

Full Length Research Paper

Antibacterial efficacy of *Mimosa pudica* against selected human pathogens: A comprehensive study

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The emergence of antibacterial resistant infections poses a significant threat to global health. As conventional antibiotics become increasingly ineffective, there is an urgent need to explore alternative therapies. Traditional medicinal plants, such as *Mimosa pudica*, have gained attention for their potential antimicrobial properties. This study aimed to evaluate the antibacterial potential of *M. pudica* extracts against a panel of pathogenic bacteria, specifically *Salmonella Typhi*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Phytochemical screening revealed the presence of bioactive compounds, including alkaloids, flavonoids, saponins, steroids, and tannins. Notably, the ethanolic extract exhibited the highest antibacterial activity, effectively inhibiting all tested pathogens. In contrast, aqueous extracts demonstrated antibacterial effects as well, but their activity was significantly lower. These findings support the traditional use of *M. pudica* in treating infections and highlight its potential as a source of novel antibacterial agents in the face of rising antibiotic resistance. However, further research is needed to demonstrate the specific mechanisms of action, optimize extraction methods, and assess the safety and efficacy of *M. pudica* extracts in clinical settings.

Key words: Antibiotic resistance, inhibitory activity, medicinal plants and phytotherapy.

INTRODUCTION

The increasing prevalence of antimicrobial resistance has necessitated the exploration of natural compounds with potential therapeutic applications. Among these, *Mimosa pudica* (Figure 1) has garnered attention due to its notable antimicrobial properties against a wide spectrum of pathogens. Traditionally, this plant has been used in various medicinal systems for treating infections and

inflammation. Recent studies have demonstrated that both aqueous and ethanolic extracts of *M. pudica* possess significant antibacterial and antifungal activities, comparable to standard antibiotics and antifungal agents such as ampicillin and fluconazole (Anand et al., 2020). This discovery positions *M. pudica* as a promising candidate in the fight against drug-resistant pathogens.

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Figure 1. *Mimosa pudica* (Touch-me-not plant).
Source: The authors.

According to Ayalew et al. (2022), *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Candida albicans* are among the pathogens affected by *M. pudica* extracts. Degu (2019) and Alshahrani et al. (2022) highlighted the effectiveness of the plant extracts in inhibiting bacterial growth. Both aqueous and ethanolic extracts were found to inhibit several fungal species, such as *Trichophyton verrucosum* and *Trichophyton soudanense*, demonstrating the plant's broad-spectrum antimicrobial potential (Alshahrani et al., 2022). Furthermore, these studies revealed moderate antifungal activity against *Microsporum ferrugineum* and *Trichophyton schoenleinii*, underscoring the relevance of *M. pudica* in addressing fungal infections.

In addition to its antimicrobial activity, *M. pudica* is rich in phytochemicals, including flavonoids, saponins, phenolic compounds, and alkaloids. These compounds play significant roles in the plant's defense mechanisms and contribute to its bioactivity. Flavonoids, for instance, are known for their antioxidant properties and their potential to modulate key enzymatic pathways (Swamy, 2020; Saini et al., 2024).

Saponins, on the other hand, serve as protective agents against microbial invasion, further enhancing *M. pudica*'s antimicrobial profile. These bioactive compounds suggest that the plant's therapeutic properties may extend beyond mere antimicrobial effects, potentially offering a holistic approach to disease management.

Moreover, phenolic compounds, as secondary

metabolites, have been extensively studied for their antioxidant activities and their role in the prevention of chronic diseases. Phenolics in *M. pudica* are also believed to contribute to its antimicrobial properties by disrupting microbial cell membranes (Franco and Vázquez, 2020). Alkaloids, another class of bioactive compounds found in *M. pudica*, exhibit a wide range of pharmacological activities, including antibacterial, anticancer, and antimalarial effects (Kurek, 2019). The synergistic effects of these phytochemicals could be key to the plant's efficacy in treating infections caused by resistant pathogens.

The present study, therefore, aims to investigate the antimicrobial efficacy of *M. pudica* extracts against a variety of pathogenic microorganisms, including *S. aureus*, *Salmonella* Typhi, *E. coli*, *P. aeruginosa*, and *Bacillus cereus*. By assessing the plant's bioactivity and identifying its phytochemical constituents, this research seeks to further explore the potential of *M. pudica* as an alternative treatment for antimicrobial-resistant infections. With rising concerns over drug resistance, such investigations are vital in the quest to discover new, effective antimicrobial agents from natural sources.

