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ANTIBACTERIAL ACTIVITY OF SAGO CATERPILLAR OIL (*Rhynchoporus bilineatus* L.) FROM MINAHASA AGAINST *Salmonella typhi* AND *Staphylococcus aureus*

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Abstract

Sago caterpillars are ethnomedically used as food and medicine by the people of several tribes in Indonesia. Sago caterpillar oil contains active compounds, especially fatty acids that have antibacterial potential. Research has been carried out which aims to obtain the bioactive content of sago worm oil and the antibacterial activity of Salmonella typhi and Staphylococcus aureus bacteria. Sago caterpillars are obtained from the Minahasa region. Four to six instar sago caterpillars are used for oil isolation. Analysis of the content of sago worm oil using the Gas Chromatography Mass Spectrophotometry method. Meanwhile, the antibacterial test used the agar diffusion method using pure cultures of *Salmonella thypii* and *Staphylococcus aureus*. The results showed that the average sago caterpillar oil was 0.26 to 0.28 ml per head. The results of the antibacterial test showed the highest inhibition of S. aureus at a concentration of 1000 mg/L, namely 8.04 mm, while the inhibition of growth of S. thypii was highest at a concentration of 1000 mg/L, namely 7.69 mm. Sago caterpillar oil has antibacterial potential.

Keywords: sago caterpillar oil, antibacterial, bioactive, *Salmonella thypii*, *Staphylococcus aureus*

INTRODUCTION

Insects are a class of Arthropods with the highest diversity of animal species (Mokosuli, 2013). The characteristics of these insects are partly due to their ability to produce secondary metabolism as a form of self-defense against the environment. Approximately one million described insect species can adapt to repeated environmental changes and their resistance to a broad spectrum of pathogens (Vallet-Gely et al., 2008; Hillyer, 2016). Sago beetle (Rhynchophorus sp.) is a species of insect with a wide distribution area on Earth.

In Indonesia, there are two main species of sago caterpillars: Rhynchophorus ferruginous and Rhynchophorus bilineatus. Sago caterpillars are categorized as pests on sago plants. This is due to the

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sago caterpillar boring the stems of the sago plant, which is rich in carbohydrates as food. Based on molecular barcoding using the CO1 gene, the most common sago caterpillar in North Sulawesi is Rhynchophorus bilineatus (Korua et al., 2019). Furthermore, sago caterpillars are classified as edible insects because they are widely consumed. Empirically, the people of Minahasa and Papua consume sago caterpillars as a side dish in food. The larvae of the sago beetle or sago caterpillar are insects that are widely consumed by people in eastern Indonesia as food and are considered to have medicinal properties.

Although sago caterpillars contain protein, most of it is fat. The content of sago caterpillars includes 67.35% water, 2.45% ash, 11.47% protein, and 18.25% fat. This shows that the high nutrient content is fat and protein in wet and dry conditions (drying at 70 ^{0} C). The high-fat content in sago caterpillars is because fat will be used as energy reserves when sago caterpillars enter the pupa (cocoon) phase. According to Indonesian Food Composition data from the Ministry of Health, 100 grams of raw sago caterpillar contains water: 65.9 grams, energy: 241 cal, protein: 5.8 grams, fat: 21.6 grams, carbohydrates: 5.8 grams, fiber: 2.8 grams, ash: 0.9 grams, calcium: 20 mg, phosphorus: 70 mg, iron: 0.5 mg, sodium: 210 mg, potassium: 210 mg, copper: 1 mg, zinc: 7.7 mg, thiamin: 0.17 mg, vitamin B2: 1.45 mg, niacin: 0.1 mg, and vitamin E.

