

The Antibacterial Effect of Sumbawa Honey (*Apis dorsata*) against The Growth of *Staphylococcus aureus* isolate (ATCC 25923)

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ABSTRACT

Introduction: *Staphylococcus aureus* is recognized as a highly pathogenic microorganism within the Gram-positive bacterial group, responsible for both nosocomial and community-acquired infections. Another issue of concern pertains to the rise and spread of nosocomial organisms exhibiting heightened resistance to antimicrobial agents. In Indonesia, the prevalence of *Staphylococcus aureus* infection accounts for 28% of the total cases of infection and exhibits an upward trend on an annual basis. The escalation in treatment and care expenses poses a significant challenge for global healthcare. Sumbawa honey (*Apis dorsata*) presents itself as a viable natural antimicrobial option that exhibits the potential to mitigate the resistance tendencies observed in *Staphylococcus aureus*, surpassing the efficacy of conventional antibiotics.

In this study, we employed various methodologies to investigate the research question at hand.

Methods : It is a laboratory experimental test to analyze the comparative effectiveness of Sumbawa honey (*Apis dorsata*) as an antibacterial therapy on *Staphylococcus aureus* isolates (ATCC 25923).

Results: The findings of the study indicate that Sumbawa honey (*Apis dorsata*) exhibited antibacterial properties against the growth of *Staphylococcus aureus* (ATCC 25923). The inhibition zone observed was 8 mm at a concentration of 100%, 18.4 mm at a concentration of 75%, 9 mm at a concentration of 50%, and 5.4 mm at a concentration of 25%. In the study, Vancomycin was employed as a positive control to elicit a positive effect in the form of an inhibition zone measuring 23 mm. The antibacterial efficacy of Sumbawa honey (*Apis dorsata*) is found to be higher at a concentration of 75% compared to 100% concentration. This can be attributed to the honey's elevated viscosity, which poses challenges in its penetration into the disc paper and subsequent diffusion into the surrounding media. Sumbawa honey (*Apis dorsata*) exhibits antibacterial properties due to the presence of tannins, flavonoids, saponins, and steroids.

Conclusion: Sumbawa Honey (*Apis dorsata*) concentration of 25%, 50%, 75%, and 100% exhibit inhibitory effects on the growth of *Staphylococcus aureus* isolates (ATCC 25923). The Sumbawa honey with a concentration of 75%, exhibits the most potent antibacterial activity in inhibiting the growth of *Staphylococcus aureus* isolates.

Keywords: Sumbawa honey (*Apis dorsata*), *Staphylococcus aureus*, zone of inhibition

INTRODUCTION

Staphylococcus aureus is widely recognized as a highly pathogenic microorganism within the Gram-positive bacterial group, known for its propensity to cause both nosocomial and community-acquired infections. *Staphylococcus aureus* is classified as an opportunistic bacterium that typically resides within the human microflora. However, it exhibits a propensity to initiate

infection when the host has impaired immune system.¹ The incidence of *Staphylococcus aureus* in Indonesia reaches 28% of all cases of infection and will continue to increase every year.²

The prevalence of *Staphylococcus aureus* infection and the emergence of antibacterial resistance contribute to elevated hospitalization expenses and heightened rates of patient morbidity and mortality. Sumbawa Honey (*Apis dorsata*) presents itself as a viable and cost-effective natural alternative that possesses the ability to mitigate the resistance patterns of *Staphylococcus aureus*, in contrast to conventional antibiotics. This is attributed to the inherent antibacterial properties of honey, which effectively hinder the proliferation of *Staphylococcus aureus*.³

Sumbawa honey (*Apis dorsata*) is known to possess antibacterial properties due to the presence of several bioactive compounds including hydrogen peroxide, flavonoids, essential oils, and a variety of other organic compounds. The antibacterial properties of honey are influenced by factors such as high osmolarity effects, low water activity, and low pH, resulting in a high level of acidity in honey.⁴ According to several studies, honey has been found to contain natural antibacterial compounds such as flavonoids, saponins, and tannins, which exhibit antibacterial properties.⁵⁻⁹

METHODS

A laboratory experiment was conducted at the Microbiology and Pharmacology laboratory, Faculty of Medicine, Universitas Mataram, from March to August 2023. The aim of the experiment was to investigate the potential antibacterial effect of Sumbawa Honey (*Apis dorsata*) against *Staphylococcus aureus* isolates (ATCC 25923). The Sumbawa Honey has obtained certification under the identification number IDM000168988. Honey samples were prepared with varying concentrations of 25%, 50%, 75%, and 100%. A positive control was included using Vancomycin, while a negative control was prepared using Aquadest. A total of four replicants have been utilized for replication purposes.

