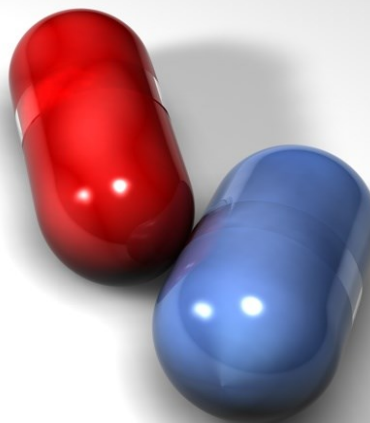


Bangladesh Journal of Pharmacology

Volume: 17; Number 1; Year 2022



Cite this article as: Sundar RDV, Arunachalam S. Anti-MRSA activity of *Pollianthes tuberosa* leaf extracts. Bangladesh J Pharmacol. 2022; 17: 11-13.



Letter to the Editor

Anti-MRSA activity of *Polianthes tuberosa* leaf extracts

Sir,

Staphylococcus aureus was known to be the most significant clinical pathogen, causing various infections ranging from benign, superficial skin lesions to life-threatening infections like pneumonia, bacteremia, toxic shock syndrome, endocarditis and infections associated with medical devices (Gowrishankar et al., 2013). All over the world, presently about 90-95% of *S. aureus* clinical strains were penicillin-resistant. The foremost antistaphylococcal penicillin-methicillin was reported in 1959. Within 2 years, the first MRSA strain emerged and now it accounts for about 60% in the US from the clinical strains isolated from intensive care units. They are very rapid and in many parts of the US, it causes soft-tissue infections in patients (Sakoulas and Moellering, 2008). The community and hospital-acquired infections were mainly caused due to Gram positive bacteria, particularly the proliferation of nosocomial pathogens such as vancomycin-resistant enterococci and MRSA (methicillin-resistant *Staphylococcus aureus*). These pathogens are stimulated by poor infection control and antibiotic selection pressure (Palombo and Semple, 2002).

Plants with medicinal properties have been studied in different parts of the world as a cure for numerous ailments (Ravi et al., 2020). Globally medicinal plants can serve as a rich source of antibacterial activities (Voravuthikunchai and Kitpipit, 2005). On comparing with synthetic drugs, plants derived antimicrobials were not associated with adverse effects. It has a wide range of potential therapeutic properties to treat various infectious diseases (Ravi et al., 2020).

Tuberose (*Polianthes tuberosa* L.) is an ornamental plant that belongs to the family Amaryllidaceae. They are widely used in the perfume industry for their intense fragrance (Barghout et al., 2020). Studies reported that chloroform, n-hexane, water and carbon tetrachloride from concentrate methanol extract where exhibited antagonistic activity against Gram negative pathogens such as *Vibrio mimicus*, *Shigella dysenteriae*, *Shigella boydii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. paratyphi*, *Vibrio parahemolyticus* and Gram positive pathogens like *Bacillus subtilis*, *S. aureus*, *Sarcina*

lutea, *B. megaterium*, *B. cereus* at 400 µg dose/disc with 9-11 mm resistance range. The methanol extract obtained from the dried flower was active against *Proteus mirabilis* with inhibition of 16 ± 1 mm at 60 µg/mL dose and 40 µg/mL dose, it was active against *E. coli* with 12.3 ± 1.5 mm inhibition ((Setiani et al., 2020). The present study aimed to screen the antagonistic activity of crude extracts obtained from *P. tuberosa* against selected bacterial pathogens.

Healthy plant leaf segments were collected in a sterile polythene bag from the Vellore district. Samples were washed thoroughly in running tap water followed by double distilled water to remove debris. Then it is shade dried for a week and powdered using an electric blender. About 10 g of powder were soaked in 100 mL of Milli Q water and different polarity solvents such as dichloromethane, butanol and ethyl acetate in a 250 mL Erlenmeyer flask. The flask was kept in a rotatory shaker at 120 rpm for 2 days. Then the content was filtered using Whatman filter paper No. 1 and dried using a rotatory evaporator. The filtrate was kept in an airtight container for further studies (Ravi et al., 2020).

Phytochemical screening was done by a standard protocol to determine the presence or absence of phytochemicals such as steroid, terpenoid, phenol, alkaloid, saponin, flavonoid, quinone and tannin in the leaf powder (Eve et al., 2020). Antibacterial properties of the leaf crude extracts were tested against methicillin-resistant *S. aureus* (ATCC 43300), *S. aureus* (ATCC 25923) and *S. aureus* (MTCC 3160). The strains were pre-cultured in tryptic soy broth. The turbidity of the test bacterial suspension was pre-adjusted with 0.5 McFarland standards in 0.85% saline. Bacterial cultures were streaked over the freshly prepared Muller Hinton agar plates. About 5 mm wells were made using a sterile cork borer. To the wells, 100 µL of different concentrations (25, 50, 75, 100 µg/mL) of crude extracts were loaded. The plates were incubated for 18 hours at 37°C and the zone of inhibition was measured in diameter to determine the antibacterial activity (Ravi et al., 2019).