Sago caterpillar contains essential amino acids and high unsaturated fatty acids, which can boost the immune system in the body. Sago caterpillar contains omega 3, 6, and 9, especially oleic acid. The results of laboratory analysis of the content of essential amino acids in sago caterpillars show a complete content, which is the same as the levels of amino acids in snakehead fish, whicyh function to boost the immune system in the human body. The content of free fatty acids in sago caterpillar oil is relatively high. The unique characteristics of sago worm oil extract compared to other animals and plants are that sago worm oil contains capric acid, palmitic acid, and oleic acid. The highest content in capric acid and oleic acid. In addition, it was reported that sago caterpillar oil contains polyhydroxysteroids known as ecdysteroids as non-saponifiable components. Ecdysteroids in invertebrates are hormones that control the skin regeneration process in Arthropods. It is known that more than 100 ecdysteroid compounds that have different structures. Ecdysteroids have the potential to be antibacterial substances.

In addition to the content of fatty acids, sago caterpillars are also rich in protein and complex peptides. Insects release antimicrobial peptide (AMP) as a typical humoral immune response when infected with bacteria. Antimicrobial peptides (AMP), small molecules consisting of 10–100 amino acid residues produced by all organisms, are attractive candidates for new antibiotic designs due to their natural antimicrobial properties and low propensity for resistance development (Bradshaw, 2003; Manniello et al., 2021). Empirically, the people of the Minahasa tribe and several tribes in Papua believe that the sago caterpillar has antibacterial activity. Ethnomedically, sago worm oil is believed to be able to treat typhus and skin infections. However, the analysis of the antibacterial activity of sago worm oil has never been reported. Thus, the exploration of potential antibacterial bioactive sources from sago caterpillars was carried out.

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RESEARCH METHODS

Samples

Sago caterpillar samples were obtained from sago trees in Tandengan Village, Eris District, Kombi Village, Kombi District and, Tolour Village, South Tondano District, Minahasa District, North Sulawesi, Indonesia. Sago caterpillars are preserved in plastic containers along with sago tree fiber as food. Sorting is done in the laboratory to determine the larval stage used in oil isolation. The larval stage is used in instars 4-6. The description of the sago caterpillar was carried out in the bioactivity and molecular biology laboratory, Department of Biology, Faculty of Mathematics, Natural and Earth Sciences, Manado State University. Antibacterial activity tests were carried out at the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, Manado State University. The compound content of sago worm oil was analyzed at the Central Laboratory of the Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung.

Research Procedure

This study used a descriptive research method to obtain the data from laboratory experiments. The stages of the research consisted of sampling sago caterpillars, descriptions of sago caterpillars, isolation of sago caterpillar oil, and antibacterial tests: 1). *Salmonella thypii* 2). *Staphylococcus aureus.*

Isolate sago caterpillar oil.

Oil from sago worms is obtained by the heating method. Sago caterpillars were heated at 95 0 C for 15 minutes, then cooled in a desiccator. After it cools down, the sago caterpillar's abdomen is pressed to release the oil. Sago caterpillar oil was then preserved in a 2 ml tube and stored at room temperature. Sago caterpillar oil isolation activities were carried out under aseptic conditions.

Antibacterial assay

Sterilization of tools and materials

Tools and materials used in research are sterilized to avoid contamination during testing. The tools to be used are sterilized first and then dried. Furthermore, the tools that have been sterilized together with the media material are sterilized using an autoclave at 121˚C for 5 minutes. Sterilized tools using an autoclave are usually made of glass, such as test tubes, Erlenmeyer, and petri dishes. Meanwhile, other tools can be sterilized by igniting a Bunsen lamp or dipping it in alcohol and passing it over a Bunsen flame.

Bacterial rejuvenation.

Nutrient agar media was prepared according to the manufacturer's protocol, namely 17.5 grams of media dissolved in 1000 ml of distilled water. After sterilizing the media by autoclaving at 121 atm and 125 0 C, the media was cooled in laminar airflow. Media that has been frozen and not contaminated for 1 x 24 hours is used for rejuvenation of bacterial isolates. Bacterial isolates were rejuvenated in petri dish media and tilted agar in test tubes. After 2 x 24 hours, the pure bacterial isolates were ready to be used as a source of bacteria for the antibacterial test.