MATERIALS AND METHODS

Sample collection and preparation

M. pudica plants (stem and leaves) were collected from an open area in Sogakope, located in the Volta Region of Ghana, and

identified by Dr. Wahab (A certified botanist and lecturer). Clinical bacterial isolates, including *S. Typhi*, *P. aeruginosa*, *B. cereus*, *E. coli*, and *S. aureus*, were obtained from the stock culture of the Microbiology Laboratory at the Applied Biology Department, University for Development Studies, Navrongo campus. The collected *M. pudica* was thoroughly washed with distilled water to remove dirt and other contaminants and then air-dried. The plant material was ground into a fine powder using a blender.

Preparation of plant extracts

Aqueous extracts

For the hot aqueous extraction, 13 g of the powdered plant was mixed with 130 ml of distilled water, boiled at 100°C for 30 min, cooled, and filtered according to (Saqib et al., 2020). For the cold aqueous extraction, 13 g of the powdered plant was soaked in 130 ml of distilled water for 24 h at room temperature and filtered using Whatman filter paper.

Ethanolic extracts

To prepare the ethanolic extract, 13 g of the powdered sample was soaked in 130 ml of ethanol for 48 h at room temperature. The solution was allowed to air-dry, and the extract was concentrated by heating at 78°C, as described by Sonam et al. (2017).

Preparation of antibiotics and culture media

Antibiotics were prepared by dissolving 250 mg of each antibiotic in 2.5 ml of distilled water to create a concentration of 100 mg/ml. Mueller-Hinton agar was prepared by dissolving 17 g of media in 500 ml of distilled water, and then heated until fully dissolved. This was autoclaved at 121°C for 15 min. After cooling, the agar was evenly distributed into sterile Petri dishes to ensure a uniform depth for solidification (Altemimi et al., 2017).

Phytochemical analysis

Phytochemical tests were performed to identify the presence of bioactive compounds in the extracts:

- i) Steroids: A chloroform solution was added to 2 ml preparation of each extract and shaken with concentrated sulfuric acid; a red color indicated the presence of steroids (Pandey et al., 2018).
- ii) Alkaloids: One milliliter of each extract was dissolved in 2 ml of hydrochloric acid and treated with Mayer's reagent; a yellow precipitate indicated alkaloids (Ali, 2018).
- iii) Saponins: One milliliter of the extract was diluted with 20 ml of distilled water, and shaken for 15 min; a 2 cm layer of foam indicated saponins (Ayalew et al., 2022).
- iv) Phenols: One milliliter of the extract was mixed with 2 ml of ferric chloride solution; a bluish-black color indicated phenols Sonam et al. (2017).
- v) Flavonoids: Extracts treated with sodium hydroxide solution formed an intense yellow color that turned colorless upon the addition of dilute hydrochloric acid (Pant et al., 2017).
- vi) Tannins: To test for this phytochemical, 0.5 ml of each extract was treated with distilled water and 2 drops of ferric chloride solution; a blue color indicated gallic tannins, while a green-black color indicated catecholic tannins (Ahmed et al., 2019).

Resuscitation of pathogens and antibiotic susceptibility testing

To prepare nutrient broth, 2.6 g of nutrient broth was suspended in 200 ml of distilled water and autoclaved at 121°C for 15 min. After cooling, 15 ml of broth was dispensed into labelled sterile test tubes, and 1 ml of each pathogen inoculum was added. The cultures were incubated at 35.5°C for 24 h, with turbidity indicating growth. For the antibiotic susceptibility test, a sterilized cork borer with a diameter of approximately 6 mm was used to create wells in the Mueller-Hinton agar plates. A total of 0.1 ml of both the aqueous and ethanolic extracts of *M. pudica* was dispensed into the wells. The plates were incubated at 35.5°C for 24 h, and the zones of inhibition were measured to assess the sensitivity of the microorganisms. One milliliter of a fresh culture of the selected human pathogens was inoculated onto sterile Mueller-Hinton agar using the spread plate method to ensure even distribution.

