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Antibacterial activity of *Orthosiphon stamineus* towards *Staphylococcus aureus* and methicilin resistant *Stahpylococcus aureus* (MRSA)

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ABSTRACT

In this study, antibacterial activity of crude extract from *Orthosiphon stamineus* was analyzed. Extraction through immersing method was performed using methanol and aqueous extraction from whole plant (except root). Spot test and MIC was conducted for both extracts to screen for their antibacterial activity against *Staphylococcus aureus* and 10 methicillin resistant *Staphylococcus aureus* (MRSA). No antibacterial activity was observed for all strain tested in the spot test assay. However, observation through MIC test showed that both extracts have antibacterial activity. The aqueous extract showed antibacterial activity in the range of 6.25 mg/mL to 50 mg/mL against *S. aureus*, MRSA strains (ATCC 4330, TM, WM2, K31, K32, K39, and K52). The antibacterial activity of methanol extract was found to be in the range of 12.5 mg/mL to 50 mg/mL). Methanol extract was found to be more active against *S. aureus* and 9 MRSA strains (ATCC 4330, BM3, TM, WM2, K31, K32, K39, K52 and K59**.** The aqueous extract tested on *S. aureus* has the highest MIC value which is 6.25 mg/mL. In conclusion, *O. stamineus* was found to have potential as an anti-MRSA agent. Further analysis has to be done in order to develop a novel anti-MRSA drug to combat multiple resistant bacteria.

KEY WORDS: *Orthosiphon stamineus*, antibacterial, MRSA, *Staphylococcus aureus.*

1. INTRODUCTION

Herbal plant is one of the most widely used sources of traditional medicine (Pytlakowska, 2012). Herbal plants are rich in bioactive compounds such as tannin, flavonol glycosides and flavonoid that have been scientifically proven attributed to many pharmacological properties especially antibacterial properties (Cowan, 1999). Therefore, study on ethnomedicinal plants will provide a novel new source of antimicrobial agents as plant extracts are rich in wide variety of secondary metabolites.

Orthosiphon stamineus (*O. stamineus*) also known as Misai Kucing is widely used in Malaysia for treatment of kidney problem, fever and jaundice (Wiart, 2002). In the study carried out by Ho (2010), good antibacterial activity of *O. stamineus* was observed. The activity observed was due to the presence of rosmarinic acid which causes formation of free radicals that act as antioxidant. The leaf extract of *O. stamineus* has shown to induce apoptosis in cancer cells (Doleckova, 2012). Another study has shown that functional recovery of the liver enzyme profiles in mice exposed to Thioacetamide when treated with the leaf extract (Sun, 2000; Alshawsh, 2011).

Staphylococcus aureus (*S. aureus*) is an ingenious bacterium with virulent factors which can cause increase in the percentage of nosocomial and community-acquired disease infection (Enright, 2000; Gould, 2012). Most of the clinical cases being reported were related to methicillin resistant *S. aureus* (MRSA) where certain MRSA have genetic factors which can cause an increase in virulence and causes clinical syndrome (Gordon & Lowry, 2008). Therefore, the discovery of new antibacterial agents is necessary to overcome this resistance. Previous studies showed that *O. stamineus* was able to inhibit growth of *S. aureus*. However, to our knowledge, this will be the first study conducted on *O. stamineus* against MRSA being reported.

2. MATERIALS AND METHODS

Preparation of plant extraction: *O. staminues* was obtained from Ladang Herba Ya'acob Berkat, Bukit Katil, Melaka in dried form. In this study, whole plant body of *O. stamineus* was used. About 1.8 kg of dried *O. stamineus* sample was grounded into powder using grinder (Cuttermill model no: CM-1) Powder was divided into two different packets with each contains 450 g of powder. Each packet of plant powder was then soaked in two different solvents, distilled water and methanol, in separate conical flasks 2 L. After the immersion, the extracts were transferred into Falcon tubes and ultracentrifugation was carried out at 1500 rpm for 5 minutes to remove sediments. For methanol extraction, rotary evaporator was used to concentrate the extract and the extract was left air dried in fume hood to obtain methanol crude extract. For aqueous extraction, the extract was transferred into Falcon tubes until one-third of the level of Falcon tubes. Aqueous extract was then frozen at -20°C before freeze-drying process was carried out. The freeze-drying process was done using freeze-dry machine. Each solution produced will be filtered and the precipitate was re-used three times for extraction and the extract was kept as stock. Each crude extract obtained was weighed and was dissolved at desired concentrations with sterile distilled water, DMSO 10% or DMSO 1% before experiments were carried out.