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Disc diffusion method antibacterial test

An antibacterial test was carried out using Muller Hinton agar (MHA) media. The antibacterial activity test was carried out by the disc diffusion method. The test microorganisms were suspended until 10 6 dilution. 100 µl of the suspension was taken and then dripped into the MHA media, which had solidified and spread evenly using an L rod until it was evenly distributed over the entire surface of the petri dish. Next, positive control discs, negative control discs, and those containing the ethanol extract of red betel leaves were placed on the surface of the media containing the suspension of the tested microorganisms. Petri dishes were incubated at 37°C for 24 hours. The Inhibitory Diameter (DDH) formed around the disc was observed and measured using a vernier caliper. The test was carried out in three repetitions.

Data analysis

The research data were analyzed: Characteristics of sago worm oil were analyzed descriptively. Antibacterial activity, namely the zone of inhibition of bacterial growth, was analyzed for variance using SPSS IBM 25.

RESULTS AND DISCUSSION

Description of sago caterpillar

Sago caterpillars used as an oil source are 5-6 instar larvae. The body parts of the larvae are yellowish white, segmented eight to twelve, and the head is blackish brown. The sago caterpillar's body is soft, round, and looks fat. From each location of origin, ten sago caterpillars were taken (Figure 2). The largest average body length was obtained from Tondano (4.08 cm). In contrast, the smallest average body length was obtained from Kombi (3.73 cm). The largest average width in the middle is the Eris sample (1.85 cm), while the smallest is the Kombi sample (1.81 cm). The largest average body weight was obtained from the Tondano sample (5.03 gr), while the smallest was from the Kombi sample (4.48 gr).

Table 1. Average length, middle width and larval weight.

No	Samples	Length (cm)	Width (cm)	weight (gr.)
1	Kombi	3,73 ± 0,38	1.81 ± 0,15	4,48 ± 0,53
2	Eris	3,92 ± 0,21	1,85 ± 0,15	4,83 ± 0,38
3	Tondano	4,08 ± 0,20	1,92 ± 0,05	5,03 ± 0,16

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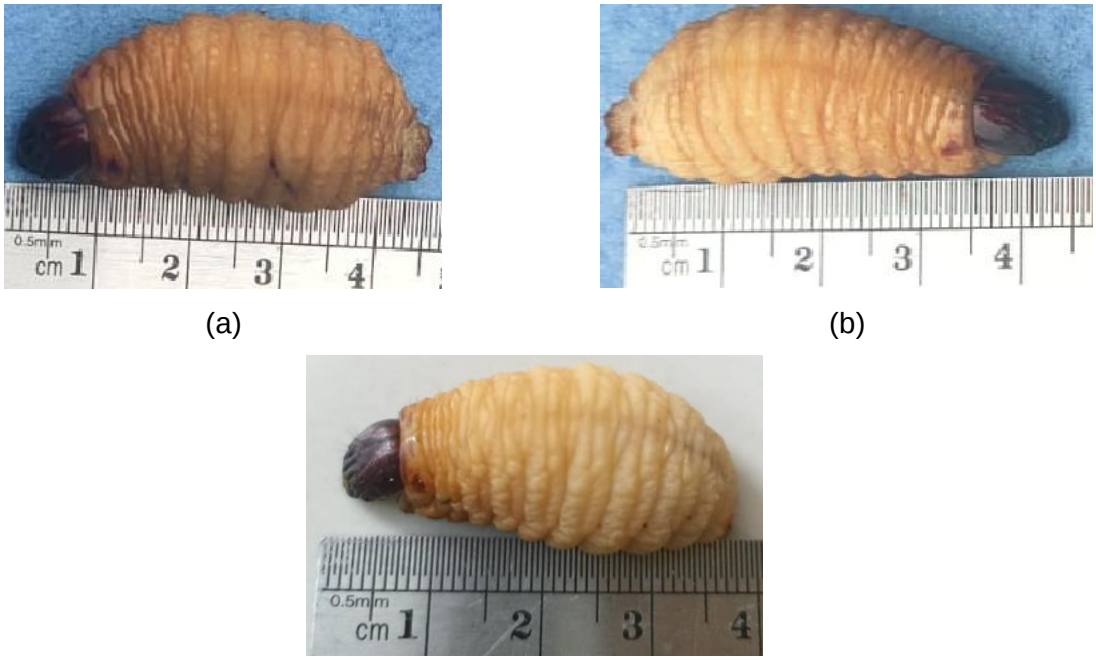


