several compounds consisting of amino acids, terpenoids, and Polyphenols. This compound has effects as an antioxidant, anticancer, anti-inflammatory, anti-allergic, antiviral, and antibacterial.[6]

No research has explored the bioactivity of the combination of these two ingredients. Therefore, this research focused on testing the antioxidant and antibacterial activity of a combination of *S. alba* leaf extract and *H. itama* propolis ethanol extract. This research will contribute to further understanding of the synergistic potential of combining the two extracts to fight free radicals and bacterial infections. It is hoped that this research can provide new insights into the development of natural therapies that effectively overcome current health problems related to free radicals and bacterial infections. Apart from that, it is also hoped that this research will open up opportunities for developing environmentally friendly natural products as an alternative to improve the quality of life and public health.

## 2. Methods

### 2.1. Tools

The extraction was performed using various tools, including a rotary evaporator (Buchi Interface I-100 from China), volumetric flasks and beaker glass (Iwaki), a UV-Vis spectrophotometer (Genesys 10s UV-Vis from Germany), a vortex mixer (Scilogex MX-S from North America), micropipettes (Scilogex from North America).

### 2.2. Materials

The materials used in this research were *S. alba* or rambai leaves, *H. itama* propolis (both collected from Sangkulirang and Samarinda), 70% ethanol, 96% ethanol, PA methanol, distilled water, Clindamycin, Staphylococcus aureus bacteria, DPPH (1,1-diphenyl-2-picrylhydrazyl).

2.3. Procedures

2.3.1. Determination

The first step of this research was to determine the species of *Sonneratia alba*, or rambai leaves, which was carried out at the Tropical Forest Biodiversity Ecology and Conservation Laboratory, Faculty of Forestry, Mulawarman University, Samarinda.

2.3.2. Sample Preparation and Extraction

*S. alba* leaves were obtained from the riverbank of Sangkulirang District, collected, and cleaned after being dried. A 70% ethanol solvent was used for extraction (3 × 24 h). The filtrate was evaporated using a rotary evaporator to obtain the crude extract.

*H. itama* propolis was obtained from Mugirejo Village and cleaned. The raw extract was crushed, put into a glass jar, and macerated using a 96% ethanol solvent for 72 hours (3 days). Then, it evaporated until the propolis was extracted.

2.3.3. Extract Yield

The yield of rambai leaf extract (*S. alba*) and ethanol extract of propolis (*H. Saitama*) was obtained through an extraction process using the maceration method. The solvent used was 70% ethanol. The yield of the thick extract obtained was calculated as follows:[7]

$$\text{Extract yield} = \frac{\text{Total Weight of Extract}}{\text{Total Weight of Simplicia}} \times 100\ \%$$

2.3.4. Antioxidant Activity Determination

The extract of rambai leaves (*S. alba*) and the ethanol extract of propolis (*H. itama*) were added in the ratio (1:1), (1:2), (2:1). That combination of *S. alba* leaves extract, and *H. itama* propolis ethanol extract made a stock solution with five concentrations each including 20, 40, 60, 80, 100 ug/mL. The ascorbic acid concentration was 200, 400, 600, 800, 1000 ug/ml. Next, a DPPH solution was prepared by weighing 5 mg of DPPH, after which methanol was added to 50 mL.

The formula was prepared into five concentrations, 3 mL of DPPH solution was added into a 50 mL measuring flask wrapped in aluminum foil, then homogenized and left to stand in a dark room at room temperature for 30 minutes. After that, the absorbance was measured at the maximum DPPH wavelength of 517 nm using a UV-visible spectrophotometer, and then it was continued to determine the percentage of inhibition.[8]

2.3.5. Antioxidant Data Analysis

Measurement of the antioxidant activity of the combination of rambai leaves extract (*S. alba*) and propolis ethanol extract (*H. itama*) was carried out by analyzing the absorbance data measured using UV-Vis Spectrophotometry with the application of the linear regression equation (y = ax + b). After this stage, antioxidant activity was calculated based on percentage (%) inhibition. Calculation of the % inhibition value can be done by applying the mathematical formula below.