The extracts that showed significant antibacterial activity was taken further to determine minimum inhibitory concentration by broth micro-dilution technique. To 96 well plate, 100 µL of Muller Hinton broth was added. The dichloromethane and ethyl acetate crude extracts were diluted at a 1 mg/mL concentration. From the stock solution, 100 µL of the crude extracts



Table I						
Antibacterial activity of <i>P. tuberosa</i>						
Pathogens	Extract concentrations ($\mu\text{g/mL}$)	Milli Q	Butanol	Dichloromethane	Ethyl acetate	Linezolid (30 mg)
		Zone of inhibition (mm)				
MRSA (ATCC 43300)	25	-	-	-	-	24
	50	7	-	-	13	
	75	10	-	10	19	
	100	11	14	18	21	
<i>S. aureus</i> (ATCC 25923)	25	-	-	9	-	24
	50	7	-	12	12	
	75	9	8	16	15	
	100	10	12	18	18	
<i>S. aureus</i> (MTCC 3160)	25	-	-	-	7	24
	50	8	14	11	9	
	75	10	15	15	11	
	100	13	17	18	13	

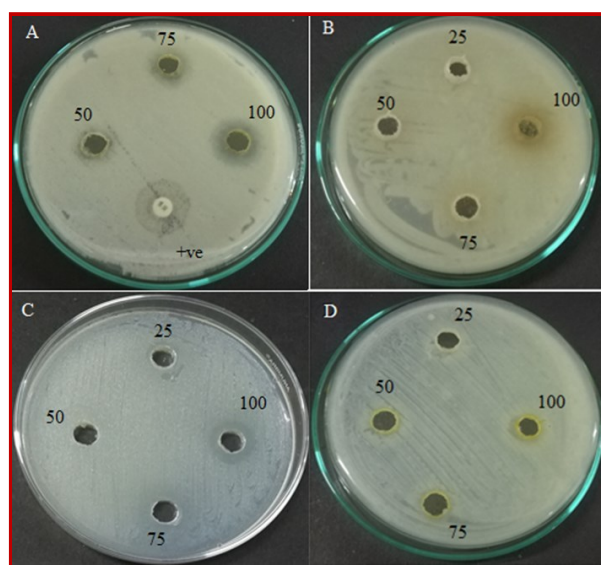


Figure 1: Ethyl acetate (A), dichloromethane (B), butanol (C), and water (D) crude extracts against MRSA

were dispensed to the first well. Followed by that two-fold serial dilution were made up of 7 wells. The concentration of the wells ranges from 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 $\mu\text{g/mL}$. About 5 μL of fresh prepared bacterial strain was added to each well. The last two wells containing Muller Hinton broth served as positive control and the well with Muller Hinton broth and bacteria served as a negative control. The plate was incubated for 12-18 hours at 37°C. After incubation growth was examined visually and data were recorded. The presence of turbidity showed bacterial growth in the well, while clear broth with no turbidity showed

Table II		
MIC of crude extracts against test pathogens		
Pathogens	Ethyl acetate	Dichloromethane
	MIC ($\mu\text{g/mL}$)	
MRSA (ATCC 43300)	6.25	12.5
<i>S. aureus</i> (ATCC 25923)	25	25
<i>S. aureus</i> (MTCC 3160)	12.5	25

inhibition of bacterial growth. The lowest concentration at which the bacterial visible growth was inhibited was considered as the minimum inhibitory concentration value. The entire test was performed in triplicates (Bussmann et al., 2010).

Phytochemical screening of leaf powder revealed the presence of phenol, alkaloid, saponin, flavonoid, quinone whereas steroid, terpenoid and tannin were absent.

All the crude extracts screened exhibited significant antibacterial activity against the tested pathogens (Figure 1). The inhibition ranged from 7 to 21 mm (Table I). The ethyl acetate crude extract demonstrated the highest antagonism against MRSA with a minimum inhibitory concentration value of 6.25 $\mu\text{g/mL}$ and a zone of inhibition of 21 mm. Followed by it exhibited 18 mm zone of inhibition against *S. aureus* (ATCC 25923) with a minimum inhibitory concentration of 25 $\mu\text{g/mL}$. The DCM extract showed antagonistic activity against MRSA with a minimum inhibitory concentration value of 12.5 $\mu\text{g/mL}$ and zone of inhibition of 15 mm at 100 $\mu\text{g/mL}$ concentration. Table II shows their MIC values.

Thus, the results presented in this study should be taken further, as it might provide a novel bioactive compound that is effective against the infections caused by multi-drug resistant *S. aureus*.

The authors thank the Vellore Institute of Technology for providing lab facilities to carry out the present study.

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