The data was subjected to analysis using a one-way analysis of variance (ANOVA) statistical test, employing the SPSS (Statistical Product of Service Solution) software. The objective was to investigate the impact of Sumbawa as an antibacterial agent on the growth of *Staphylococcus aureus* (ATCC 25923).

RESULTS

In general, the outcomes of antibacterial research experiments indicated that different concentrations of the Sumbawa honey exhibited inhibitory effects on *Staphylococcus aureus* (ATCC 25923). The zone of inhibition test demonstrates the inhibition of bacterial growth.

Table 1. The zone of inhibition test of the Sumbawa Honey (*Apis dorsata*) against *Staphylococcus aureus* (ATCC 25923)

Sample Test	Replication				Total	Median (mm)
	I	II	III	IV		
Honey 25%	3 mm	4,5 mm	6,5 mm	7,5 mm	21,5 mm	5,4 mm
Honey 50%	6 mm	7 mm	8 mm	15 mm	36 mm	9 mm
Honey 75%	16,5 mm	16,5 mm	19 mm	21,5 mm	73,5 mm	18,4 mm
Honey 100%	4 mm	8 mm	10 mm	10 mm	32 mm	8 mm
Vancomycin	22 mm	23 mm	23 mm	24 mm	92 mm	23 mm
Aquadest	0	0	0	0	0	0

The inhibitory effects of Sumbawa Honey at concentrations of 25%, 50%, 75%, and 100% were observed in relation to the growth inhibition of *Staphylococcus aureus* (ATCC 25923). The highest level of antibacterial efficacy is observed in Sumbawa Honey when used at a concentration of 75%. The findings from the organoleptic tests (Table 2) indicate that the aroma, flavor, color, and viscosity of Sumbawa Honey (*Apis dorsata*) meet the quality standards established by the National Standardization Agency in Indonesia. These standards serve as a reference to ensure the quality and safety of honey available in the market.

Table 2. Organoleptic tests on Sumbawa Honey (*Apis dorsata*)

Test Type	Characteristics
Smell	Typical honey
Taste	Typical honey
Color	Typical honey
Consistency	Typical honey

Phytochemical screening was conducted to identify the constituent compounds present in Sumbawa Honey (*Apis dorsata*). The tube test method was employed to observe alterations in color and the formation of precipitates subsequent to the introduction of test reagents.

Table 3. Phytochemical Screening on Sumbawa Honey (*Apis dorsata*)

Test	Reagen	Color	Results
Tanin	H ₂ O+FeCl ₃	Black	+
Flavonoid	Mg metal	Dark red	+
Saponin	Hydrochloric Acid 2 N	Violet	+
Steroid	Chloroform + Anhydrous Acetic Acid + Sulfuric Acid	Chocolate	+

According to the results of the phytochemical screening test, it was determined that Sumbawa honey (*Apis dorsata*) contains flavonoid compounds. This was confirmed by the presence of black tannins, which indicated a positive result, as well as the formation of dark red flavonoids, also indicating a positive result. Additionally, the presence of violet/brown saponins, accompanied by the formation of stable froth, further supported a positive result. The presence of a brown ring formation serves as an indicator for a positive outcome in the detection of steroids.

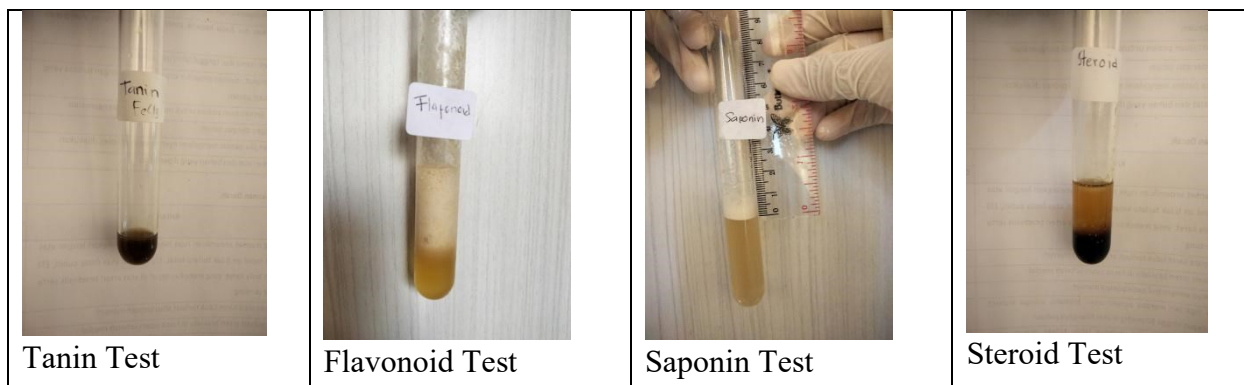


Figure 1 Phytochemical Screening on Sumbawa Honey (*Apis dorsata*)