Preparation of nutrient broth

Media preparation

- i) Weighed 2.6 g of nutrient broth powder and dissolved it in 200 mL distilled water.
- ii) Sterilized by autoclaving at 121°C for 15 min.
- iii) Allowed cooling to room temperature (~25 to 30°C).

Inoculation

- i) Aseptically dispensed 15 mL broth per sterile test tube.
- ii) Inoculated with 1 mL of standardized pathogen suspension (adjusted to 0.5 McFarland standard, $\sim 1.5 \times 10^8$ CFU/ml).
- iii) Incubated at 35.5°C for 18 to 24 h until turbidity indicated sufficient growth.

Standardization of bacterial suspension (McFarland Standard)

Adjusting turbidity

- i) Compared broth culture turbidity against 0.5 McFarland standard (a reference for $\sim 1.5 \times 10^8$ CFU/ml).

Verification

- i) Performed colony counting (CFU/ml) via serial dilution and plating.

Inoculation of Mueller-Hinton Agar (MHA) for susceptibility testing

Spread plate method

- i) Dipped a sterile swab into the standardized bacterial suspension.
- ii) Streaked evenly over Mueller-Hinton agar (MHA) plates in three directions (rotating 60° each time).
- iii) Plates dried for 5 to 10 min before adding antibiotic/extract wells.

Well diffusion assay

- i) Used a sterile cork borer (6 mm diameter) to punch wells.
- ii) Added 0.1 ml of test extracts (aqueous/ethanolic) into wells.

Table 1. Phytochemical analysis of cold aqueous extract, hot aqueous extract and ethanolic extract of the whole *M. pudica* plant.

Phytochemical	Cold aqueous extraction	Hot aqueous extraction	Ethanolic extraction
Alkaloids	+	++	-
Flavonoids	++	+	+
Saponin	++	++	+
Phenol	-	-	++
Steroids	++	+	+++
Tannins	+	+	-

+ indicates Positive; - indicates Negative, + = low amount; ++ = moderate; +++ = high amount.

Table 2. Antibacterial activity of cold aqueous extract, hot aqueous extract and ethanolic extract of *M. pudica* on some selected human pathogens.

Sample	Zones of inhibition (mm)				
	<i>S. Typhi</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i> (mm)
Cold aqueous extract <i>M. pudica</i>	2.50 ± 0.71	14.00 ± 2.8	3.00 ± 0.00	2.00 ± 1.41	0.00
Hot aqueous extract	4.00 ± 1.41	5.50 ± 2.12	4.00 ± 1.41	0.00	2.00 ± 0.00
Ethanolic extract	4.00 ± 1.41	11.5 ± 2.12	5.50 ± 0.71	4.50 ± 0.71	8.5 ± 0.71
Control					
Ciprofloxacin	30.50 ± 6.39	62.00 ± 0.00	25.50 ± 2.12	30.0 ± 1.41	22.00 ± 1.41
Amoxicillin	17.00 ± 0.00	18.00 ± 1.41	22.00 ± 0.00	16.00 ± 0.00	8.00 ± 0.00
Chloram-phenicol	10.00 ± 0.00	11.5 ± 2.12	28.00 ± 1.41	26.00 ± 4.24	3.00 ± 0.00

iii) Incubated at 35.5°C for 16 to 24 h.

Quality control measures

- Positive control: Used known antibiotic to confirm test validity.
- Negative control: Used sterile solvent (water/ethanol) to ensure no contamination.
- Incubation conditions: Maintained at 35.5±1°C.

RESULTS

Phytochemical analysis of the hot aqueous, cold aqueous and ethanolic extracts of *M. pudica* revealed varying active components. The hot aqueous extraction yielded alkaloids, flavonoids, saponins, steroids, and tannins, while the cold aqueous extraction similarly produced alkaloids, flavonoids, saponins, steroids, and tannins. The ethanolic extraction, however, revealed the presence of saponins, phenols, and steroids. The antibacterial activity of the cold aqueous extract demonstrated inhibition against *S. Typhi*, *B. cereus*, *S. aureus*, and *E. coli*, with *P. aeruginosa* showing resistance. Among the pathogens tested, *B. cereus* exhibited the highest susceptibility to the cold extract,

followed by *S. aureus*, *E. coli*, and *S. Typhi*. Table 1 shows the phytochemical analysis of cold aqueous extract, hot aqueous extract and ethanolic extract of the whole *M. pudica* plant.