(a) (b)

(c)

Figure 1. Sago caterpillar (a). Kombi (b). Tondano (c). Eris

The highest average of sago caterpillar oil produced from each of the ten heads was from the Tondano sample, which was 0.28 ml per head, while the lowest average was from the combi sample, 0.26 ml per head. All the sago caterpillar oil produced has the same characteristics, namely golden yellow color with a distinctive aroma of sago worms (Figure 2).

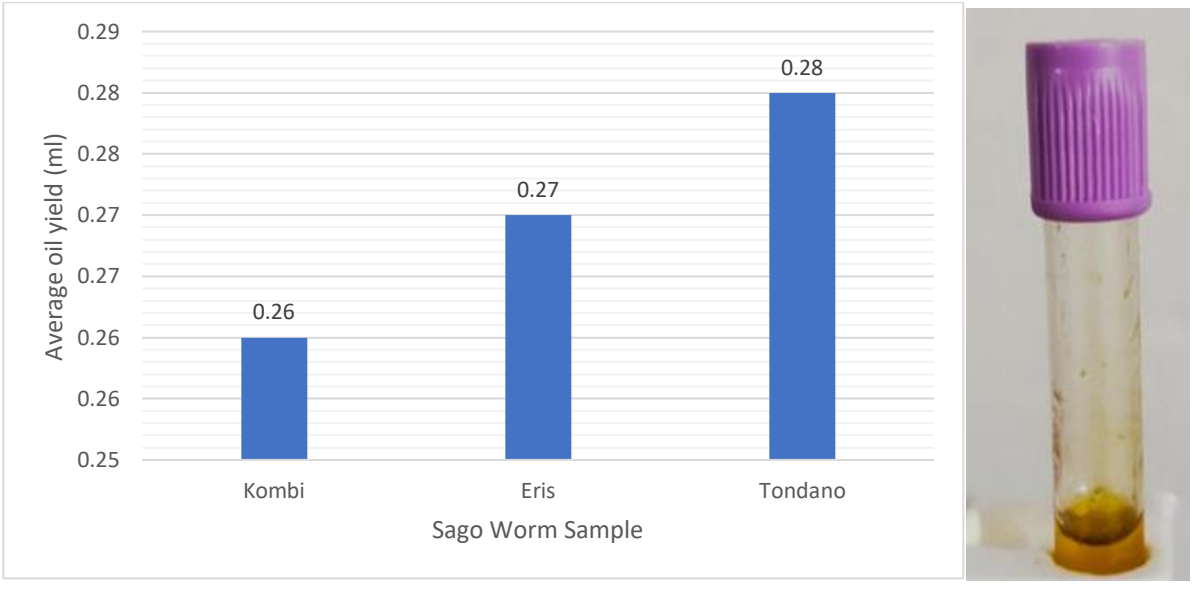
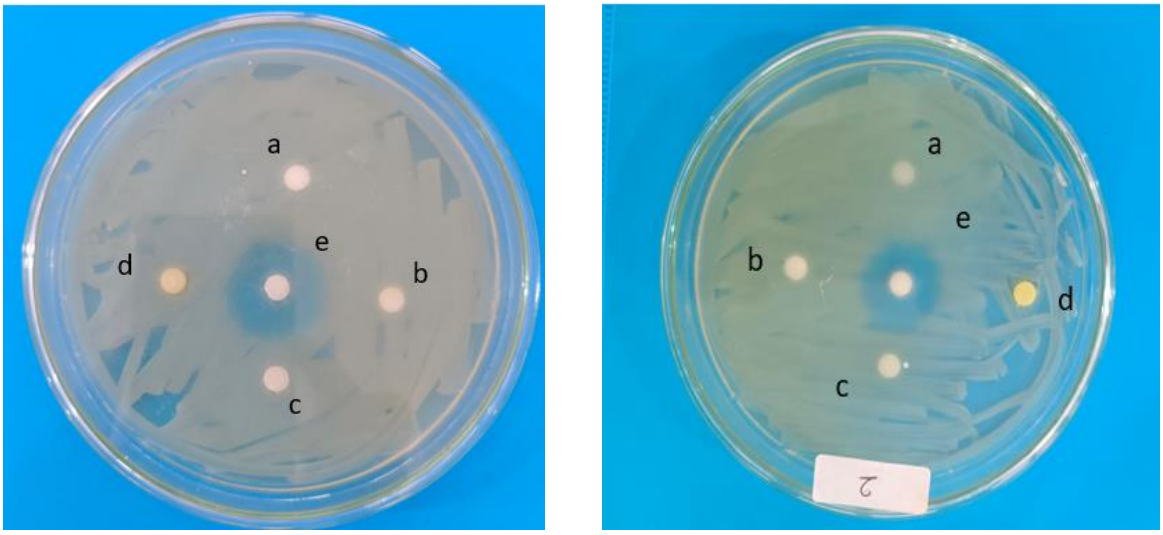


