BT-201: INVESTIGATION OF THE SPIDER WEB FOR ANTIBACTERIAL ACTIVITY

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Abstract

Spiders build their webs with a material called silk. Spider silk contain protein fiber that have many advantages and functions. One of them is to capture their prey such as flies, insects, and others. The needs on the research of antibacterial activity are important for human health because of importance of finding a new cure for some diseases that occur because of microorganisms. Some of the microorganisms, especially bacteria are becoming resistant to many antibacterial agents. The purpose of this new investigation was to determine if spider webs exhibit antibacterial properties. In order to determine antibacterial properties, the spider webs were extracted using different solvents such as methanol, ethanol, acetone, and water in different conditions (extraction time, and concentration used for optimization). These extracts were screened for antibacterial activity using disc diffusion assay. Two bacteria were used in the antibacterial assay namely *Bacillus subtilis*, and *Escherichia coli*. The determination of spider webs exhibiting antibacterial properties was based, at least in this study, solely upon the definite appearance of inhibition zone around the well of plates. In screening, acetone solvent was shown the best for antibacterial activity compare to other solvents with 10 mm of diameter of inhibition zone for *Bacillus subtilis* and 9 mm of diameter of inhibition zone for *Escherichia coli*. In optimization, the maximum inhibition zone on the *Bacillus subtilis* was 15 mm at a time of 48 hours and concentration of 0.035 g/ml. Meanwhile, the maximum diameter of inhibition zone on the *Escherichia coli* was 12 mm at a time of 48 hours and concentration of 0.035 g/ml. Therefore, this study showed that spider webs could be potential source of new antibacterial agents.

Key word: *Antibacterial, Bacillus subtilis, Escherichia coli, Inhibition zone, Spider web.*

INTRODUCTION

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan, as well as killing viruses. The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before 1941, the year where penicillin was discovered, there were no true cure existed for some diseases such as gonorrhea (a venereal disease involving inflammatory discharge from the urethra or vagina), strep throat, or pneumonia (a lung infection in which the air sacs fill with pus). Often, the infected wound patients have a wounded limb removed, or face death from the infection.

The needs on the research of antibacterial activity are important for health of millions of human in the world because of important of finding a new cure for some diseases that occur because of microorganisms. Some of the microorganisms, especially bacteria are becoming resistant to more and more antibacterial agents. By making a new research, microorganisms that can kill or inhibit the growth of other bacteria can be found (Cosgrove *et al*., 2003). Now, most of these infections can be cured easily with a short course of antibacterial

However, the effectiveness of antimicrobial therapy is somehow in doubt. Microorganisms, especially bacteria are becoming resistant to more and more antimicrobial agents. They are becoming resistant more quickly than new drugs that are being made available. Therefore, future research in antimicrobial therapy may focus on finding the new antimicrobials that can overcome this problem, or treat infections with alternative means.

Thus, in this paper I will do a research regarding the effect of antimicrobial activity using the spider house and test into the Gram positive and Gram negative bacteria. There are many types of spider house that can be test but I only focus on the common spider and nontoxic spider such as jumping spider, orb weaver spider, America house spider and others. However, there is no consistent relationship between the classification of spiders and the types of web they build because some species in the same genera may build very similar or significantly different webs.

Spiders use a variety of strategy to capture prey. Some species are web builders that use webbing to ensnare their prey. Others are active hunters that actively search for their prey. Passive hunters are spiders that lay in wait for their prey rather than searching for it. (Kim *et al*., 2005) Some of the preys contain bacteria and then the bacteria will stick on the web. So, from this knowledge we can start the investigation for spider house on antimicrobial activity. In this paper, I report the antimicrobial activity of spider house from various samples which are from nontoxic spiders.

The needs on the research of antimicrobial activity are important for health of millions human in the world because we want to find a new cure for some diseases that occur because of microorganisms. Some of the microorganisms, especially bacteria are becoming resistant to more and more antimicrobial agents. By making a new research, we can find microorganisms that can kill or inhibit the growth of other bacteria. The techniques to test the antimicrobial activity on spider house are still not developing yet. However, recently the research on antimicrobial activity have been done on spider by tested the venom of spider which is *Lycosa carolinensis* where lycotoxins I and II have shown potent antimicrobial activity against both prokaryotic and eukaryotic cells. (Fogaca *et al*., 1999). The objectives of this project are to prepare samples of spider house from various sources and to test samples for antimicrobial effect.

