ISSN 2582 – 6050

Available Online at www.ijpba.info
International Journal of Pharmaceutical & Biological Archives 2025; 16(1):11-16

RESEARCH ARTICLE

Evaluation of the Antibacterial Activity of Methanolic Extracts of *Hemigraphis Alternata* and *Ocimum Sanctum* Against Wound-Infecting Bacteria: A Preliminary Investigation

Parvathy Prasad[1], Divya Grace Dilip[2], J. I. Mehjabin[2], Meera Kamila[2], A. R. Sree Nandana[2]

[1]*Department of Research and Development, Bioroot Exploration India Private Ltd, Thiruvananthapuram, Kerala, India,* [2]*Department of Zoology, All Saints'College, Thiruvananthapuram, Kerala, India*

Received: 21-02-2025; Revised: 07-03-2025; Accepted: 14-03-2025

ABSTRACT

The antibacterial activities of *Hemigraphis alternata* and *Ocimum sanctum* methanolic extracts were evaluated against wound pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The agar well diffusion method was used as a qualitative assessment to observe the antibacterial effect of the methanolic extract of *H. alternata* and *O. sanctum* against wound-infecting bacteria. *H. alternata* exhibited a significant zone of inhibition at 500 µg/mL against *P. aeruginosa* (16 mm) and *S. aureus* (14 mm), with a minimum inhibitory concentration (MIC) of 100 µg/mL. Conversely, *O. sanctum* showed a zone of inhibition of 12 mm against *P. aeruginosa* at 1000 µg/mL but exhibited no activity against *S. aureus*, with an MIC of 1000 µg/mL. These findings suggest that methanolic extracts of *H. alternata* and *O. sanctum* have potential as components in wound healing formulations to enhance antibacterial efficacy.

Keywords: Antibacterial, *Hemigraphis alternate*, *Ocimum sanctum*, methanolic extract, wound-infecting bacteria

INTRODUCTION

Aromatic herbs are a significant source of biologically active compounds with applications in agriculture and medicine). One such herb, *Ocimum tenuiflorum* (also known as *Ocimum sanctum*, Tulsi, or Holy Basil), from the *Lamiaceae* family, has been described as the "Queen of plants" and the "mother medicine of nature" due to its perceived medicinal qualities.[1] Tulsi has been used to treat various ailments such as poisoning, stomachaches, colds, headaches, malaria, inflammation, and heart disease. Its pharmacological properties, including analgesic, anti-inflammatory, and immunomodulatory effects, along with antioxidant capabilities, contribute to its role in promoting wound healing.

***Corresponding Author:**
Parvathy Prasad,
E-mail: *paruprasad@gmail.com*

Similarly, *Hemigraphis alternata* (commonly known as red ivy or purple waffle plant), belonging to the family *Acanthaceae*, is renowned for its wound-healing properties. It has been used in folk medicine for treating conditions such as ulcers, diabetes, blood dysentery, and anemia.[2,3] The plant's antibacterial and antioxidant properties further enhance its effectiveness in wound care. Traditionally, *H. alternata* leaves have been applied directly to open wounds to stop bleeding and promote healing.[4]

A wound is an injury to living tissue caused by a cut, blow, or any other impact. It causes damage to the underlying tissue.[5] Wound pathogens are microorganisms that cause wound infection by invading the wound. These microorganisms find the wounded area suitable for their growth and start to colonize near the wound.[6] In severe cases, they enter lymphatic vessels and blood vessels and cause sepsis.[7] These pathogens affect the immune

system of our body thus making it difficult for the wound to heal properly. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the two most prominent bacteria found on the skin. They are known for their multidrug resistance (MDR).[8]

S. aureus is a Gram-positive coccus found on the skin as part of the normal flora but can cause infections when entering the bloodstream or tissues through wounds. It is responsible for a range of conditions including abscesses, cellulitis, and septicemia in severe cases.[9] Methicillin-resistant *S. aureus* presents additional challenges due to its resistance to multiple antibiotics.

P. aeruginosa is a Gram-negative rod-shaped bacterium that frequently infects immune-compromised individuals, such as those with cystic fibrosis or burn wounds.[10] Its ability to form biofilms increases its resistance to environmental stress and antimicrobial agents. Both *S. aureus* and *P. aeruginosa* are major contributors to chronic wound infections due to their biofilm-forming capabilities, which hinder effective treatment.

With the growing concern of antibiotic resistance among wound pathogens, there is an urgent need for alternative treatments derived from natural sources. Plants such as *O. sanctum* and *H. alternata* have been reported to contain bioactive compounds with potent antimicrobial properties, making them potential candidates for treating wound infections.