Figure 2. (a). Average sago caterpillar oil produced (b). Sago caterpillar oil

Antibacteria assay

The antibacterial test of sago worm oil was carried out on two pure bacteria cultures, E. coli and S. aureus. As a positive control, 100 mg/L chloramphenicol was used. Incubation was carried out for 48

hours and then the diameter of the inhibition zone formed was measured. Incubation was carried out at a constant temperature of 25 ^{0} C in UV Teck (Figure 3).



(1) (2)

Figure 3. Bacterial growth inhibition test (1). S. typhi (2). S. aureus. A: concentration of 125 mg/L, b: concentration of 250 mg/L, c: concentration of 500 mg/L, d L concentration of 1000 mg/L) and e: chloramphenicol control.

S. aureus

Sago caterpillar oil extract in four test concentrations was used to obtain the diameter of the inhibition zone against pure culture of S. aureus bacteria. The average inhibition zone for bacterial growth after 48 hours of treatment showed the highest inhibition at a concentration of 1000 mg/L, namely 8.04 mm. In comparison, the lowest inhibition at a concentration of 125 mg/L is 6.11 mm. Although compared to the control chloramphenicol with an average inhibition of 14.29 mm, the inhibition of the highest concentration of sago oil is still relatively low. The average DMSO control inhibition showed in figure 4.

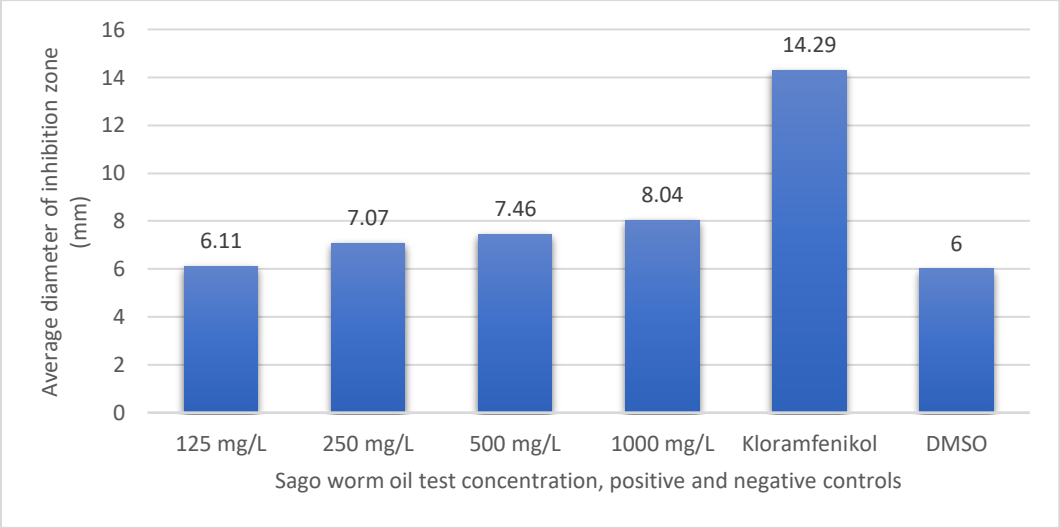


Figure 4. The average inhibition of S. aureus bacteria in sago caterpillar oil compared to controls.

