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RESEARCH ARTICLE

Evaluation of Antibacterial Activity of Chloroform and Acetone Extracts from *Tabernaemontana divaricata*

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Abstract: The widespread emergence of antibiotic resistance in pathogenic bacteria has necessitated the exploration of alternative antimicrobial agents, with plant-derived compounds gaining significant attention. This study evaluated the antibacterial activity of chloroform and acetone extracts obtained from the stem of *Tabernaemontana divaricata*, a plant widely used in traditional medicine. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, tannins, and saponins in the extracts. The antibacterial activity was assessed against Gram-positive *Staphylococcus aureus* (ATCC-25923) and Gram-negative *Pseudomonas aeruginosa* (ATCC-27853) using the agar well diffusion method. The zones of inhibition were compared to those of standard antibiotics, azithromycin (for Gram-positive) and metronidazole (for Gram-negative). Both chloroform and acetone extracts exhibited concentration-dependent antibacterial activity against the tested strains. The chloroform extract exhibited more potent activity, with larger zones of inhibition compared to the acetone extract. At the highest tested concentration of 1000 µg/mL, the chloroform extract exhibited zones of inhibition of 13.73 mm and 12.25 mm against S. aureus and P. aeruginosa, respectively. The findings suggest that the stem of *T. divaricata* possesses bioactive compounds with potential antibacterial properties, warranting further investigation for the identification and isolation of the active principles responsible for the observed activity.

Keywords: *Tabernaemontana divaricata*; Antibacterial activity; Chloroform extract; Agar well diffusion method; Phytochemicals; Antibiotic resistance.

1. Introduction

Plants have been an invaluable source of therapeutic agents since ancient times, and their use in traditional medicine systems has been widely documented across various cultures. With the increasing prevalence of antimicrobial resistance and the emergence of new infectious diseases, there is an urgent need to explore alternative sources of antimicrobial compounds [1,2]. Natural products derived from plants have gained significant attention in recent years due to their potential to provide novel bioactive compounds with unique mechanisms of action. *Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult., commonly known as "Crape Jasmine" or "Nandhivriksha," is an evergreen shrub belonging to the Apocynaceae family.

Figure 1. Image of *Tabernaemontana divaricata* plant

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This plant is widely distributed in tropical and subtropical regions of Asia, including India, and has been extensively used in traditional medicine systems for treating various ailments. The plant is rich in phytochemicals, such as alkaloids, flavonoids, terpenoids, steroids, and phenolic compounds, which are known to possess diverse biological activities, including antimicrobial, anti-inflammatory, antioxidant, and cytotoxic properties. [3,4] Several studies have reported the antibacterial activity of *T. divaricata* leaf extracts against a range of pathogenic bacteria, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Escherichia coli, and *Klebsiella pneumoniae*. [5] However, limited information is available regarding the antibacterial potential of the stem extracts of this plant. The present study focuses on evaluating the antibacterial activity of chloroform and acetone extracts obtained from the stem of *T. divaricata* against clinically relevant bacterial strains, *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative).

2. Materials and methods

2.1. Plant material collection and authentication

The stems of *Tabernaemontana divaricata* were collected from the college garden of Pullareddy Institute of Pharmacy, Domadugu, Hyderabad, India. The plant material was authenticated by a qualified botanist, and a voucher specimen was deposited in the institutional herbarium for future reference [6, 7].

2.2. Extraction procedure

The collected stems were washed, air-dried under shade, and ground into a coarse powder using a mechanical grinder. The powdered material was subjected to successive solvent extraction using chloroform and acetone in a Soxhlet apparatus. [8] The extracts were filtered, and the solvents were evaporated under reduced pressure using a rotary evaporator to obtain the dried chloroform and acetone extracts, respectively.

2.3. Phytochemical screening

The chloroform and acetone extracts were subjected to preliminary phytochemical screening to detect the presence of various phytochemical constituents, including alkaloids, flavonoids, glycosides, tannins, saponins, and carbohydrates, following standard qualitative tests described in the literature [9, 10]

2.4. Test organisms and culture conditions

The antibacterial activity of the extracts was evaluated against two clinically relevant bacterial strains: *Staphylococcus aureus* (ATCC-25923, Gram-positive) and *Pseudomonas aeruginosa* (ATCC-27853, Gram-negative). The bacterial strains were obtained from the American Type Culture Collection (ATCC) and maintained on Nutrient Agar (NA) slants at 4°C. Prior to the experiment, the cultures were revived by subculturing on fresh NA plates and incubating at 37°C for 24 hours [11]