O. sanctum has demonstrated significant antibacterial activity against various bacterial strains, including *Escherichia coli* and *S. aureus*. Its antioxidant properties also make it effective in promoting wound healing. Similarly, *H. alternata* has been used traditionally for its wound-healing properties and has shown antibacterial activity, further supporting its use in treating skin infections. The combination of antibacterial, antioxidant, and healing properties in these plants makes them valuable alternatives for managing wound infections, particularly considering increasing antibiotic resistance.

This study aims to evaluate the antibacterial activity of *O. sanctum* and *H. alternata* against wound pathogens *S. aureus* and *P. aeruginosa*. By investigating the potential of these plant extracts as alternative treatments, the study seeks to address the growing issue of antibiotic resistance in wound care.

MATERIALS AND METHODS

Sample Collection and Extraction

The leaves of *O. sanctum* and *H. alternata* were collected and thoroughly washed with distilled water. After washing, the leaves were shade-dried, avoided direct sunlight, and further dried in a hot air oven at 50°C. The dried leaves were ground into fine powder using a blender. For *O. sanctum*, 2 g of powdered leaves were weighed, and 50 mL of methanol was added to a beaker. The mixture was intermittently stirred and allowed to macerate for 24 h. Similarly, 1 g of *H. alternata* powder was soaked in 25 mL of methanol, stirred intermittently, and macerated for 24 h. After 24 h, both mixtures were filtered using filter paper or cheesecloth, and the filtrates were collected. The excess methanol was evaporated using a water bath at 60°C. The concentrated extracts were then stored at 4°C.

Preparation of Media

Nutrient broth

0.13 g of nutrient broth was weighed and mixed in 100 mL DW into a conical flask. 5 mL of nutrient broth was taken in two test tubes. Inoculate a loopful of bacteria, *S. aureus* in the first test tube and *P. aeruginosa* in the second test tube. Incubate both the test tubes at 37°*C* for 18–20 h.

Nutrient agar

1.3 g of nutrient broth was mixed with 100 mL DW and 1.5 g of agar. The mixture was autoclaved at 121°C and 15 lbs pressure for 15 min. The media was then poured into sterile petri dishes and allowed to solidify.

Agar Well Diffusion Method

Bacterial cultures were uniformly spread on nutrient agar plates using sterile cotton swabs. Sterile pipette tips were used to punch wells into the agar. Ciprofloxacin was used as the positive

control (PC), whereas methanol served as the negative control (NC).

30 µL of *O. sanctum* and *H. alternata* extracts, prepared at different concentrations of 250, 500, and 1000 µg/mL, were added into the agar wells. The plates were then incubated at 37°C for 24 h. After incubation, the diameters of the zone of inhibition were measured to evaluate the antibacterial activity of the extracts.

Broth Dilution Method

Media preparation

0.975 g of nutrient broth was dissolved in 75 mL DW, dispensed into 5 mL aliquots, and autoclaved for bacterial culture and minimum inhibitory concentration (MIC) determination.

Stock preparation

10 mg of plant extract was dissolved in 1 mL methanol to prepare a 10 mg/mL stock solution. Working concentrations of 100, 250, 500, 750, and 1000 µg/mL were prepared.

Broth dilution

1 mL of each extract concentration was added to test tubes with nutrient broth, followed by 20 µL of bacterial inoculum. Ciprofloxacin (0.2%) served as the PC, whereas methanol as NC, and uninoculated tubes as sterile controls. After 24 h at 37°C, absorbance was measured at 600 nm to assess bacterial growth inhibition. The percentage of bacterial growth inhibition was calculated using the following equation:

% Inhibition of bacterial growth =Ac-As/Ac × 100

Ac = Absorbance of control

As = Absorbance of sample.

RESULTS

Extraction of *O. sanctum*

The *O. sanctum* and *H. alternata* are extracted using the Soxhlet extraction process with methanol as the solvent. The methanolic extract of *O. sanctum* and *H. alternata* is shown in Figures 1 and 2.

