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Antimicrobial Efficacy of *Strongylodon macrobotrys* A. Gray (Jade Vine) Stem Extract

Rogerlyn G. Maning[1], Lucky Kris M. Tarrayo[1], Angelica Ana Alarcon[1], George V. Admirable[1], Cristian Demier V. Dollano[1], and Cielo Emar M. Paraoan[1,2]

[1]Science Department, College of Science, Bulacan State University, Malolos, Bulacan 3000 the Philippines
[2]Institute of Biology, College of Science, University of the Philippines Diliman, Diliman, Quezon City 1101 the Philippines

***Strongylodon macrobotrys* A. Gray is known for its ornamental woody vines and magnificent blooms consisting of dangling clusters of claw-shaped flowers. It is known as the Philippine jade vine, locally known as "tayabak." Studies have shown that this plant has antimicrobial efficacy; however, there were no studies conducted locally focusing on the antimicrobial properties of the plant. Hence, this study utilizes the plant, particularly the stem to identify the antimicrobial efficacy of *S. macrobotrys* in inhibiting the growth of representative pathogens such as *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 13311), and *Staphylococcus aureus* (ATCC 6538) using methanol and ethanol as solvents with various concentrations. The Kirby-Bauer method was used to assess the antimicrobial efficacy of different extracts by measuring the diameter of the zone of inhibition (ZOI) and determining optimal extract concentrations. Between the two extracts studied, the methanol extract had stronger antimicrobial activity, having the highest ZOI (12.0 ± 1.73) at a 95% concentration level of methanol as used to extract the stem of *S. macrobotrys*. Additionally, the methanol extract of *S. macrobotrys* stem proved to be more potent against the Gram-positive bacterial strains, particularly *B. subtilis* [$F(2,33) = 1971$, $p < 0.001$] and *S. aureus* [$F(2,33) = 27.7$, $p < 0.001$] than the inactive Gram-negative bacterial strains *E. coli* and *S. typhimurium*. The findings indicate that the extract has a changeable level of inhibition capacity depending primarily on the resistance of bacterial strains. This is the first study about the antimicrobial efficacy of *S. macrobotrys* conducted locally. This firmly established that *S. macrobotrys* stem continues to contribute to the interest in natural products as potential treatments, particularly in combating antimicrobial resistance.**

Keywords: antimicrobial resistance, ethanol extract, Kirby-Bauer method, methanol extract, Philippine jade vine

INTRODUCTION

Plants have a lot of purposes and roles in our environment. Almost everything that people intake originates from plants, either directly or indirectly. As such, one-quarter of all prescription medications are derived directly from plants or are plant derivatives that are used in medical treatment and pharmaceutical development, and play an important role in maintaining a healthy lifestyle (Kelly 2014).

Strongylodon macrobotrys A. Gray, also known as the Philippine jade vine or locally known as "tayabak," is one of the endemic plants that can be seen in places of the Philippines, particularly in damp tropical woods and

*Corresponding author: rogerlynmaning01@gmail.com

near rivers and ravines of Luzon (Eusebio 2014). It has the taxonomic classification that belongs to the Kingdom Plantae, Phylum Tracheophyta, Class Magnoliopsida, Order Fabales Bromhead, Family Fabaceae, and Genus *Strongylodon* Vogel (Banki *et al.* n/d) with claw-shaped turquoise or greenish blue jade flowers. This tough and fast-growing vine can reach lengths of up to 59 ft (18 m), whereas the large flower clusters can reach 10 ft (3 m) in length (Sain *et al.* 2022). It has a mutualistic relationship with bats, where they pollinate the flowers' nectar by hanging it upside-down. In the process, pollen is deposited on the bat's head and transferred to the next flower it visits in the wild, which is drawn to the glowing luminescence of the blossoms after dusk (Ott 2018). Ragasa *et al.* (2013) conducted a study focusing on the chemical constituents of *S. macrobotrys*, a native Philippine ornamental plant with no reported biological activity. It is shown by chemical investigation that the stems, flowers, and leaves of the plant provided a variety of biologically active compounds. The dichloromethane extract of *S. macrobotrys* stem led to the isolation of taraxerone, stigmasterol, b-sitosterol, and triglyceride. It is revealed that among these isolated compounds, triglyceride exhibited antimicrobial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, and *T. mentagrophytes*. Triglycerides demonstrated a direct connection between toxicity and higher unsaturation, which contributed to increased susceptibility to oxidation. The fatty acid compound is known to have bactericidal and antifungal effects (Kabara *et al.* 2011). Linoleic acid, which is one of the fatty acids esterified to triglycerides, inhibits the growth of the bacteria by significantly increasing the membrane permeability, which is interpreted as the formation of pores. This increased permeability also prevents macromolecular synthesis and coupling of the electron transfer chain (Greenway and Dyke 1979). Therefore, this compound yielded in the stem of *S. macrobotrys* has the potential to be an antimicrobial agent against pathogens.