2.5. Inoculum Preparation

Fresh bacterial inocula were prepared by transferring a loopful of the overnight culture into sterile Nutrient Broth (NB) and incubating at 37°C for 4-6 hours. The turbidity of the bacterial suspension was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1×10^8 colony-forming units per milliliter (CFU/mL). [12]

2.6. Antibacterial Activity Assay

The antibacterial activity of the chloroform and acetone extracts was evaluated using the agar well diffusion method, as described by Perez et al. (1990). Briefly, Müller-Hinton Agar (MHA) plates were inoculated with the standardized bacterial suspension by spreading it evenly over the surface using a sterile cotton swab. Wells of approximately 6 mm in diameter were punched into the agar using a sterile cork borer. The extracts were dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions of 10 mg/mL. Serial dilutions of the stock solutions were prepared to obtain various concentrations ranging from 100 to 1000 µg/mL. Each well was filled with 50 µL of the respective extract solution, and DMSO served as the negative control. [13] Azithromycin and metronidazole were used as positive controls for Gram-positive and Gram-negative bacteria, respectively.

The plates were incubated at 37°C for 24 hours, and the diameters of the zones of inhibition (ZOI) around the wells were measured using a digital caliper. The experiments were performed in triplicate, and the mean values were calculated. [14]

2.7. Statistical Analysis

The data obtained from the antibacterial activity assay were analyzed using appropriate statistical methods. The results were expressed as mean ± standard deviation (SD). Differences between groups were evaluated using analysis of variance (ANOVA) or Student's t-test, as applicable. A p-value of less than 0.05 was considered statistically significant [15]

3. Results

3.1. Phytochemical Screening

The preliminary phytochemical screening of the chloroform and acetone extracts from the stem of *Tabernaemontana divaricata* revealed the presence of various phytochemical constituents. The results are summarized in Table 1. The chloroform extract showed the presence of alkaloids, glycosides, tannins, flavonoids, and saponins, while the acetone extract was found to contain alkaloids, carbohydrates, tannins, and flavonoids.

Table 1. Results of phytochemical screening

S No	Plant constituents	Chloroform extract	Acetone extract
1	Alkaloids	+	+
2	Carbohydrates	-	+
3	Glycosides	+	-
4	Tannins	+	+
5	Flavonoids	+	+
6	Saponins	+	-

+ Positive - Negative

3.2. Antibacterial Activity

The chloroform and acetone extracts exhibited varying degrees of antibacterial activity against the tested bacterial strains, *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative), as evidenced by the formation of zones of inhibition around the wells. [16]

3.3. Antibacterial Activity against *Staphylococcus aureus*

The antibacterial activity of the extracts against *Staphylococcus aureus* is presented in Table 2. Both the chloroform and acetone extracts exhibited concentration-dependent antibacterial activity, with larger zones of inhibition observed at higher concentrations. [17]

Table 2. Results of antibacterial activitiy against *S. aureus*

Sno	Concentrations (ug/ml)	Alithromycin [+ ve]	Chloroform extract	Acetone extract
1	100 ug/ml	10.26 mm	9.15 mm	8.05 mm
2	200 ug/ml	12.7 mm	9.57 mm	9.0 mm
3	300 ug/ml	12.9 mm	10.13 mm	9.23 mm
4	400 ug/ml	12.48 mm	10.49 mm	9.63 mm
5	500 ug/ml	12.88 mm	10.57 mm	10.48 mm
6	600 ug/ml	13.6 mm	10.98 mm	10.57 mm
7	700 ug/ml	13.73 mm	11.07 mm	11.07 mm
8	800 ug/ml	14.17 mm	11.21 mm	11.97 mm
9	900 ug/ml	14.58 mm	12.47 mm	12.42 mm
10	1000 ug/ml	15.53 mm	13.73 mm	12.48 mm

The chloroform extract displayed superior activity compared to the acetone extract, with zones of inhibition ranging from 9.15 mm to 13.73 mm for concentrations ranging from 100 to 1000 µg/mL. At the highest tested concentration of 1000 µg/mL, the chloroform extract exhibited a zone of inhibition of 13.73 mm, which was comparable to the standard antibiotic azithromycin (15.53 mm). The acetone extract also showed promising antibacterial activity, with zones of inhibition ranging from 8.05 mm to 12.48 mm for the same concentration range. However, its activity was slightly lower than that of the chloroform extract. [18]

