Antibacterial activity of ethanolic extract from Derris scandens against human pathogenic bacteria

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ABSTRACT: The purpose of this study was to assess the antibacterial activity of an ethanolic extract of *Derris scandens* against clinically isolated human pathogenic bacteria. **Materials and methods:**The ethanolic extract was assessed for antibacterial activity against ten human pathogenic bacteria at different concentrations (25 - 100 µg/ml) through the agar well diffusion method. Group 1 (25µg/ml), Group 2 (50µg/ml), Group 3 (75µg/ml) Group 4 (100µg/ml), Group 5 (positive control), and Group 6 (negative control) and calculated the zone of inhibition. **Results and discussion:** The ethanolic extract showed antibacterial activity against 8 clinical bacterial strains of the 10 pathogens tested. The highest concentration (100µg/ml) of the ethanolic extract showed a maximum of 20 and 22mm inhibition zone against *E. coli* and *S. typhi*. The mean values were 0.130, 0.141, 0.117, 0.194, and 0.120 The sample size was calculated with a pretest G power of 80%. The sample size per group is 6, and the total sample size is 60. **Conclusion:** The effective bacterial inhibition rate of *Derris scandens* might provide a promising beneficial agent against bacterial infection and help to develop future infectious disease drugs.

Keywords: *Derris scandens*, novelmethanolic extract, infection, antibacterial, human health, drugs, pathogenic bacteria, health

INTRODUCTION

Infectious diseases remain a major public health concern, accounting for 41% of the worldwide disease burden measured in Disability Adjusted Life Years (DALYS). One of the primary causes of this problem is the rapid spread of acquired bacterial resistance to antibiotics, which has created a severe threat to worldwide public health (Xuan et al. 2023). Despite the fact that pharmaceutical corporations have developed a number of novel antibiotics during the last three decades, microorganism resistance to these medications has increased. Bacteria, in general, have the genetic potential to transmit and acquire resistance to medications used as therapeutic agents (Barczyński et al. 1999).

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Microbial resistance is on the rise, and the future utility of antimicrobial medications remains unknown. As a result, steps must be taken to address this issue, such as limiting antibiotic use, conducting research to better antibiotics and understand the genetic underpinnings of resistance, and continuing trials to produce new antibiotics, either synthetic or natural. The ultimate goal is to provide appropriate and efficient antibacterial medications to patients. Plants have long been a great source of natural products for sustaining human health, particularly in the recent decade, with more extensive investigations for natural remedies. In Brazil, the utilization of plant substances for pharmacological reasons has increased (Zhu et al. 2022). Scientists have extracted biologically active compounds from orchids using solvents such as ethanol, methanol, chloroform, ethyl acetate, and n-butanol (Srivastava et al. 2023).

These compounds include alkaloids, bibenzyl derivatives, flavonoids, and phenanthrenes, which may have therapeutic benefits. Orchid bioactive compounds have antimicrobial activity and are powerful inhibitors of both gram-positive and gram-negative bacteria (Perue et al. 2023). The utilization of plant extracts and phytochemicals, both of which have antibacterial properties, can be extremely beneficial in therapeutic treatments. A number of studies have been undertaken in various nations over the last few years to demonstrate such efficacy. Many plants have been employed because of their antibacterial properties, which are related to chemicals synthesized in the plant's secondary metabolism. These items are distinguished by their active ingredients, such as the phenolic compounds found in essential oils and tannin (Fukumoto et al. 2021). The methanolic extract of Acanthephippium bicolor orchid leaves demonstrated antibacterial activity against a variety of pathogenic bacteria. The current study investigated the antibacterial activity of an ethanolic extract of *Derris scandens* leaves against clinical pathogens.

MATERIALS AND METHODS

Experimental design: At various doses of 25–100 g/ml, the antibacterial activity of the ethanol extract was evaluated against ten human pathogenic microorganisms. In order to compute the zone of inhibition, the various concentrations of the sample and standard were separated into six groups: Group 1 (25 g/ml), Group 2 (50 g/ml), Group 3 (75 g/ml), Group 4 (100 g/ml), Group 5 (Positive control), and Group 6 (Negative control). Pretest G power of 80% was used to compute the sample size. There are 6 samples each group, for a total of 60(Faul et al. 2007)

Collection of leaves and preliminary treatment: Derris scandens leaves were collected from Puthukottai district, Tamil Nadu, India and washed with tap and distilled water before being shade dried and powdered in a mixer grinder.