DISCUSSION

The experiment conducted involved testing the antibacterial activity of various concentrations of Sumbawa honey (*Apis dorsata*) against *Staphylococcus aureus* (ATCC 25923). The results indicated that there was a discernible variation in the diameter of the inhibition zone. The zone of inhibition zone resulting from the concentration of Sumbawa honey (*Apis dorsata*) increases proportionally with its concentration, except for a honey concentration of 100%. A honey concentration of 75% exhibits a larger zone of inhibition compared to a honey concentration of 100%. A honey concentration of 75% exhibits superior antibacterial activity compared to a honey concentration of 100%. The viscosity of Sumbawa honey, when compared to a concentration of 100%, renders it superior due to its limited ability to permeate the disc paper and disperse within the medium. The highest level of antibacterial efficacy is observed in Sumbawa honey (*Apis dorsata*) when it is utilized at a concentration of 75%. The findings align with the research conducted by Huda (2015), which demonstrated that the antibacterial efficacy of Musi Rawas Forest honey exhibited an increase in the inhibition zone when used at concentrations ranging from 10% to 70%. However, at concentrations of 80% and 90%, a decrease in the zone of inhibition was observed.⁴

The formation of a clear zone, known as the zone of inhibition, is attributed to the presence of flavonoids, saponins, and tannins in honey. The antibacterial properties of flavonoids can be attributed to their lipophilic nature, which leads to the disruption of microbial membranes and interference with the activity of peptidoglycan transpeptidase. Consequently, the formation of the bacterial cell wall is disrupted, resulting in cell lysis. Saponin compounds are known to exhibit antibacterial properties by interacting with the bacterial wall, causing its disruption and the subsequent breakdown of surface tension. This disruption facilitates the entry of antibacterial substances into the bacterial cell, ultimately leading to bacterial death. In the interim, tannin compounds exhibit antibacterial properties by impeding the activity of reverse transcriptase and DNA topoisomerase enzymes, thereby impeding the formation of bacterial cells. Tannins possess the ability to impede bacterial proliferation through their interference with protein transportation, as well as their inactivation of cell adhesins and enzymes within bacterial cells.¹⁰⁻¹³

The formation of the zone of inhibition is attributed to the elevated levels of sugar present in honey, which effectively hinder the growth and progression of *Staphylococcus aureus* by exerting osmotic pressure. The presence of high sugar molecules, a low acidic pH ranging from 3 to 4, and a low water content ranging from 0.562 to 0.620 has been observed to result in the loss of bacterial viability to survive.¹⁴ Moreover, honey is known to possess radical hydrogen peroxide (H_2O_2) compounds that exhibit antibacterial properties, capable of eliminating bacteria and various

pathogenic microorganisms. These compounds exhibit reactivity towards the functional groups present in biomolecules within bacterial cells, resulting in their damage. The catalase enzyme present in honey rapidly catalyzes the decomposition of hydrogen peroxide (H₂O₂), a substance commonly employed for bactericidal purposes, into water and oxygen.¹⁵ Variations in the duration of honey storage can also impact the magnitude of the clear zone observed in honey concentrations. This phenomenon is influenced by the composition of honey's reducing sugar content, which in turn affects the functional characteristics of honey. Specifically, it affects the water-holding capacity of honey, and its ability to prolong its life, and the hygroscopic properties derived from glucose and fructose. Consequently, in cases where honey exhibits low compound content and insufficient honey storage during the incubation process at 37°C for a duration of 24 hours, it is expected that the diameter of the inhibition zone will decrease at varying concentrations of honey.¹⁶

CONCLUSION

There is antibacterial effect of Sumbawa Honey (*Apis dorsata*) at concentrations of 25%, 50%, 75%, and 100% against of *Staphylococcus aureus* isolates. The best antibacterial effect against *Staphylococcus aureus* isolates is Sumbawa Honey (*Apis dorsata*) with a concentration of 75%.

Conflict of interest

All author declares there is no conflict of interest regarding publication of this report.

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Author contribution

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