

# Antibiotics Based from Insect Potency of Various Insect Species in Bali as an Antibiotic

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## 1. Introduction

Antibiotics are chemical substances produced by fungi and bacteria, which had a lethal efficacy or inhibit the growth of bacteria. The ability of antibiotic drugs as antibacterial encourages excessive use of antibiotics with improper dosage. As a result, the development of antibiotic resistance by bacteria process is now beginning to emerge. Antibiotic resistance condition is characterized by the lost of its efficacy for some diseases caused by bacteria.

Effort to find an active substance that is antibacterial continues to be done. Insects have been used as the first standard traditional medicine of various ethnic groups around the world for wound healing. Based on that information, Reseacher interested in investigate the potential of maggots or larvae of the green bottle fly (*Luciliasericata* (Meigen)), coconut caterpillars or larvae of the red palm beetle (RPW) (*Oryctesrhinocherus* L.), Kroto (mixture of larvae and pupae of ants (*Oecophyllasmaragdina*)) and larvae of silkworms (*Bombyxmori*) to be employed as a source of antibiotics.

## 2. Problem Statement

There is no research comparison about maggots or larvae of the green bottle fly (*Luciliasericata* (Meigen)), coconut caterpillars or larvae of the red palm beetle (*Oryctesrhinocherus* L.), Kroto (mixture of larvae and pupae of ants (*Oecophyllasmaragdina*)) and larvae of silkworms (*Bombyxmori*) to be employed as a source of antibiotics.

## 3. Purpose of the Research

The purposes of this study include were (1) to review the potency of insect producing antibiotic published in scientific papers; (2). To identify the types of insects and their larvae (maggots or larvae of the green bottle fly (*Luciliasericata* (Meigen)), coconut caterpillars or larvae of the red palm beetle (RPW) (*Oryctesrhinocherus* L.), Kroto (*O. smaragdina*) and larvae of silkworms (*Bombyxmori*) with the most potential to produce antibacterial or antibiotic substance.

## 4. Research Methodology

Coconut caterpillars (*Oryctes rhinoceros* L), Kroto (*O. smaragdina*), Maggots (*Luciliasericata*), and silkworm (*Bombyxmori*) were collected from sources available in Bali such as abattoir, cadaver, (maggots; collection of Biomedical Lab Vetereinary Faculty of Udayana University), rotten coconut tree (coconut caterpillar), bird markets Denpasar (Kroto), and Agro silkworm, SibangKaja Village Badung Regency (silkworm). Various scientific papers on natural antibiotic from insect are reviewed.

Research has been conducted in the Microbiology Lab of Faculty of Medical Udayana University.

25<sup>th</sup> January – 12<sup>th</sup> February ,2016. This study used an experimental method of completely randomized design, which consists of six (6) treatments, namely: P-0: NaCl physiologic (negative control), P-1: Penicilin-Gentamycin suspension of 25.000µg penicillin and 25.000 µg Gentamycin/milliliter (positive control), P-2: Maggots (*Luciliasericata* (Meigen)), P-3: Kroto (*O. smaragdina*), P-4: Coconut Caterpillars (*Oryctes rhinoceros* L), P-5: Silkworm (*Bombyxmori*). Each of bacteria contain 6 kinds of treatment and there are 4 plates for each bacteria so the total of research objects on both plates of *Escherichia coli* and *Staphylococcus aureus* are 192, So Each treatment was repeated four times.

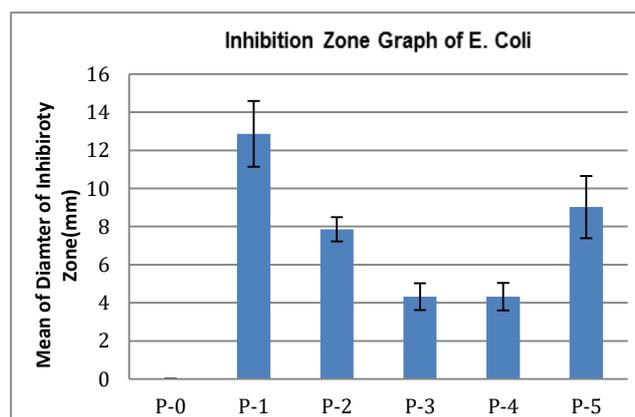
A total of 10 grams of all insect larvae was crushed to a final concentration of 100% with physiological NaCl. Grinding is done using micro-pastel. After grinding, the suspension of hemolymph was centrifuged at a speed of 10,000 RPM for 5 minutes. Supernatant was taken and stored in sterile Eppendorf tubes and frozen until testing is done.

Gram negative bacteria of *Escherichia coli* and Gram-positive of *Staphylococcus Aureus* grown in agar were provided by Microbiology Lab of Faculty of Medical Udayana University. Antibacterial activity was tested in Mueller Hinton agar. The presence of inhibition zone was observed and measured.