3.4. Antibacterial Activity against *Pseudomonas aeruginosa*

The results of the antibacterial activity against *Pseudomonas aeruginosa* are summarized in Table 3. Both extracts demonstrated concentration-dependent antibacterial activity, albeit with relatively lower efficacy compared to their activity against *Staphylococcus aureus*. The chloroform extract exhibited zones of inhibition ranging from 8.05 mm to 12.25 mm for concentrations between 100

and 1000 µg/mL, respectively. At the highest tested concentration of 1000 µg/mL, the chloroform extract showed a zone of inhibition of 12.25 mm, which was lower than the standard antibiotic metronidazole (14.58 mm). The acetone extract displayed zones of inhibition ranging from 8.54 mm to 11.97 mm for the same concentration range, with a maximum zone of inhibition of 11.97 mm at 1000 µg/mL. The results indicate that both the chloroform and acetone extracts possess antibacterial activity against Gram-positive and Gram-negative bacterial strains, with the chloroform extract exhibiting superior activity compared to the acetone extract. The activity was more pronounced against the Gram-positive *Staphylococcus aureus* than the Gram-negative *Pseudomonas aeruginosa*. [19]The visual representation of the zones of inhibition for the extracts and standard antibiotics is depicted in Figures 2.

Table 3. Results of antibacterial activitiy against *P aeuroginosa*

Sno	Concentrations(µg/ml)	Metronidazole (-ve)	Chloroform extract	Acetone extract
1	100 ug/ml	11.1 mm	8.05 mm	8.54 mm
2	200 ug/ml	11.6 mm	8.54 mm	9.45 mm
3	300 ug/ml	11.97 mm	9.45 mm	9.23 mm
4	400 ug/ml	12.06 mm	9.57 mm	9.63 mm
5	500 ug/ml	12.25 mm	10.41 mm	10.13 mm
6	600 ug/ml	13.1 mm	10.48 mm	10. 41mm
7	700 ug/ml	13.6 mm	10.98 mm	10.57 mm
8	800 ug/ml	13.73 mm	11.22 mm	11.6 mm
9	900 ug/ml	14.0 mm	11.61 mm	11.63 mm
10	1000 ug/ml	14.58 mm	12.25 mm	11.97 mm