Spot Test: *Staphylococcus aureus* ATCC 25923 and MRSA ATCC 43300 used in this study was obtained from School of Bioscience and Biotechnology (PPBsBt), Universiti Kebangsaan Malaysia (UKM) culture collection with

the accession numbers SKUK1016 and SKUK1008 respectively. Nine strains of methicillin resistant *S. aureus* (MRSA) were isolated from different hospitals around Selangor area designated BM3, K59, K31, K52, K32, K39, TM, BM2 and WM2. The test was carried out following the protocol of Tomé et al. (2006) with slight modifications. *S. aureus* and ten strains of MRSA were sub-cultured on nutrient agar (NA). A few colonies of inoculum were transferred into 5 mL of Muller Hinton broth (MHB) and the turbidity was adjusted to 0.5 McFarland standards. Then bacteria lawn was produced on the surface of Muller Hinton agar (MHA) with sterile cotton buds. Different concentrations of plant extracts were prepared as previously described. 5 µL of different concentrations of *O. stamineus* extract were spotted on the bacterial lawn at indicated locations. Commercial antibiotic discs, vancomycin, was used as positive control while 5 µL of DMSO 10% or 5 µL of sterile distilled water was used as negative control. The bacteria were then incubated at 37°C for 24 hours. Three replicates of the experiment were performed for each pathogen. After 24 hours of incubation, diameter of inhibition zones produced on the plates were observed, measured and recorded.

Minimum inhibitory concentration: Microdilution method was used to determine the minimum inhibitory concentration (MIC) value of *O. stamineus* extracts according to Eloff (1998). A series of two-fold dilution was performed on 96 well microtitre plate for both of the extracts with starting concentration of 50 mg/mL. After that, 50 µL of bacterial suspension was inoculated into wells containing *O. stamineus* extract solutions of different concentrations. 50 µL of rifampicin at concentration of 10 mg/mL and 50 µL of MHB together with 50 µL of bacterial inoculum were used as positive and negative controls, respectively. Solvents or diluents for the extracts were also used as a control to ensure no antibacterial effect was contributed by the solvents. Extracts of different concentrations which were not inoculated with bacteria were used as colour control. This procedure was performed to give a better visualization of the change in colour after colorimetric MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) solution was added into the wells. The entire plate was then incubated for 24 hours at 37°C followed by MTT assay. MTT assay was performed to determine MIC of each extract towards tested pathogens. 50 µL of MTT solution was added into all wells and the plate was then wrapped with aluminium foil and was incubated at 37°C for two hours. The change in colour of the MTT solution in each well was observed.

3. RESULTS

Plant extracts of *Orthosiphon stamineus*: Methanol and aqueous extraction of *O. stamineus* were selected for this study. The percentage yields of methanol and aqueous extracts for *O. stamineus* were found to be 8.00% and 4.54% on dry weight basis, respectively.

Antibacterial activity of *O. stamineus* crude extracts: The antibacterial activity of different plant extracts from whole plant parts of *O.stamineus* was first screened through spot test. Results are shown in Table.2. Observation from this test showed that none of the plants extracts tested elicited any bacterial growth inhibition.

Table.1. Total percentage yields of methanol and aqueous extracts for *O. stamineus*

Extracts	Extract yield (%)
Methanol	8.00
Aqueous	4.54

Table.2. Antibacterial activity of crude methanol and aqueous extracts of *O. stamineus* by spot test

Tested pathogen	Concentration of extract (mg/mL)	Zone of Inhibition (mm)				
		Methanol	Aqueous	Vancomycin	10% DMSO	H_2O
S. aureus	50.00	0.00	0.00	19.67 ± 0.57	0.00	0.00
	25.00	0.00	0.00			
	12.50	0.00	0.00			
	6.250	0.00	0.00			
	3.125	0.00	0.00			
MRSA ATCC 43300	50.00	0.00	0.00		0.00	0.00
	25.00	0.00	0.00			
	12.50	0.00	0.00			
	6.250	0.00	0.00			
	3.125	0.00	0.00	17.67 ± 0.57		
MRSA BM2	50.00	-	-	18.33 ± 1.53	-	-
	25.00	-	-			
	12.50	-	-			
	6.250	-	-			
	3.125	-	-			