Preparation of ethanolic extract: The procedure outlined for making the extract was (Saravanan, Murugan, and Kumaravel 2020) In a nutshell, 100g of powder samples were extracted in 1L of ethanol three times by soaking for a whole night at room temperature. Whatman No. 1 filter paper, which is sterile, was used to filter the final product. In a rotary evaporator set at 40°C, the extracts from three sequential soakings were mixed and evaporated under decreased pressure. The concentrated extract (ethanolic extract) was then vacuum-dried in desiccators and stored in the refrigerator at -20°C until it was needed for additional study.

Antibacterial activity-Bacterial strains:Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Vibrio cholerae, Klebsiella oxytoca, Escherichia coli, Salmonella

paratyphi, Proteus mirabilis, Vibrio parahaemolyticus, and Streptococcus pyogenes were the ten human clinical pathogens chosen for this investigation from the Microbial Culture Maintenance Laboratory, Department of Medical Microbiology, Saveetha Medical College and Hospital (SMCH), Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India.

Inoculum Preparation: In test tubes, nutrient broth was made and autoclaved for 15 minutes at 15 lbs of pressure. Each of the 10 bacterial strains was separately injected into the sterile nutrient broth, which was then incubated for 24 hours at 37° C..

Antibacterial activity-Agar well diffusion method:According to the instructions provided by, the ethanolic extract's antibacterial activity was assessed using the agar well diffusion technique (Jeyaseelan et al. 2012). After 24 hours, the cultures were aseptically swabbed with a sterile cotton swab on nutritional agar plates. On swabbed plates, the wells were punched using a sterile 5 mm well cutter. In 10% dimethyl sulfoxide, a stock solution of ethanolic extract was created at a concentration of 1 mg/ml (DMSO). 25, 50, 75, and 100 g/ml were the four distinct concentrations that were employed. Standard tetracycline (1 mg/ml) and control (10% DMSO) were injected into the designated wells. At 37 oC, the plates were incubated for 24 hours. Each well's inhibitory zone diameter was measured and converted to millimeters to yield the results.

Statistical analysis

All experimental data were subjected to a one way analysis of variance (ANOVA) test to check the statistical significance using IBM SPSS version 28.0.0 to determine the difference among means at the level of 0.05.

RESULTS

Yield of ethanolic extract

The yield of the ethanolic extract from the *D. scandens* flower was found to be 0.85% (w/w) on a dry weight basis in the current study.

Antibacterial activity of ethanolic extract

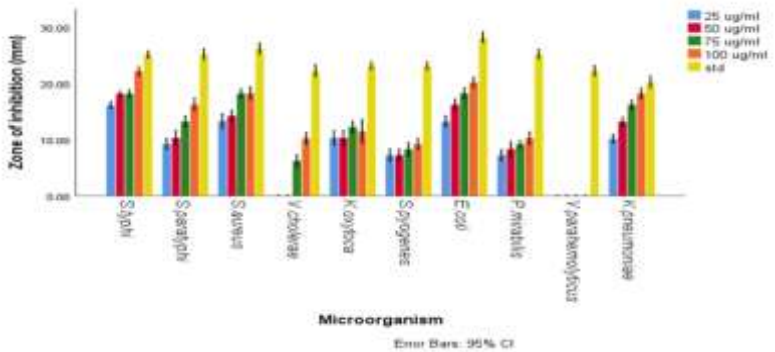

Fig. 1. Antibacterial activity of ethanolic extract from the plant of *D. scandens* against human pathogens, where X axis represents the microorganisms, and the Y axis represents the zone of inhibition

The ethanolic extract from D. scandens showed antibacterial efficacy against 8 clinical bacterial strains out of the 10 pathogens studied. The ethanolic extract showed the highest degree of antibacterial activity at a concentration of 100 g/ml (Table 1). A 100 g/ml concentration of the ethanolic extract showed an inhibitory zone of up to 20 and 22 mm against S. typhi and E. coli, respectively. The lowest inhibitory zone measured 9 mm for S. pyogenes. The ethanolic demonstrated the most activity with an 18 mm inhibition zone against S. typhi, S. aureus, and E. coli in the case of a 75 g/ml concentration, while S. pyogenes showed the lowest activity with a 9 mm inhibition zone.

The ethanolic extract's maximal inhibition zone against S. typhi was 18 mm, and its lowest inhibition zone against P. mirabilis was 8 mm at 50 g/ml concentration. At a concentration of 25 g/ml, the ethanolic extract exhibited a 16 mm inhibition zone against S. typhi, but only 7 mm and 7 mm inhibition zones against S. pyogenes and P. mirabilis, respectively. P 0.05 for the average values.(Table 1-3 and Fig. 1).