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Salmonela thypii

The average growth inhibition zone of sago caterpillar oil against S. thypii after 48 hours of treatment showed the highest inhibition at a concentration of 1000 mg/L, namely 7.69 mm. Meanwhile, the lowest inhibitory power was at a concentration of 125 mg/L, namely 6.08 mm. The average inhibition of the chloramphenicol control was 14.77 mm; thus the inhibition of the highest concentration of sago oil was still below the inhibition value of the positive control. The average inhibitory power of the DMSO control was 6.00, indicating that there was no inhibition zone in the DMSO control (Figure 5).

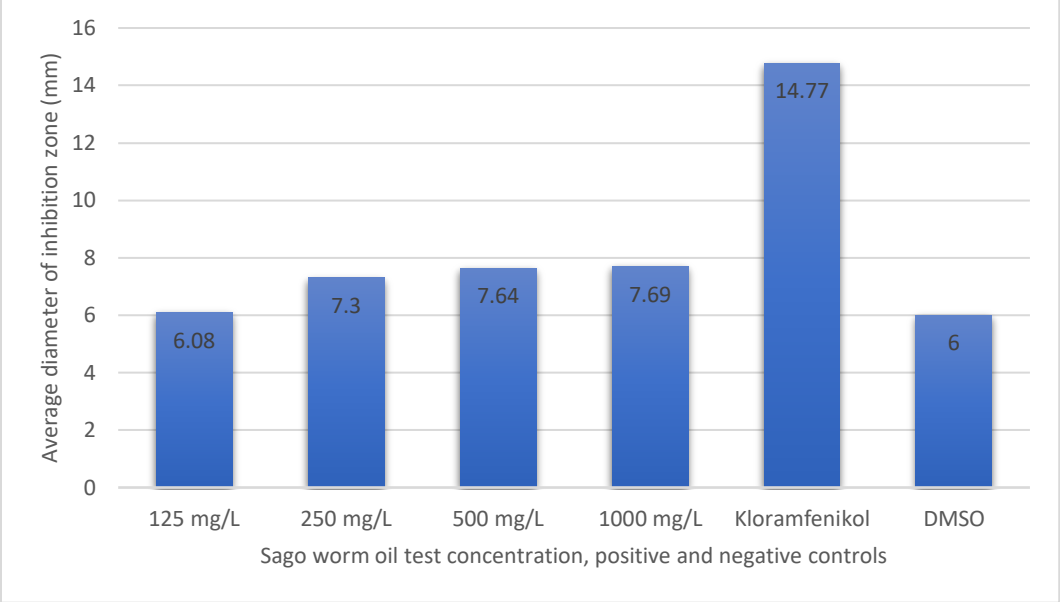

Figure 5. Average growth inhibitory power of the sago worm oil bacterium S. typii compared to the control.

Analysis of variance

Staphylococcus aureus

Based on statistical analysis using SPSS 25, the average inhibitory power of a concentration of 125 mg/L is 6.11 mm with a standard deviation of 0.11. The average inhibitory power of the test concentration of 250 mg/L was 7.07 mm, with a standard deviation of 0.06. The average inhibitory power for bacterial growth at a concentration of 500 mg/L was 7.46 mm, with a standard deviation of 0.10. Meanwhile, the average inhibitory power for bacterial growth at a test concentration of 1000 mg/L was 8.04, with a standard deviation of 0.11 (Table 2). Analysis of variance showed that there was an effect of extract concentration on bacterial growth inhibition ($p<0.05$) with Fcount 193.33 (Table 3).