$$(\%)\ \text{Inhibition} = \frac{\text{DPPH Abs.} - \text{Sample Abs.}}{\text{DPPH Abs.}} \times 100\ \%$$

The antioxidant activity of the combination of rambai leaf extract (*S. alba*) and propolis ethanol extract (*H. itama*) was measured using the $IC_{50}$ (50% inhibition concentration) parameter. The $IC_{50}$ value indicates the sample concentration required to capture 50% DPPH free radicals during the absorbance measurement period (operating time).[9]

2.3.6. Antibacterial Determination

To determine antibacterial activity, Staphylococcus aureus bacteria were streaked on the media and incubated at 37°C for 24 hours.[10] Clindamycin was used as a positive control.

The method used for antibacterial activity in this research involved the disc diffusion method for bacteria *S. aureus* 15-20 mL of Nutrient Agar (NA), which is poured into each petri dish and allowed to solidify.[11] Next, 0.1 mL of the S. aureus bacterial suspension was inoculated onto the surface of the agar medium and then spread evenly using a cotton swab. Disc paper with a diameter of 6 mm was soaked in a test solution of a combination of rambai leaves extract and propolis ethanol extract with various concentrations of 60%,

**Table 1.** Category of Bacterial Inhibition Zone (mm)[14]

| Category | Inhibition Zone (mm) |
|---|---|
| Very strong | > 20 mm |
| Strong | 10 - 20 mm |
| Currently | 5 - 10 mm |
| Weak | < 5 mm |

80%, and 100% (w/v). As a positive control, clindamycin 2% (w/v) gel was used, while for the negative control, sterile distilled water was used with equivalent concentrations, namely 60%, 80%, and 100%. The soaked paper disc is then placed on the surface of the media inoculated with *S. aureus* bacteria using sterile tweezers, then slightly pressed, and incubated at 37°C for 24 h. This process is repeated up to 3 times.[12] The diameter of the inhibition zone at each concentration and the positive control and negative control were observed as an indicator of antibacterial activity. The clear zone formed around the disc paper was measured using a caliper. Inhibition zone categories are classified based on the results of clear zone diameter measurements (Table 1).[13] The formula used to calculate the bacterial inhibition diameter:

$$\text{Inhibition Zone (mm)} = \frac{(DV-DC)+(DH-DC)}{2}$$

Information :
DV = Horizontal Area
DH = Vertical Area
DC = Disc Area (Paper Disc)

2.3.7. Antibacterial Data Analysis

The results of the antibacterial activity test data for a combination of *S. alba* rambai leaves extract, and *H. itama* propolis ethanol extract obtained were processed statistically using the GraphPad application with the one-way ANOVA method.

## 3. Result

3.1. Extraction Results

The extraction results of samples of rambai leaves extract (*S. alba*), and propolis ethanol extract (*H. itama*) are expressed in yield values (%). Table 2 displayed the total % yield value of the combination of *S. alba* leaves extract and *H. itama* propolis ethanol extract.

3.2. Antioxidant Activity

The antioxidant activity of the combination of *Sonneratia alba* rambai leaves extract and H. itama propolis ethanol extract (EDREP) was measured by measuring DPPH free radicals by adding the extracts in several ratios (1:1, 1:2, and 2:1) using a UV-Vis Spectrophotometer. The results of the antioxidant activity are shown in Figure 1.

The results of the $IC_{50}$ value of ascorbic acid showed an inhibitory activity of 12.293 ppm. Meanwhile, measuring the $IC_{50}$ results for a combination of rambai leaves extract (*Sonneratia alba*) and propolis ethanol extract (*Heterotrigona itam*a) in ratio 1:1 showed an $IC_{50}$ value of 12.669 ppm, a Formula (1:2) showed an $IC_{50}$ value of 32.756 ppm. A of Formula (2:1) showed an $IC_{50}$ value of 19.694 ppm. It can be noted that the three EDREP combination formulas that have the best $IC_{50}$ are Formula 1:1. This can be interpreted that the smaller the $IC_{50}$ value (< 50 µg/ml) in the extract, the greater the antioxidant potential of the extract in inhibiting free radicals.