MRSA BM3	50.00 25.00 12.50 6.250 3.125	- - - - -	- - - - -	18.00 ± 0.00	-	-
MRSA TM	50.00 25.00 12.50 6.250 3.125	0.00 0.00 0.00 0.00 0.00	0.00 0.00 0.00 0.00 0.00	18.67 ± 0.58	0.00	0.00
MRSA WM2	50.00 25.00 12.50 6.250 3.125	0.00 0.00 0.00 0.00 0.00	0.00 0.00 0.00 0.00 0.00	17.67 ± 0.58	0.00	0.00
MRSA K31	50.00 25.00 12.50 6.250 3.125	0.00 0.00 0.00 0.00 0.00	0.00 0.00 0.00 0.00 0.00	17.33 ± 0.058	0.00	0.00
MRSA K32	50.00 25.00 12.50 6.250 3.125	0.00 0.00 0.00 0.00 0.00	0.00 0.00 0.00 0.00 0.00	19.00 ± 0.00	0.00	0.00
MRSA K39	50.00 25.00 12.50 6.250 3.125	0.00 0.00 0.00 0.00 0.00	0.00 0.00 0.00 0.00 0.00	18.67 ± 1.15	0.00	0.00
MRSA K52	50.00 25.00 12.50 6.250 3.125	0.00 0.00 0.00 0.00 0.00	0.00 0.00 0.00 0.00 0.00	18.00 ± 0.00	0.00	0.00
MRSA K59	50.00 25.00 12.50 6.250 3.125	0.00 0.00 0.00 0.00 0.00	0.00 0.00 0.00 0.00 0.00	18.00 ± 1.73	0.00	0.00

Values are mean ± standard deviation was analysed individually in triplicate.

Determination of MIC value of *O.stamineus* extracts: The MIC values of methanol and aqueous extract of the whole plant of *O. stamineus* against *S. aureus* and MRSA are presented in Table.3, indicated that methanol extract of *O. stamineus* were found to be more active in inhibiting the growth of *S. aureus* and nine MRSA strains. The lowest MIC value was seen for *S. aureus* (12.5 mg/mL for methanol extract and 6.25 mg/mL for aqueous extract).

Table.3. Minimum Inhibitory Concentration (MIC) of *O. stamineus* extracts against *Staphylococcus aureus* and MRSA strains

Tested pathogen	Minimum inhibitory concentration (mg/mL)	
	Methanol	Aqueous
Staphylococcus aureus	12.5	6.25
MRSA ATCC 4330	50.0	12.5
MRSA BM2	-	-
MRSA BM3	25.0	-
MRSA TM	50.0	50.0
MRSA WM2	25.0	-
MRSA K31	50.0	50.0

MRSA K32	25.0	50.0
MRSA K39	50.0	50.0
MRSA K52	25.0	50.0
MRSA K59	50.0	-

DISCUSSION

The study showed that the whole plant of *O. stamineus* can be used as an antibacterial agent against *S. aureus* and MRSA strains. Therefore, *O. stamineus* could be introduced as natural antibacterial substance. In previous study, Alshawsh (2012), showed that the lowest MIC value of *O. stamineus* leaf extract at 1.56 mg/mL while in this present study, the aqueous extract from the whole plant showed the lowest MIC value of 6.25 mg/mL. The antibacterial activity in their study is higher compared to this study because different part of the plant was used. Different parts of plant possess different secondary metabolites and also the amount of each secondary metabolite (Fenny, 1976). Result of MIC test indicated that both extracts have antibacterial activity against *S. aureus* and MRSA strains. The results are comparable to the results reported by Kong (2014). In their study, aqueous extract of *O. stamineus* was observed to be able to protect *Caenorhabditis elegan* (nematode) from *S. aureus* and MRSA infection. These finding indicated that *O. stamineus* leaf extract can modulate immune response of the nematodes against *S. aureus* and MRSA infection. Although there have been some reports on antibacterial activity of *O. stamineus* leaf extract, little information is available on its antibacterial activity of *O. stamineus* whole plant extract against *S. aureus* and MRSA strains. On the other hand, methanol *O. stamineus* whole plant extract were previously reported to be active against food-borne bacteria, *Vibrio parahaemolyticus*. According to previous studies by Ho (2010) and Akuwoah (2005), the presence of rosmarinic acid in *O. stamineus* extract directly contribute to antibacterial action.

4. CONCLUSION

As a conclusion, both methanol and aqueous extract of *O. stamineus* have been found to have antibacterial activity against *Staphylococcus aureus* and MRSA. *O. stamineus* methanol extract however has more profound activity against MRSA compared to *O. stamineus* aqueous extract. Thus, *O. stamineus* methanol extract have potential to be developed as anti-MRSA agent. However, further study need to be done to further characterise different component of the extract that conferred towards this antibacterial activity.

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