MATERIALS AND METHODS

Collection of the spider webs from any places (e.g.: ceiling, garage, & etc).

Screening of different solvents to extract sample (methanol, ethanol, acetone and distilled water) were used for the determination of antimicrobial effectiveness against specific pathogen as essential to appropriate therapy.

Preparation of media and cell culture

Antibacterial test by disc diffusion assay was used to test the extract for antibacterial activity. The examples of gram-positive bacteria are *Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Bacillus subtili,s* and others. In this study only *Bacillus subtili,s* was used as example of gram-positive bacteria. The examples of gram-negative bacteria are *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium,* and others. However, E. coli is used in this study as example for gram-negative bacteria. The result was recorded by measuring the diameter of inhibition zone.

Optimization (Parameter: time and concentration). Design of experiment (DOE); Central Composite Design – two (2) parameters (time, and concentration), three (3) Levels (Table 1). Full design were applied followed by analysis of the result obtained from DOE.

Table 1: Design of experiments

	Std	Run	Block	Factor 1 A:Temperature degree celcius	Factor 2 B:Time hours	Factor 3 C:Agitation Spe rpm	Response 1 diameter mm
	17	1	Block 1	31.00	36.00	250.00	
	1	2	Block 1	25.00	24.00	200.00	
	8	3	Block 1	37.00	48.00	300.00	
	6	4	Block 1	37.00	24.00	300.00	
	15	5	Block 1	31.00	36.00	250.00	
	7	6	Block 1	25.00	48.00	300.00	
	14	7	Block 1	31.00	36.00	300.00	
	2	8	Block 1	37.00	24.00	200.00	
	12	9	Block 1	31.00	48.00	250.00	
	11	10	Block 1	31.00	24.00	250.00	
	4	11	Block 1	37.00	48.00	200.00	
	3	12	Block 1	25.00	48.00	200.00	
	9	13	Block 1	25.00	36.00	250.00	
	19	14	Block 1	31.00	36.00	250.00	
	13	15	Block 1	31.00	36.00	200.00	
	20	16	Block 1	31.00	36.00	250.00	
	16	17	Block 1	31.00	36.00	250.00	
	18	18	Block 1	31.00	36.00	250.00	
	5	19	Block 1	25.00	24.00	300.00	
	10	20	Block 1	37.00	36.00	250.00	

RESULTS AND DISCUSSION

Screening: Figure 1 shows the results on Petri dish for both gram-positive and gram-negative bacteria. The inhibition zones were measured as stated in Table 2. Four solvents were used for screening include methanol, ethanol, acetone and distilled water. As in Table 2 the distilled water extract showed no inhibition.

Figure 1: Screening process show inhibition zone for acetone (high inhibition zone) on *B. subtilis* and *E. coli*

Table 2: Screening results

Solvents	**Diameter of Inhibition Zone (mm)**	
	Bacillus subtilis	***Escherichia coli***
Ethanol	7.0	7.0
Methanol	7.5	8.0
Acetone	10.0	9.0
Distilled water	-	-

Spider webs had significant potential as an antibacterial compound. Acetone which is the less polarity shows the best antibacterial activity. Gram positive bacteria (*B. subtilis*) are higher inhibition zone compared to gram negative bacteria (*E. coli*).

Optimization: The optimization was carried on for both gram positive and gram-negative bacteria using acetone extract and the parameters used were time and concentration as shown in Table 3 and Figure 2.