The chemical investigation made by Ragasa *et al.* in 2013 was the only study conducted locally. Furthermore, no study was conducted focusing on the antimicrobial properties of the plant, which is why there is a need for further research, development, and cultivation of jade vine to utilize more the uses and importance of the plant. Hence, this study will emphasize the plant's stem to identify the antimicrobial efficacy of *S. macrobotrys* and its potential use in inhibiting the growth of selected representative bacterial ATCC strains.

MATERIALS AND METHODS

Sampling

The fresh stems of *S. macrobotrys* were collected from the Center for Ecozoic Living and Learning (CELL) located at Silang, Cavite, the Philippines, and were identified by the Bureau of Plant Industry (BPI). The bacteria that were used in this study are the Gram-negative bacteria *E. coli* (ATCC 25922) and *S. typhimurium* (ATCC 13311), as well as the Gram-positive bacteria *B. subtilis* (ATCC 6633) and *S. aureus* (ATCC 6538).

Extraction of *Strongylodon macrobotrys*

Ethanol (AR Grade, Chemsupply EA043) and methanol (AR Grade, Labscon AR1115) as extraction solvents in three different concentrations (50, 70, and 95%) were used to evaluate the effect on the extraction yield in the plants against the microorganisms a modified method from Hikmawanti *et al.* (2021). The extraction procedure was done by the Pharmaceuticals Section Chemicals and Energy Division of the Department of Science and Technology–Industrial Technology Development Institute (DOST-ITDI) in Taguig City, whereby ethanol 18.50g (8.8%) and methanol 7.87g (3.75%) crude extracts were obtained and stored in labeled amber bottles. Their procedures were as follows: 210 g of dried jade vine stem were pulverized with a Wiley mill and soaked in 3 L of 95% ethanol and methanol for 48 h. The mixture was filtered and concentrated for 2 h in a rotary evaporator. An evaporating dish and water bath were used to concentrate the extract.

Culture Media Preparation and Inoculation with Bacteria

Four bacterial strains were utilized in this study: *E. coli* (ATCC 25922) and *S. typhimurium* (ATCC 13311) (Gram-negative), and *B. subtilis* (ATCC 6633) and *S. aureus* (ATCC 6538) (Gram-positive). Stock cultures were prepared and cultured in Mueller-Hinton agar (MHA) and broth (MHB), following a modified method from Dulay *et al.* (2016). The modification involved culturing each bacterial strain in 9 mL of MHB medium at 37 °C for 24 h. Cultures were subsequently adjusted to match the turbidity of 0.5 McFarland standard (1.5×10^8CFU/mL). Thereafter, sterile cotton swabs were used to transfer the standardized bacterial suspension onto MHA plates for even distribution with a glass spreader.

Determination of Antimicrobial Property

The crude extract of *S. macrobotrys* was carried out using the Kirby-Bauer method. Sterile 5-mm diameter paper discs were soaked in ethanol and methanol crude extracts with 50, 70, and 95% concentrations. Water was used as

a negative control, whereas ciprofloxacin was used as a positive control. Paper discs were placed equidistantly on the medium and were incubated at 37 °C for 24 h (Dulay *et al.* 2016). Antimicrobial activities were then determined by measuring the zone of inhibition (ZOI) in millimeters using vernier calipers. The procedure was conducted in triplicates.

Statistical Analysis

One-way independent univariate analysis of variance (ANOVA) was conducted to compare the effect of different treatments on the ZOI. Two-way ANOVA with interaction was used to test the main effects and interaction effects of the type of solvent (ethanol and methanol) and level of concentration (50, 70, and 95%). Data were considered statistically significant based on a $p < 0.05$.

RESULTS

The jade vine stem extract was used for antimicrobial testing against the four selected bacterial strains. The average size of the ZOI by treatment is presented in Table 1. There were no inhibition zones observed when negative control (water) was utilized in four selected bacterial strains. On the other hand, the ZOI was larger in *B. subtilis* (35.0 ± 1.00mm) and *S. aureus* (29.6 ± 0.79mm) when positive control (ciprofloxacin) was used compared to when the extract of *S. macrobotrys* applied as an antimicrobial agent in *B. subtilis* (10.7 ± 1.56 mm) and *S. aureus* (8.94 ± 2.26 mm). One-way independent univariate ANOVA was conducted to compare the effect of different treatments on the ZOI of selected Gram-positive bacteria. The results in Table 2 showed that the effect of different treatments on the ZOI of *B. subtilis* and *S. aureus* was significant [F (2,33) = 1971, $p < 0.001$ and F (2,33) = 27.7, $p < 0.001$], respectively. In contrast, *S. macrobotrys* stem extract was inactive in Gram-negative bacteria.