3.3. Antibacterial Activity

The antibacterial activity of the combination of *S. alba* leaves extract and *H. itama* propolis ethanol extract (EDREP) was determined three times by measuring the diameter of the inhibition zone for the growth

**Table 2.** Extract Sample Yield

| Sample | Simplicia (g) | Extract (g) | Yield (%) | Organoleptic |
|---|---|---|---|---|
| *S. alba* leaves | 342.2 | 184.84 | 9.4 | Greenish brown, Thick |
| *H. itama* propolis | 684.9 | 205.80 | 7.1 | Brown, thick |

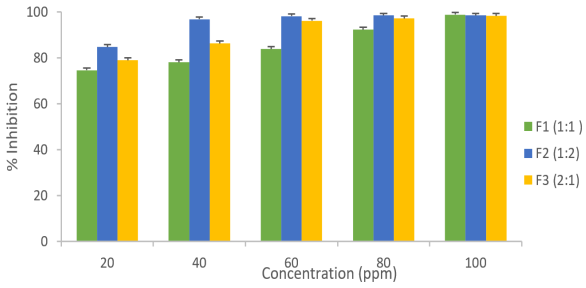



**Figure 1.** Antioxidant Activity of Formula Combination from *S. alba* leaves extract: *H. itama* propolis (EDREP)

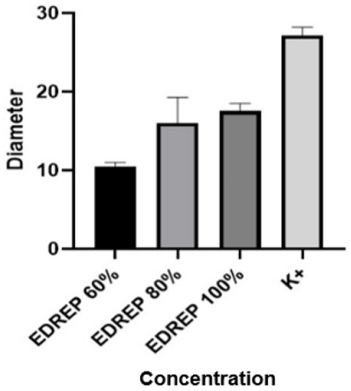



**Figure 2.** Inhibition Zone of EDREP against *Staphylococcus aureus* (K+ = Clindamycin)
(* significantly different, ($p<0.05$))

of *Staphylococcus aureus* bacteria at various extract concentrations (60%, 80%, and 100%) using the disc diffusion method. For the positive control test, 0.1% clindamycin was used, and sterile distilled water was used for the negative control test.

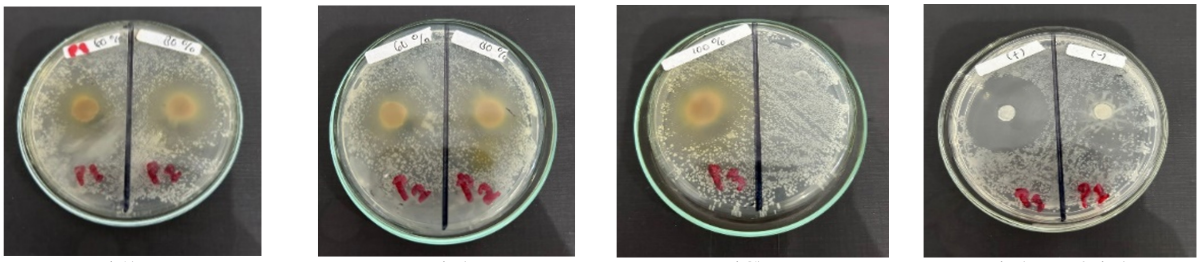
The diameter of the inhibition zone is the sensitivity of the tested bacteria. The larger the inhibition zone, the greater the antibacterial activity. In Figure 2, there are differences in the diameter of the inhibition zones for each group. Clindamycin had an average inhibitory zone diameter of 27.5 mm, while the EDREP group with concentrations of 60%, 80%, and 100% had an inhibitory zone diameter of 10.5 mm, 16 mm, and 17.5 mm. Visualization of the inhibition zone of the formula can be seen in Figure 3.