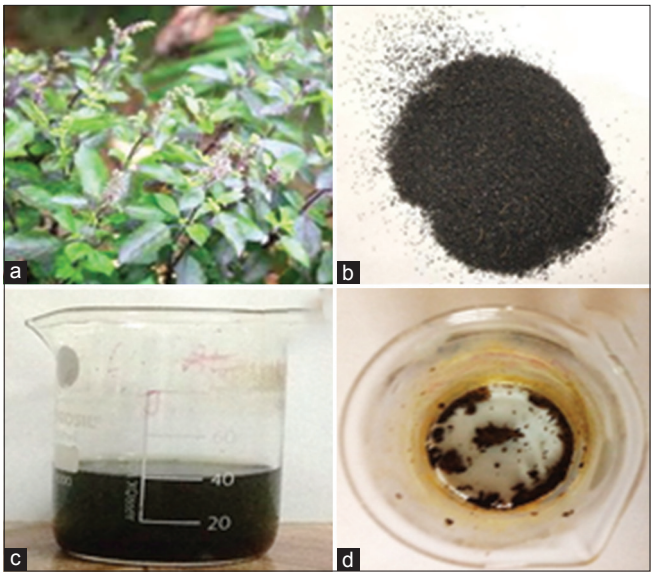

Figure 1: (a) *Ocimum sanctum* (b) shade dried powdered (c) methanolic extract (d) dried extract

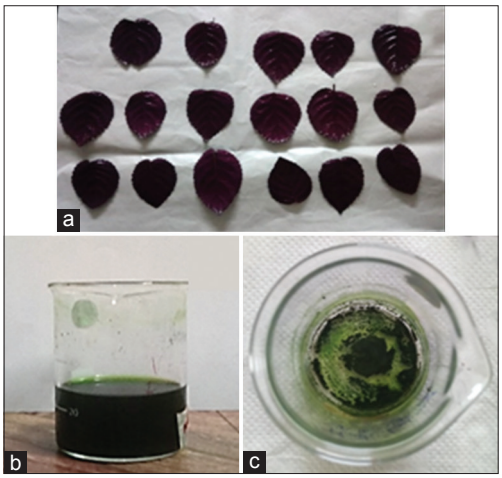

Figure 2: (a) Shade drying of leaves (b) methanolic extract (c) crude methanolic extract after evaporation of *Hemigraphis alternata*

Antimicrobial Analysis of Methanolic Extract

Agar well diffusion method

The antibacterial activity of *H. alternata* and *O. sanctum* (Tulsi) was tested against the wound-infecting bacteria *P. aeruginosa* and *Staphylococcus aureus* using various concentrations shown in Figures 3 and 4.

The Zone of inhibition for *H. alternata* and *O. sanctum* is displayed in Tables 1 and 2.

For *H. alternata*, concentrations of 250 µg/mL, 500 µg/mL, and 1000 µg/mL were used, with *P. aeruginosa* and *S. aureus* showing the greatest zone of inhibition at 500 µg/mL. Ciprofloxacin was used as PC and methanol as NC. For *O. sanctum*,

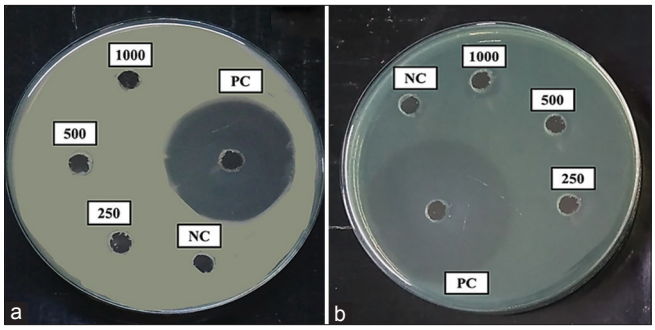

Figure 3: Agar well diffusion of methanolic extract of *Ocimum sanctum* against (a) *Pseudomonas aeruginosa* and (b) *Staphylococcus aureus*

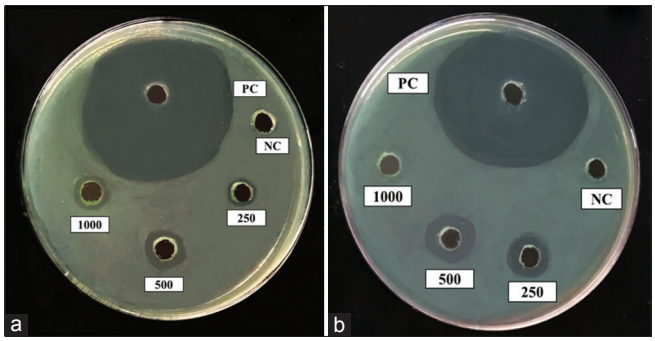

Figure 4: Agar well diffusion of methanolic extract of *Hemigraphis alternata against* (a) *Staphylococcus aureus* and (b) Pseudomonas *aeruginosa*