Six (6) filter disks were submerged in each plate (positive and negative controls as well as four insect larvae suspensions). The disks were than applied in the surface of Mueller Hinton agar plate with *Escherichia coli* and *Streptococcus* sp. The plates were incubated in 37°C overnight. The presence of inhibition zone was observed and measured by vernier caliper.

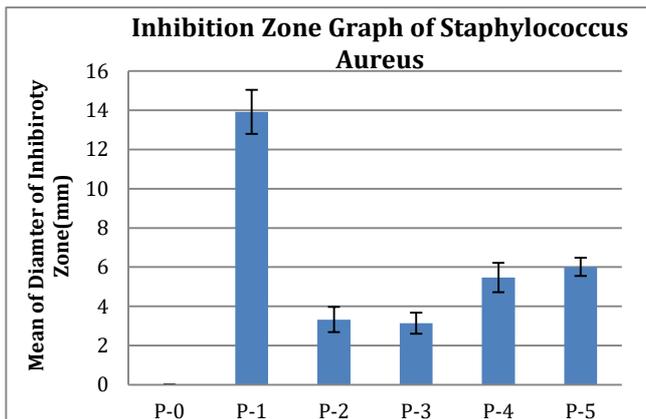
## 5. Results & Analysis

Observation of the inhibition zone diameter is shown in Graph 1. As shown, the suspension of the silkworm extract had shown the widest inhibition zone diameter towards gram negative bacteria *Escherichia Coli* compared to the other treatment except P-1. Inhibition zone is the widest towards gram negative bacteria *Escherichia Coli* but Silkworms extract also showed the widest inhibition zone after the P-1 which also followed by the other extract with their own inhibition zone itself



**Graph 1. Mean of Inhibition Zone Diameter of Escherichia Coli (millimeter)**

Observation of the inhibition zone diameter is shown in Graph 2. As shown, the suspension of the silkworm extract had shown the widest inhibition zone diameter towards gram positive bacteria *Staphylococcus aureus* compared to the other treatment except P-1. Inhibition zone is the widest towards gram positive bacteria *Staphylococcus aureus* but Silkworms extract also showed the widest inhibition zone after the P-1 which also followed by the other extract with their own inhibition zone itself



**Graph 2. Inhibition Zone Diameter of Staphylococcus Aureus (millimeter)**

From the graphic that shown above, we can take a conclusion which is each extract shown an inhibition zone and the significant of each data is very different because on this research, we want to know if there's a potency on each treatment and the data also showed that P-1 showed the widest inhibition zone than the other treatment but P-5 which is Silkworm extract showed a big inhibition zone towards each gram positive and negative bacteria.

**6. Discussion**

**Table 1 Comparing with Control Negative in Escherichia coli**

	Variable 1	Variable 2
Mean	9.025	0
Variance	2.664666667	0
Observations	16	16
Hypothesized Mean Difference	0	
Df	15	
t Stat	22.11493959	
P(T<=t) one-tail	3.6594E-13	
t Critical one-tail	1.753050356	
P(T<=t) two-tail	7.31881E-13	
t Critical two-tail	2.131449546	

On the discussion, we discuss the data in statistic, as what it shows on the statistic we can conclude that the data is valid and there's a potency of each treatment can be use as antibacterial and antibiotic. Silkworm as an example, because silkworm has the biggest inhibition zone which showed that it's the most potential as an antibacterial also antibiotic than the rest of the treatment, after compared with the control negative on bacteria *Escherichia coli*, the T stat showed the extract is statistically significant at  $\alpha \leq 0.05$  due to no inhibition observed in the negative control of NaCl. Then the inhibition zone produced by the treatment must be due to the active compound contained in the extract.

**Table 2. Comparing with Control Negative in Staphylococcus aureus**

	Variable 1	Variable 2
Mean	6.0125	0
Variance	0.2105	0
Observations	16	16
Hypothesized Mean Difference	0	
Df	15	
t Stat	52.41903609	
P(T<=t) one-tail	1.04062E-18	
t Critical one-tail	1.753050356	
P(T<=t) two-tail	2.08123E-18	
t Critical two-tail	2.131449546	

On the discussion, we discuss the data in statistic, as what it shows on the statistic we can conclude that the data is valid and there's a potency of each treatment can be use as antibacterial and antibiotic. Silkworm as an example, because silkworm has the biggest inhibition zone which showed that it's the most potential as an antibacterial also antibiotic than the rest of the treatment, after compared with the control negative on bacteria *Staphylococcus aureus*, the T stat showed the extract is statistically significant at  $\alpha \leq 0.05$  due to no inhibition observed in the negative control of NaCl. Then the inhibition zone produced by the treatment must be due to the active compound contained in the extract.

**10. Conclusion.**

The conclusion that can we gather from the paper is P2-P5 can be used as antibacterial also antibiotic, because all of the extract from the insect showed a inhibition zone on both of gram positive and negative bacteria which is *Staphylococcus aureus* and *Escherichia coli*, P-1 showed the biggest inhibition zone but P-5 which is Silkworms extract showed a big inhibition zone toward the bacteria itself, and the rest of the treatment also showed some inhibition zone which proved there's a potency for them to be an antibiotic