## 4. Discussion

Most free radicals are formed in many types. One of the free radicals that can damage biological systems is known as reactive oxygen species (ROS), the oxygen-derived free radicals resulting from the homolytic breakdown of the covalent bonds of a molecule or the lone pair of electrons of an atom.[15] Endogenous and exogenous factors can be sources of ROS. Endogenous factors include cell metabolism, mitochondria, and inflammatory cell activation. Meanwhile, exogenous factors include exposure outside the body, such as environmental pollution, radiation, bacterial infections, fungi, and viruses.[16]

The differences between the results of the combination of extract and ascorbic acid may be due to the unique properties of ascorbic acid, a natural antioxidant compound often used as a comparison in antioxidant tests. Ascorbic acid is a powerful antioxidant that can efficiently neutralize free radicals. It has four hydroxyl groups, while vitamin E has only one, and vitamin A has no hydroxyl groups. Therefore, the antioxidant activity of ascorbic acid is considered strong compared to that of vitamins A and E.[17]

Based on the results of the one-way



***(A)*** ***(B)*** ***(C)*** ***(D) and (E)***

**Figure 3.** Inhibition Zone of Extract Combination from *S. alba* Leaves and *H. itama* Propolis against *Staphylococcus aureus* Bacteria. (A) EDREP 60%, (B) EDREP 80%, (C) EDREP 100%, (D) Clindamycin 0.1% (left side), (E) Control (-) (right side)

ANOVA analysis of concentrations among the combinations of EDREP, positive control, and negative control, significant values were obtained. Meanwhile, 80% concentration and 100% concentration obtained insignificant results ($p>0.05$). Overall, there is a substantial difference in antibacterial inhibition between treatment groups.[18]

Several factors that influence the formation of inhibition zones include the turbidity of the bacterial suspension. If the bacterial suspension is more turbid, the diameter of the resulting inhibition zone will tend to be larger, and vice versa. Temperature or incubation temperature also plays a role in forming the inhibition zone. If the incubation temperature is less than 35°C, the diameter of the inhibition zone formed tends to be more significant. Another influencing factor is the thickness of the agar medium, where the effective medium thickness for forming an inhibition zone is usually around 4 mm.[19]

The activity of the combination formula of *S. alba* and *H. itama* propolis shows good antioxidant and antibacterial activity. The content of phenol compounds in propolis has the potential for bioactivity that increases with the addition of concentration.[20] Stingless bee propolis takes its raw materials from the sap exudate of various trees processed by the bees into a mixture.[21] Therefore, the content of compounds in the propolis is diverse and complementary, and combining the two natural ingredients can cause a synergistic effect that increases its bioactivity. The combination formula can be developed into a cosmetic preparation sourced from natural ingredients.

## 5. Conclusion

The combination of *S. alba* leaves extract and *H. itama* propolis ethanol extract from three ratios, 1:1, 1:2, and 2:1, each has antioxidant activity. Formula 2 (1:2) has higher antioxidant activity 84 % since 20 ppm concentration, compared to Formula 3 (2:1) 79 % and Formula 1 (1:1) 75 %. Antibacterial activity of the combination of *S. alba* leaves extract and ethanol extract of *H. itama* propolis from a concentration of 60% has an inhibition zone of 10.5 mm in the medium category (5-10 mm), while a concentration of 80% and a concentration of 100% have an inhibition zone of 16 mm and 17.5 mm in the strong category (10-20 mm). Combining the two extracts shows potential antibacterial activity against *Staphylococcus aureus*.

**Acknowledgements**

The authors thank Rendri Arista Avimaro for providing a propolis sample. This work was financially funded by the KedaiReka 2023 grants (Contract ID: 62/E1/HK.02.02/2023).

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