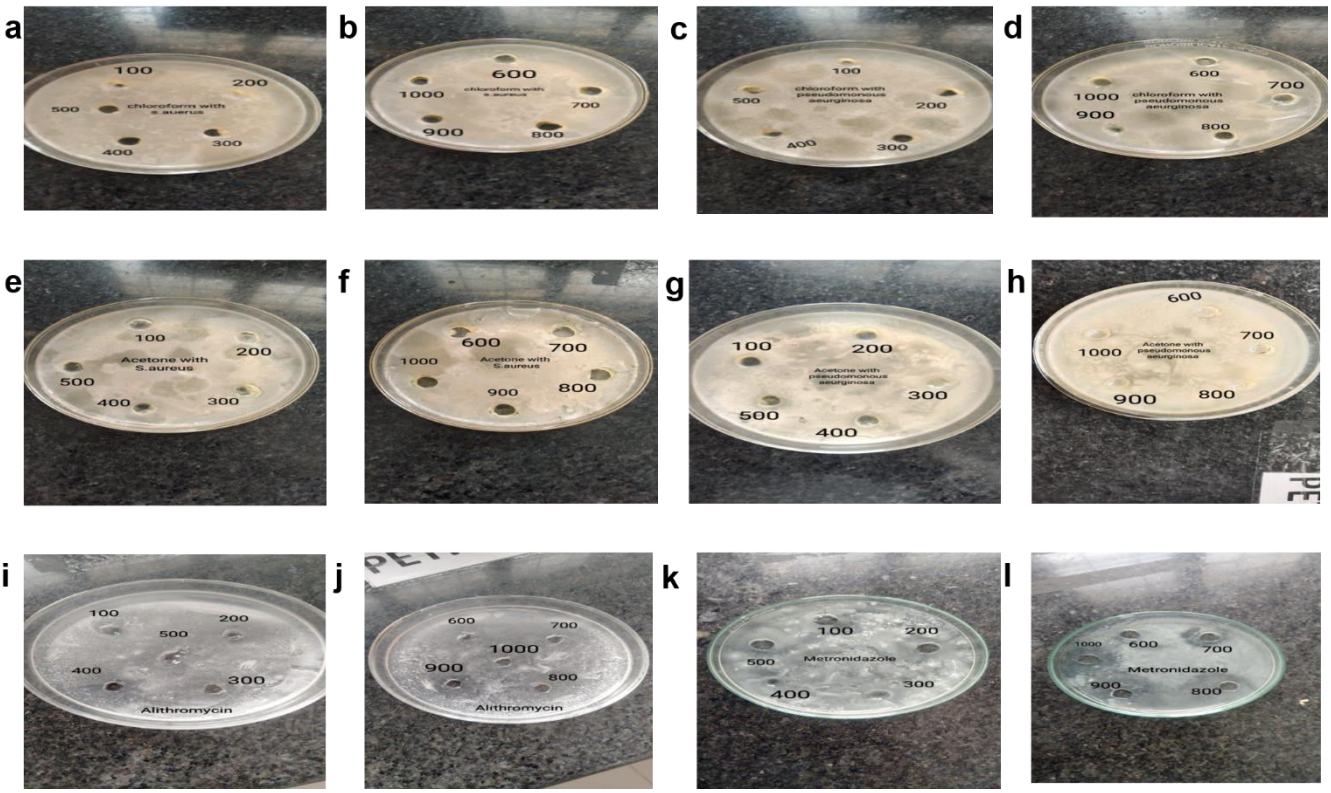

Figure 2. Results of antibacterial activity; a, b: Chloroform with Staphylococcus aureus, c, d: Chloroform with Pseudomonas aeruginosa; e, f: Acetone with Staphylococcous aureus; g,h: Acetone with Pseudomonas aeruginosa; i,j: Alithromycin [Standared drug (+ve)]; k,l: Metronidazole [standared drug(-ve)].

4. Discussion

The present study investigated the antibacterial activity of chloroform and acetone extracts obtained from the stem of *Tabernaemontana divaricata* against clinically relevant bacterial strains, *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative). The results demonstrated that both extracts exhibited promising antibacterial activity, with the chloroform extract exhibiting superior activity compared to the acetone extract. [20]

The preliminary phytochemical screening revealed the presence of various secondary metabolites, including alkaloids, flavonoids, glycosides, tannins, and saponins, in the chloroform and acetone extracts. These phytochemical constituents are known to possess diverse biological activities, including antimicrobial properties. The presence of these compounds in the extracts could contribute to the observed antibacterial activity. The antibacterial activity of the extracts was evaluated using the agar well diffusion method, which is a widely accepted and reliable method for determining the antimicrobial potential of plant extracts and isolated compounds. The results showed that both the chloroform and acetone extracts exhibited concentration-dependent antibacterial activity, with larger zones of inhibition observed at higher concentrations. [21]

Against *Staphylococcus aureus*, the chloroform extract displayed superior activity compared to the acetone extract, with zones of inhibition ranging from 9.15 mm to 13.73 mm for concentrations ranging from 100 to 1000 µg/mL. Notably, at the highest tested concentration of 1000 µg/mL, the chloroform extract exhibited a zone of inhibition of 13.73 mm, which was comparable to the standard antibiotic azithromycin (15.53 mm). This suggests that the chloroform extract contains potent bioactive compounds with antibacterial activity against Gram-positive bacteria. [22, 24] The antibacterial activity against *Pseudomonas aeruginosa* was relatively lower compared to *Staphylococcus aureus*, which is consistent with the general observation that Gram-negative bacteria are more resistant to antimicrobial agents due to their complex cell wall structure. However, both extracts demonstrated promising activity, with zones of inhibition ranging from 8.05 mm to 12.25 mm for the chloroform extract and 8.54 mm to 11.97 mm for the acetone extract at concentrations between 100 and 1000 µg/mL, respectively.

The observed antibacterial activity of the chloroform and acetone extracts could be attributed to the synergistic effects of various phytochemical constituents present in the extracts. [25] These compounds may act through different mechanisms, such as disrupting the bacterial cell membrane, inhibiting enzyme activities, or interfering with protein synthesis, ultimately leading to the inhibition of bacterial growth. The findings of this study are in line with previous reports on the antibacterial activity of various plant extracts, including those from the genus Tabernaemontana. Several studies have demonstrated the antibacterial potential of leaf extracts from *T. divaricata* against various bacterial strains, further supporting the traditional use of this plant for treating infections

5. Conclusion

The present study demonstrated the antibacterial activity of chloroform and acetone extracts obtained from the stem of *Tabernaemontana divaricata* against Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa*. The chloroform extract exhibited superior antibacterial activity compared to the acetone extract, particularly against *Staphylococcus aureus*. The observed activity could be attributed to the presence of various phytochemical constituents, such as alkaloids, flavonoids, glycosides, tannins, and saponins, in the extracts. These findings provide scientific validation for the traditional use of *T. divaricata* in treating bacterial infections and highlight the potential of this plant as a source of novel antimicrobial agents. Further studies are warranted to isolate and identify the specific bioactive compounds responsible for the antibacterial activity and elucidate their mechanisms of action.

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