Table 1. Average size of the zone of inhibition by treatment.

	B. subtilis	**S. *aureus***
Jade vine extract	10.7 ± 1.56	8.94 ± 2.26
Water (negative control)	0.00 ± 0.00	0.00 ± 0.00
Ciprofloxacin (positive control)	35.0 ± 1.00	29.6 ± 0.79

The displayed data were mean size ± standard deviation of the zone of inhibition (mm) in triplicates

Table 3. Average size of the zone of inhibition by type and concentration level of solvent used in extracting *S. macrobotrys'* stem.

		B. subtilis	***S. aureus***
Ethanol			
	50%	9.33 (1.15)	8.67 (1.53)
	70%	11.0 (0.00)	10.0 (1.00)
	95%	11.3 (1.53)	9.33 (0.58)
Methanol			
	50%	10.3 (2.31)	5.67 (4.04)
	70%	10.3 (1.53)	10.0 (1.00)
	95%	12.0 (1.73)	10.0 (1.00)

The displayed data were the mean sizes of the zone of inhibition (mm) and standard deviation in parenthesis

The largest ZOI after applying an extract of *S. macrobotrys* as an antimicrobial agent was observed in *B. subtilis* (12.0 ± 1.73 mm), whereas the smaller ZOI was observed in *S. aureus*, having 5.67 ± 4.04 mm (Table 3). It was also observed that the ZOI was highest when a 95% concentration level of methanol (12.0 ± 1.73) was used to extract the stem of *S. macrobotrys* compared to the 95% concentration level of ethanol in *B. subtilis* (11.3 ± 1.53) (Table 3). Two-way ANOVA with interaction was conducted to test the main effects and interaction effect of the type of solvent and level of concentration at 0.05 level of significance. There was no significant interaction effect between the type of solvent used in extracting *S. macrobotrys'* stem and the level of concentration [F (2,66) = 0.015, $p = 0.985$] (Table 4). A two-way ANOVA without interaction was conducted to re-examine the main effects. It can be seen that the variation in type of solvent [F (1,68) = 0.012, $p = 0.912$] and level of concentration [F (2,68) = 0.118, $p = 0.889$] have no significant main effect in sizes of the ZOI (Table 4).

Table 2. Difference in size of the zone of inhibition of B. subtilis and S. aureus across types of treatments.

B. subtilis	**SS**	**df**	**MS**	**F**	***p***
Treatment (jade vine extract, water, popular antibiotic)	5926	2	2963	1971	< 0.001
Residual	49.6	33	1.50		
S. aureus	**SS**	**df**	**MS**	**F**	***p***
Treatment (jade vine extract, water, popular antibiotic)	2439	2	1220	27.7	< 0.001
Residual	1451	33	44.0		

Legend: [SS] sum of squares; [df] degrees of freedom; [MS] mean square; [F] F-value; [*p*] *p*-value

Table 4. Difference in size of the zone of inhibition across types of solvent and concentrations of *S. macrobotrys* extract.

	SS	df	MS	F	*p*
Solvent (main effect)	0.003	1	0.003	0.012	0.913
Concentration (main effect)	0.066	2	0.033	0.114	0.892
Solvent-Concentration (interaction effect)	0.009	2	0.004	0.015	0.985
Residuals	18.991	66	0.288		
Solvent (main effect)	0.003	1	0.003	0.012	0.912
Concentration (main effect)	0.066	2	0.033	0.118	0.889
Residuals	18.999	68	0.279		

Legend: [SS] sum of squares; [df] degrees of freedom; [MS] mean square; [F] F-value; [*p*] *p*-value