For the hot aqueous extract, antibacterial activity was observed against *S. Typhi*, *B. cereus*, *S. aureus*, and *P. aeruginosa*; while *E. coli* remained insensitive. Once again, *B. cereus* showed the greatest susceptibility. In contrast, the ethanolic extract exhibited antibacterial activity against all tested pathogens, including *S. Typhi*, *B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa*. This extract proved to be the most effective among the three, indicating a higher antibacterial potency. Across all the extracts, *B. cereus* showed the highest susceptibility to inhibition. Full results are presented in Table 2.

The data of the activity of the various extracts were analyzed using statistical models, Analysis of variance (ANOVA) to verify if there is evidence that the mean zone of inhibition is same for all test organisms. The ANOVA results show that the test statistic is less than the critical value ($F < F_a$) for columns (among pathogens) except for individual concentrates, which showed a statistical difference. The data suggests that the extracts have different antibacterial effects depending on the bacterial

strain and the concentration used. The Anova results indicate that for rows (treatments), $f = 7.1064$, $p\text{-value} = 0.0014$, and f critical = 2.9013. Since the $p\text{-value}$ is less than 0.05 and f exceeds f critical, the differences among rows are statistically significant. For columns (organisms), $f = 2.23$, $p\text{-value} = 0.127$, and f critical = 3.287. Since the $p\text{-value}$ is greater than 0.05, the differences among columns are not statistically significant.

DISCUSSION

The present study aimed to investigate the antibacterial potential of *M. pudica* against a panel of pathogenic bacteria. The phytochemical screening revealed a diverse array of bioactive compounds, including alkaloids, flavonoids, saponins, steroids, and tannins. These compounds have been extensively studied for their antibacterial, antioxidant, and anti-inflammatory properties (Stan et al., 2021). The observed antibacterial activity of the *M. pudica* extracts can be attributed to the synergistic action of these compounds, which may interfere with bacterial cell wall synthesis, membrane integrity, and protein synthesis as described by Khameneh et al. (2019).

The ethanolic extract, in particular, exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria. This can be attributed to its higher content of saponins, steroids, and phenolic compounds, which are known for their antimicrobial properties as described by Stan et al. (2021). According to Liao et al (2021), saponins, for instance, can disrupt bacterial cell membranes, leading to cell lysis. Phenolic compounds, on the other hand, can inhibit bacterial enzymes and lead to oxidative stress as mentioned by Timilsena et al. (2023).

While the study demonstrates the promising antibacterial potential of *M. pudica* extracts, further research is needed to elucidate the specific mechanisms of action and to optimize the extraction process for maximum therapeutic efficacy. Additionally, it is crucial to assess the safety and toxicity of the extracts, particularly in long-term use. The increasing prevalence of antibiotic resistance highlights the urgent need for novel antimicrobial agents. Natural products, such as those derived from medicinal plants, offer a sustainable and environmentally friendly alternative to synthetic antibiotics. However, it is essential to balance the potential benefits of plant-based therapies with ethical considerations, such as sustainable harvesting practices and fair trade.

Conclusions

The present study demonstrated the antibacterial potential

of *M. pudica* extracts against a panel of pathogenic bacteria. The phytochemical screening revealed a diverse array of bioactive compounds, including alkaloids, flavonoids, saponins, steroids, and tannins, which contributed to the observed antibacterial activity. While the plant extracts exhibited promising antibacterial properties, their efficacy was lower compared to standard antibiotics. Nevertheless, the natural origin of *M. pudica* and its potential for synergistic effects with conventional antibiotics make it a promising candidate for further investigation. To fully realize the therapeutic potential of *M. pudica*, future research should focus on understanding the specific mechanisms of action of its bioactive compounds, optimizing extraction techniques to improve the yield and quality of bioactive compounds, and developing standardized formulations for effective delivery.

Additionally, exploring the synergistic effects of *M. pudica* extracts with conventional antibiotics and conducting clinical trials to evaluate their safety and efficacy in human subjects are crucial. By addressing these aspects, we can contribute to the development of sustainable and effective antimicrobial strategies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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