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Tabel 2. Descriptives

inhibition

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
125 mg/L	3	6.1100	.11533	.06658	5.8235	6.3965	6.00	6.23
250 mg/L	3	7.0733	.06429	.03712	6.9136	7.2330	7.00	7.12
500 mg/L	3	7.4633	.10066	.05812	7.2133	7.7134	7.37	7.57
1000 mg/L	3	8.0433	.11590	.06692	7.7554	8.3313	7.91	8.12
Total	12	7.1725	.74021	.21368	6.7022	7.6428	6.00	8.12

Tabel 3. Analysis of Varians results

inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.945	3	1.982	193.334	.000
Within Groups	.082	8	.010		
Total	6.027	11			

The results of the Duncan Multiple Range Test showed that there were differences between concentration treatments on the growth inhibition of E. coli bacteria. Each treatment was different in response to the inhibition of E. coli bacteria growth (Table 4).

Tabel 4. DMRT Test

Duncan[a]

Extract concentration	N	Subset for alpha = 0.05			
		1	2	3	4
125 mg/L	3	6.1100 (a)			
250 mg/L	3		7.0733 (b)		
500 mg/L	3			7.4633 (c)	
1000 mg/L	3				8.0433 (d)
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

S. thypii

Based on statistical analysis using SPSS 25, the average inhibitory power of a concentration of 125 mg/L is 6.08 mm with a standard deviation of 0.06. The average inhibitory power of the test concentration of 250 mg/L was 7.30 mm, with a standard deviation of 0.10. The average inhibitory power for bacterial growth at a concentration of 500 mg/L was 7.64 mm with a standard deviation of 0.13. Meanwhile, the average inhibitory power for bacterial growth at a test concentration of 1000 mg/L was 7.69, with a standard deviation of 0.16 (Table 5). Analysis of variance showed that there was an effect of extract concentration on the inhibition of bacterial growth ($p<0.05$) with an Fcount of 112.32 (Table 5).

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Tabel 5. Descriptives

inhibition

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
125 mg/L	3	6.0800	.06928	.04000	5.9079	6.2521	6.00	6.12
250 mg/L	3	7.3000	.10000	.05774	7.0516	7.5484	7.20	7.40
500 mg/L	3	7.6400	.13528	.07810	7.3040	7.9760	7.50	7.77
1000 mg/L	3	7.6900	.16523	.09539	7.2796	8.1004	7.50	7.80
Total	12	7.1775	.68815	.19865	6.7403	7.6147	6.00	7.80

Tabel 6. ANOVA

inhibition

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.088	3	1.696	112.323	.000
Within Groups	.121	8	.015		
Total	5.209	11			

The results of the Duncan Multiple Range Test showed that there were differences between the treatments with a concentration of 125 mg/L and a concentration of 250 mg/L. However, there was no difference between the treatment concentrations of 500 mg/L and 1000 mg/L/ (Table 7).

Tabel 7. Uji DMRT

Duncan[a]

Extract concentration	N	Subset for alpha = 0.05		
		1	2	3
125 mg/L	3	6.0800		
250 mg/L	3		7.3000	
500 mg/L	3			7.6400
1000 mg/L	3			7.6900
Sig.		1.000	1.000	.632

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

Discussion

Sago worm oil was successfully isolated from sago worms from three locations in Minahasa Regency. Differences in average body length, middle body width, and weight of sago worms are caused by the availability of nutrients in the sago plant stems. The stems of sago plants contain starch and other nutrients, which are food for sago worms. However, the nutritional content of sago plant stems differs based on the geographical location where the sago plant grows. Differences in nutritional content are shown, among other things, by differences in the diameter of sago plant stems observed during the isolation of sago worms. The Tondano sample showed a higher average body length, midbody width, and

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weight than the other samples. This shows that the sago caterpillar isolated from Tondano lived on sago stalks with a higher availability of nutrients, especially starch, compared to the other two areas. The morphology of sago caterpillars from the three regions is relatively the same: a golden yellow body with a blackish brown head. The body is soft and segmented.

The sago worm oil produced showed differences between the three sample locations. In direct proportion to body size and weight, the volume of oil produced was highest in the Tondano sago worm samples. A sizeable average body weight indicates a high-fat content. However, the physical characteristics of the oil produced are relatively the same, namely golden yellow with the distinctive aroma of sago worms.