DISCUSSION

Antimicrobial activity was consistent having an increased ZOI at various solvent concentrations. In this study, *S. macrobotrys* extract exhibited inhibitory activity against the Gram-positive bacteria (*B. subtilis* and *S. aureus*) but showed no activity against the Gram-negative bacteria (*E. coli* and *S. typhimurium*). This result showed that Gram-positive bacteria were easier to inhibit, which is most likely due to an ion channel film that can change cell permeability, which may lead to cell death. The susceptible membrane receptor activates its role upon binding of bacteriocins that are beneficial, small antimicrobial peptides produced by specific bacteria that could replace conventional antibiotics (Lu *et al.* 2018). Thus, antibiotics can pass through the peptidoglycan cell walls of both *B. subtilis* and *S. aureus* despite their rigidity (Nikolaidis *et al.* 2014). In contrast to Gram-negative bacteria, the physical and chemical properties of the outer membrane act as selective filter protection against substances that enter cells (Handayani *et al.* 2019). This is explained by the specific cell wall structure, particularly the existence of the outer envelope, which makes these microorganisms resistant to biocides and antibiotics and, occasionally, controls and prevents their movement to the target area (Denyer and Maillard 2002). In addition, it keeps the cell from bursting due to turgor and keeps the cell shape (Reshes *et al.* 2007). Hence, jade vine extract does not have antibacterial activity against *E. coli* and *S. typhimurium*.

The study revealed that the use of methanol as a solvent in extracting the plant shows more efficacy than ethanol (Table 3). The methanol extract showed a larger ZOI, exhibiting more antimicrobial activity than the ethanol extract. This might be due to the high concentration of phenolic, flavonoid, alkaloid, and terpenoid components in this extract (Ruiz-Ruiz *et al.* 2017). Ethanol extract, however, also had likewise noticeable antimicrobial activity than the methanol extract. The polyphenols in ethanol extracts like tannins and flavonoids have been discovered also to have a significant antimicrobial action (Chance 2016). The antibacterial action of tannins may be related to their capacity to inactivate microbial adhesions, enzymes, and cell envelope proteins, whereas that of flavonoids is attributed to their capacity to bind with extracellular and soluble protein and to complex with bacterial cell walls (Mogana *et al.* 2020). These actions are essential in preventing bacterial proliferation. In addition, increasing the concentration of S. *macrobotrys* stem extract disrupts the cell membrane, resulting in leakage of cytoplasmic components and leading to the death of bacteria. It is reasonable to speculate that the high concentration of extract has significant damage to the cell wall and membrane integrity, which causes rapid destruction of microorganisms (Mikusanti *et al.* 2009). This might be because the bioactive compounds of S. *macrobotrys* stem extract disrupt the enzyme reaction (Tortora *et al.* 2007).

S. macrobotrys' Fabaceae or Leguminosae family may contribute to its effectiveness as an antimicrobial agent. Legumes produce a wide range of secondary metabolites to defend against herbivores and microbes (Wink 2013). According to the study of Mmbengwa *et al.* (2008), the important characteristic of essential oils is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Similarly, linoleic, linolenic, and oleic acids were found to be the main fatty acid components in the Fabaceae or Leguminosae species (Berber *et al.* 2014). Linoleic acid, which is one of the fatty acids esterified to triglycerides, is an omega-6 fatty acid. The linoleic acid inhibits the growth of the bacteria by significantly increasing the membrane permeability, which is interpreted as the formation of pores (Greenway and Dyke 1979). In addition, fatty acids are known to have bactericidal and antifungal effects, which shows a direct relationship between the increasing unsaturation and toxicity of triglycerides, correlated with their oxidation

susceptibility (Kabara *et al.* 2011). Hence, these compounds were isolated in *S. macrobotrys* A. Gray stems that yielded exhibit diverse biological activities, including antimicrobial properties in the stem (Ragasa *et al.* 2013).

CONCLUSION

The present study concludes that *S. macrobotrys* stem extract shows minimal inhibitory activity against the Gram-positive bacterial strains *B. subtilis* and *S. aureus*. On the other hand, the Gram-negative bacterial strains *E. coli* and *S. typhimurium* were found to be resistant to the *S. macrobotrys* stem extract on a substantially broader scale than Gram-positive bacteria. This indicates that the extract's capacity to inhibit bacterial growth varies primarily on the resistance of bacterial strains.

In addition, it revealed that the methanol extracts yield much higher quantity levels than ethanol extracts. Among the three \ concentrations, it has been determined that 95% of methanol extract (12.0 ± 1.73) was the most effective among the tested solvents and concentration for solubilizing antimicrobial compounds from the tested plant materials. It was demonstrated that as concentration increases, the ZOI also increases.

Hence, statistical analysis showed that there were no significant interaction effects between the type of solvent used [F (1,68) = 0.012, p = 0.912] and the level of concentration [F (2,68) = 0.118, p = 0.889]. Thus, the *S. macrobotrys* stem continues to add toward the interest as a valuable source of natural products in potential treatments, particularly in combating antimicrobial resistance, leading to new choices for the treatment of infectious diseases and further utilization of the jade vine plant.

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STATEMENT ON CONFLICT OF INTEREST

The authors declare no conflict of interest.

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