Table 1. Antibacterial activity of ethanolic extract from the plant of *D. scandens* against human pathogens

S. No	Name of the strains	Zone of inhibition (mm)					
		25µg/ml	**50µg/ml**	**75µg/ml**	**100µg/ml**	**+ve**	**-ve**
1	*S. typhi*	16	18	18	22	25	-
2	*S. paratyphi*	9	10	13	16	25	-
3	*S. aureus*	13	14	18	18	26	-
4	*V. cholerae*	-	-	6	10	22	-
5	*K. oxytoca*	10	10	12	11	23	-
6	*S. pyogenes*	7	7	8	9	23	-
7	*E. coli*	13	16	18	20	28	-
8	*P. mirabilis*	7	8	9	10	25	-
9	*V. parahaemolyticus*	-	-	-	-	22	-
10	*K. pneumoniae*	10	13	16	18	20	-

		Summ of squares	df	Mean square	F	Sig.
25ug/ml	Between groups	772.937	9	85.882	662.039	.000
	Within groups	2.594	20	.130		
	total	775.532	29			
50ug/ml	Between groups	1028.065	9	114.229	812.193	.000
	Within groups	2.813	20	.141		
	total	1030.878	29			
75ug/ml	Between groups	999.398	9	111.044	951.944	.000
	Within groups	2.333	20	.117		
	total	1001.731	29			
100ug/ml	Between groups	1194.725	9	132.747	683.817	.000
	Within groups	3.883	20	.194		
	total	1198.607	29			
std	Between groups	145.528	9	16.170	134.748	.000
	Within groups	2.400	20	.120		
	total	147.928	29			

Table 2. Comparison of th antibacterial activities of ethanolic extracts

Table 3. Means for groups in homogeneous subsets of ethanolic extract.

Microorganisms	N	1	2	3	4	5
K.pneumoniae	3	20.2367				
K. oxytoca	3		22.2433			
S.typhi	3		22.2500			
S. aureus	3		23.1500			
V.parahemolyticus	3		23.1500			
E.coli	3			25.1467		
S.paratyphi	3			25.1833		
V.cholerae	3			25.2133		
S.pyogenes	3				26.2233	
P.mirabilis	3					28.2100
Sig.		1.000	1.000	1.000	1.000	1.000

Means for in group in homogeneous subsets are displayed.
Uses Harmonic mean sample size=3.000

DISCUSSION

With inhibition zones of 20 and 22 mm against E. coli and S. typhi at higher concentrations of 100 g/ml and 7 mm against S. pyogenes and P. mirabilis at lower concentrations of 25 g/ml, the ethanolic extract from the leaves of D. scandens demonstrated the highest antibacterial activity in the current study. In the current investigation, ethanolic extract from Dendrobium crumenatum had the highest antibacterial activity when compared to the prior methanolic extract, which had a 13mm zone of inhibition against S. typhi at a concentration of 1000g/ml (Jeyaseelan et al. 2012). Similarly, the Hibiscus rosa-sinensis flower's ethanolic extract's antibacterial activity demonstrated a 14 mm inhibition zone against S. aureus and a 7 mm inhibition against K. pneumoniae, leaving the other bacterial species did not show any inhibition(Jeyaseelan et al. 2012)

The antibacterial activity of methanolic extract from the orchid *Sarcanthuspauciflorus* showed 18, 24, 13 and 20mm inhibition zone at 50mg/ml concentration against *S. aureus, B. subtilis, E. coli* and *Pseudomonas aeruginosa.*Bhalodia and Shukla (2011) reported the antibacterial activity of hydroalcoholic and chloroform extract of flowers from *Cassia fistula* was (19, 19, 19 & 17mm) and (21, 19, 18 & 16) at 250µg/ml against *S. aureus*, *S. pyogenes*, *E. coli* and *P. aeruginosa*. In the present study, the result of the antibacterial

activity via zone of inhibition in ethanolic extract may be attributed to the presence of hydroxyl and carbonyl functional groups.

CONCLUSION

In conclusion, the ethanolic extract of *D. scandens* leaves showed good inhibition against several clinical pathogens, with respectable concentrations. As a result, *D. scandens* ' effective bacterial inhibition rate may provide a promising beneficial agent against bacterial infection and help to develop future infectious disease drugs.

DECLARATIONS

Conflict of Interests

No conflict of interests in this manuscript

Authors Contribution

Author BSC was involved in data collection, data analysis, manuscript writing. Author PS was involved in the Action process, Data verification and validation, and Critical review of manuscript.

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