Antibacterial tests on S. typii and E. coli showed different inhibitory responses. The most significant average bacterial body inhibitory power in S. typii was found at a test concentration of 1000 mg/L, 7.69 mm. Compared with the positive control of chloramphenicol 14.77 mm, the inhibitory power of sago worm oil against S. typii is still relatively weak. No inhibition was found in the sterile DMSO control (6 mm). Analysis of variance showed that the concentration of sago worm oil affected the growth inhibition of S. typii bacteria ($p>0.05$). The DMRT test results showed differences in the bacterial growth inhibitory response of the four concentration treatments. The inhibitory power of bacterial growth is relatively weak in S. typii because this bacterial species is a species of pathogenic bacteria with high antibiotic resistance. S thpii in Indonesia has experienced resistance to many antibiotics. Multidrug-resistant Salmonella typhi (MDRST) is a condition where Salmonella typhi is resistant to three first-line antibiotics used for the management of typhoid fever, namely Ampicillin, Trimethoprim-Sulfamethoxazole, and Chloramphenicol. Extensively drug-resistant (XDR) Salmonella typhi is a condition where Salmonella typhi is resistant to Ampicillin, Trimethoprim-Sulfamethoxazole, Chloramphenicol, fluoroquinolone antibiotics, and third-generation cephalosporin antibiotics (Akram et al., 2020; WHO, 2019; Semuel et al., 2022). Research conducted in Indonesia in 2020, which examined 27 isolates of Salmonella typhi to determine MDR phenotypically, obtained data that 3.7% of the isolates were MDR (Jamilah et al., 2020). Multidrug-resistant Salmonella typhi (MDRST) and extensively drug-resistant (XDR) Salmonella typhi occur due to genetic mutations that cause bacterial resistance to antibiotics (Dutta et al., 2014; Rombot et al., 2023).

Antibacterial tests on S. aureus showed a better response compared to S. type. The most significant average inhibition of bacterial growth at a test concentration of 1000 mg/L was 8.04 mm. Compared with the positive control chloramphenicol with an inhibitory power of 14.29 mm, the antibacterial activity of sago worm oil against S. aureus is still relatively weak. DMSO negative control showed no inhibition zone. Analysis of variance showed an effect of extract concentration on the growth inhibition of S. aureus bacteria ($p>0.05$). The DMRT test showed that the concentrations of 500 mg/L and 100 mg/L were not significantly different, while the concentration was 125 mg/L. concentration of 250 mg/L is significantly different. S. aureus is a bacteria that is resistant to several antibiotics. Methicillin-resistant Staphylococcus aureus (MRSA). S. aureus is a bacterium that causes human diseases, ranging from skin infections to invasive severe infections such as pneumonia, regenerative soft tissue infections, heart

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valves, and septicemia. S. aureus resistance to methicillin (penicillin group), then called Methicillin Resistance Staphylococcus aureus (MRSA), is related to a plasmid carrying the blaZ gene that encodes β-lactamase. Additionally, S. aureus resistance is also influenced by the expression of penicillin-binding protein 2a (PBP2a), which exports penicillin out of the cell (7). Cases of resistance of S. aureus to penicillin groups occurred in more than 86% of cases (8). This case of resistance causes the failure of therapy using amoxicillin in S. aureus infection.

Because the two species of bacteria tested, both S. typii and S. aureus, are resistant to many types of antibiotics, it is suspected that this will greatly influence the antibacterial activity of sago worm oil. Antibacterial activity of lipid-derived compounds by interfering with the permeability of bacterial cell membranes. It is suspected that both bacterial species have adapted to compounds that interfere with the permeability of their membranes. However, compounds from sago caterpillar oil affect the growth of bacteria. This is evidenced by the presence of an inhibition zone that is formed. The inhibition zone formed showed that the compounds in sago caterpillar oil affected the growth of both S. thypii and S. aureus bacteria.

CONCLUSION

Sago caterpillar oil is golden yellow with the highest average oil yield from the Tondano sample. Sago worm oil showed weak category of antibacterial activity on S. aureus and S. thypii.

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