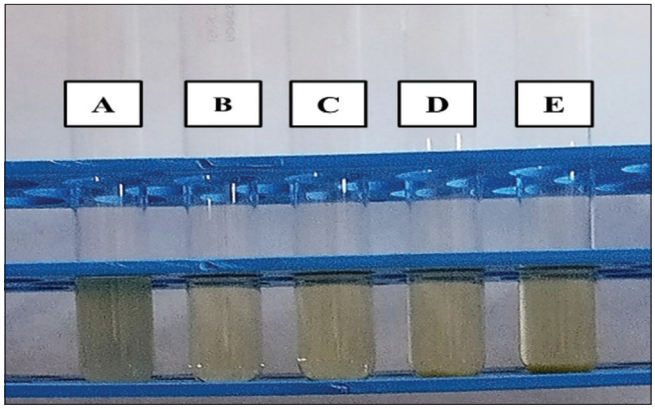

Figure 5: Minimum inhibitory concentration of *Pseudomonas aeruginosa* in *Ocimum sanctum* extract

concentrations of 250 µg/mL, 500 µg/mL, and 1000 µg/mL were tested, with the highest inhibition zone of 12 mm observed at 1000 µg/mL against *P. aeruginosa*. No inhibition was seen against *S. aureus*, whereas the PC showed 44 mm as the zone of inhibition.

MIC

The MIC is calculated using the spectrophotometry method.

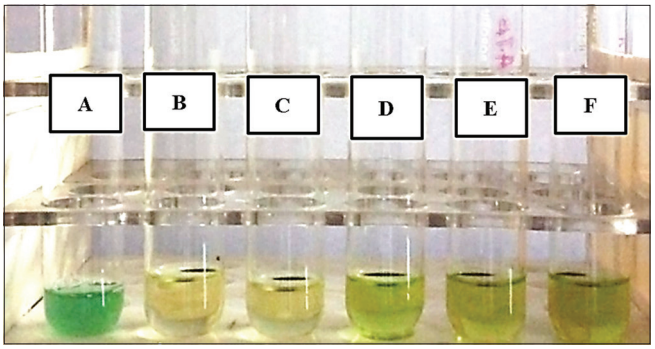

Figure 6: Minimum inhibitory concentration method of *Pseudomonas aeruginosa* (a)-growth control, (b)- 100 µg/mL, (c)-250 µg/mL, (d)-500 µg/mL, (e)-750 µg/mL, (f)-1000 µg/mL

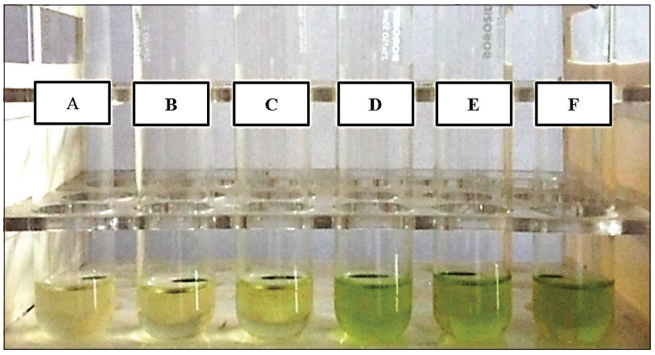

Figure 7: Minimum inhibitory concentration method of *Staphylococcus aureus* (a)-growth control, (b)- 100 µg/mL, (c)-250 µg/mL, (d)-500 µg/L, (e)-750 µg/mL, (f)-1000 µg/mL

The methanolic extract of *O. sanctum* demonstrated antibacterial activity against *P. aeruginosa* in a dose-dependent manner [Figure 5]. At 500 µg/mL, the extract exhibited the highest inhibition of 98%. Higher concentrations (1000, 2500, and 5000 µg/mL) showed consistent inhibition at 95% [Table 3]. The MIC of the methanolic extract of *H. alternata* against *P. aeruginosa* was determined based on the results [Figure 6]. The extract exhibited a dose-dependent inhibition, with significant activity observed across all tested concentrations. At 100 µg/mL, the inhibition was 98.85%, and maximum inhibition of 99.55% was achieved at 1000 µg/mL. The MIC was determined at 100 µg/mL, this concentration effectively inhibited bacterial growth (99.02%), showing a level of inhibition comparable to the PC, ciprofloxacin [Table 4]. These findings suggest that *H. alternata* extract is a potent natural antibacterial agent against *P. aeruginosa*.