Table 3: Optimization results

Time (hours) Concentration (g/ml)	**Diameter of Inhibition Zone (mm)**					
	Bacillus subtilis			***Escherichia coli***		
	24	**36**	**48**	**24**	**36**	**48**
0.060 (A)	0	11	0	0	10	0
0.035 (B)	0	11	15	0	10	12
0.010 (C)	0	13.5	12	0	0	11

The concentration of the extracts and extraction time had a relatively relationship to the antibacterial activity. At 24 hours: no inhibition because low concentration of DMSO. (Solution: use 50% DMSO instead of 20% DMSO) maximum inhibition zone:

Gram positive bacteria = 15 mm (time: 48 hours & concentration: 0.035 g/ml)
Gram negative bacteria = 12 mm (time: 48 hours & concentration: 0.035 g/ml)

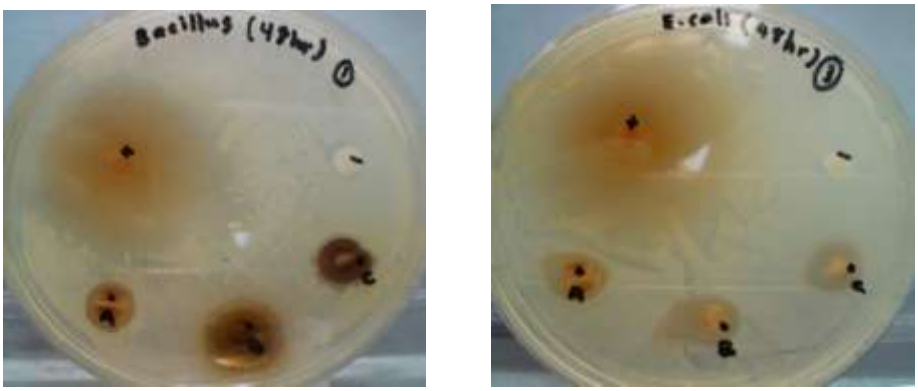

Figure 2: The maximum inhibition zone at concentration of 0.035g/ml and time of 48 hours for *B. subtilis* and *E. coli*

Figure 3 shows the three dimension plot of DOE analysis for both types of bacteria using the two parameters of time and concentration. The ANOVA result for the DOE analysis showing the R-squired and the F-value is shown in Table 4. This result showed that the inhibition by using crude acetone extract is comparable to the positive standard (tetracycline) used in this study.

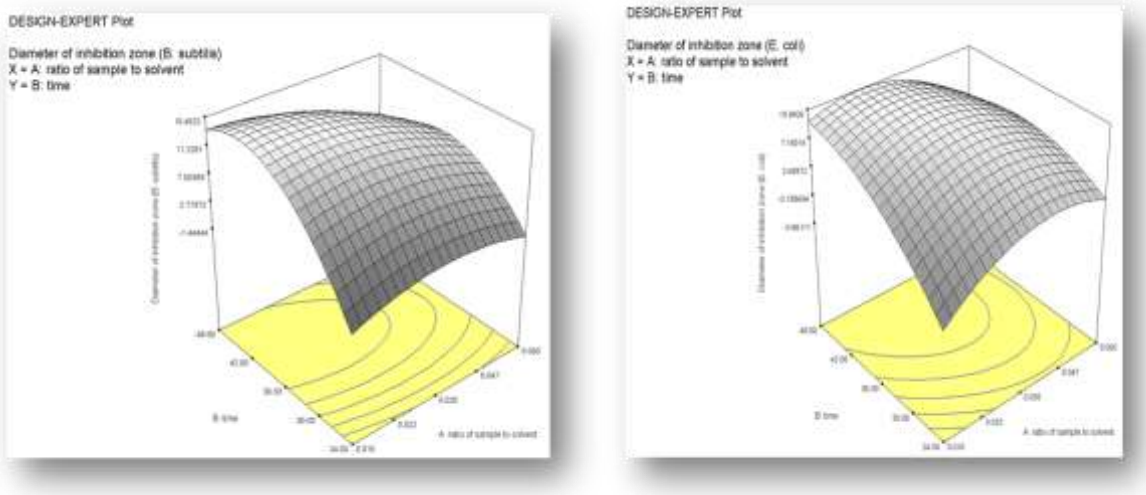

Figure 3: 3D plot response surface for *B. subtilis* and *E. coli*

Table 4: ANOVA results from design expert

	Response 1	**Response 2**
R-squared	0.8726	0.632
F-value	4.11	1.03

CONCLUSION

The results obtained show that spider webs had significant potential as an antibacterial compound. Acetone was the best solvents for screening. The maximum diameter of inhibition zone for both bacteria was at time of 48 hours and concentration of 0.035 g/ml.

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