The MIC of the methanolic extract of *H. alternata* against *S. aureus* was determined to be 96.38% as inhibition was observed at 100 µg/mL

Table 1: Zone of inhibition of methanolic leaf extract of *O. sanctum*

Concentration (µg/ml)	Zone Of Inhibition (mm)	
	P. aeruginosa	***S. aureus***
PC (Ciprofloxacin)	44	40
NC (Methanol)	-	-
1000	12	-
500	-	-
250	-	-

Table 2: Zone of inhibition of methanolic leaf extract of *Hemigraphis alternata*

Concentration (µg/ml)	Zone Of Inhibition (mm)	
	P. aeruginosa	***S. aureus***
PC (Ciprofloxacin)	36	40
NC (Methanol)	-	-
1000	-	12
500	16	14
250	11	10

Table 3: Minimum Inhibitory Concentration of *Pseudomonas aeruginosa* in *Ocimum sanctum* extract

Concentration (µg/ml)	Absorbance (600nm)	% Inhibition
Growth control	0.972	-
Positive control	0.084	91%
500 µg/ml	0.020	98%
1000 µg/ml	0.052	95%
2500 µg/ml	0.049	95%
5000 µg/ml	0.039	95%

Table 4: Minimum Inhibitory Concentration of *Pseudomonas aeruginosa* in *H. alternata* extract

Concentration (µg/ml)	Absorbance (600nm)	% Inhibition
Positive control	0.011	99.02
Growth control	1.134	0
100	0.013	98.85
250	0.011	99.02
500	0.010	99.11
750	0.008	99.29
1000	0.005	99.55

concentration [Figure 7]. Higher concentrations resulted in slightly increased inhibition, reaching a maximum of 98.07% at 1000 µg/mL. The inhibition at 750 µg/mL was (97.59%) comparable to PC (ciprofloxacin, 97.59%) [Table 5]. These results suggest that the MIC of *H. alternata* extract is 100 µg/mL, highlighting its potential as a natural antibacterial agent against *S. aureus*.

Table 5: Minimum Inhibitory Concentration of *S.aureus* in *H. alternata* extract

Concentration (µg/ml)	Absorbance (600nm)	% Inhibition
Positive control	0.011	99.02
Growth control	1.134	0
100	0.013	98.85
250	0.011	99.02
500	0.010	99.11
750	0.008	99.29
1000	0.005	99.55

DISCUSSION AND CONCLUSION

The present study was conducted to analyze the antibacterial activity of methanolic extracts of *O. sanctum* and *H. alternata* against wound- infecting bacteria, namely *P. aeruginosa* and *S. aureus*. The antibacterial activity was evaluated using the agar well diffusion method at different concentrations of the extracts (250 µg/mL, 500 µg/mL, and 1000 µg/mL) against both pathogens.

For *O. sanctum*, the antibacterial activity showed that the methanolic extract at a concentration of 1000 µg/mL inhibited the growth of *P. aeruginosa*. However, *S. aureus* did not exhibit any zone of inhibition at any tested concentration. The methanolic extract of *O. sanctum* showed better antibacterial activity compared to ethanol likely due to better diffusion into the medium, enhancing the contact with the organisms.[11] Yadav *et al*. also reported that the methanolic extract of *O. sanctum* demonstrated antimicrobial activity against MDR and pan-sensitive strains of *P. aeruginosa*.[12] Hussain *et al*. found that the ethanolic extract of *O. sanctum* did not show any zone of inhibition against *S. aureus*.[13] The MIC of *O. sanctum* against *P. aeruginosa* was found to be 1000 µg/mL. However, there is a lack of comparable studies on the MIC of *O. sanctum*, further investigation must be conducted to better understand its antibacterial potential. For *H. alternata*, antibacterial activity was tested by the agar well diffusion method, revealing inhibition zones. The maximum zone of inhibition was observed at 500 µg/mL for both *P. aeruginosa* and *S. aureus*. Interestingly, no zone of inhibition was seen at 1000 µg/mL against *P. aeruginosa*. In contrast, Anitha *et al*. reported no zone of inhibition at higher concentrations (10 mg

and 20 mg) of *H. alternata* extract. Anitha *et al*., also observed the absence of a zone of inhibition, for different solvent extracts of *H. alternata* leaves against *P. aeruginosa*.[14] Green silver nanoparticles synthesized from the extract of *H. alternata* showed antibacterial activity against *P. aeruginosa* and *S. aureus* with a zone of inhibition of 11 and 10 mm, respectively. Aqueous and ethanolic extracts of *H. alternata* showed a comparable zone of inhibition against *S. aureus* and *P. aeruginosa*.[15] Aqueous and ethanolic extracts of *H. alternata* have shown comparable zones of inhibition against both bacteria. The MIC of *H. alternata* extract against both pathogens was found to be 100 μg/mL, but similar studies on its MIC are limited, indicating the need for further research.

In conclusion, the methanolic extracts of both *O. sanctum* and *H. alternata* exhibited antibacterial properties, but more studies are required to fully understand their potential as antibacterial agents, especially with respect to their efficacy and mechanisms at different concentrations.

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