# In Vitro Evaluation of the Antibacterial Activity and Interactive Effects of Sweet Basil and Hemp Oils Against Pseudomonas aeruginosa

**Vaishali Negi**
Graphic Era University
**Anuj Gaur**
anujgaur1403@gmail.com

Gurukul Kangri Vishwavidyalaya

**Research Article**

**Keywords:** Pseudomonas aeruginosa, sweet basil oil, hemp oil, antibacterial activity, essential oil, minimum inhibitory concentration, agar well diffusion, plant-derived antimicrobials

**Posted Date:** September 2nd, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-10889181/v1



**Additional Declarations:** No competing interests reported.

## Abstract

# Background

The increasing prevalence of antimicrobial resistance in *Pseudomonas aeruginosa* has intensified the search for alternative and complementary antibacterial agents. Plant-derived oils and extracts have attracted considerable interest because of their diverse bioactive constituents and potential antimicrobial properties. However, the antibacterial activity of different plant oils may vary considerably, and their combined effects require experimental evaluation.

# Objective

The present study aimed to evaluate the in vitro antibacterial activity of sweet basil oil and hemp oil, individually and in combination, against *Pseudomonas aeruginosa* and to compare their activity with a conventional antibacterial agent.

# Methods

An experimental laboratory-based in vitro study was performed using a *P. aeruginosa* isolate obtained from a research laboratory in Dehradun, Uttarakhand, India. The isolate was revived and confirmed by Gram staining and standard biochemical tests. Antibacterial activity was evaluated using the agar well diffusion method on Mueller–Hinton agar. Sweet basil oil, hemp oil, their combination, and streptomycin were evaluated, with DMSO used as a negative control. Each treatment was tested in triplicate. Minimum inhibitory concentration (MIC) was further assessed using a broth microdilution assay with resazurin as a growth indicator.

# Results

The agar well diffusion assay demonstrated differential antibacterial activity among the tested agents. Hemp oil produced no measurable zone of inhibition (0 mm), whereas sweet basil oil produced a mean inhibition zone of 14 mm. Streptomycin showed the highest antibacterial activity, producing a mean inhibition zone of 33 mm, while DMSO produced no inhibition zone. MIC assessment showed growth inhibition by sweet basil oil at higher concentrations, whereas hemp oil showed limited antibacterial activity. The combination of sweet basil oil and hemp oil did not demonstrate a marked enhancement of antibacterial activity compared with sweet basil oil alone.

# Conclusion

Under the experimental conditions of this study, sweet basil oil demonstrated greater antibacterial activity against *P. aeruginosa* than hemp oil, whereas streptomycin remained substantially more active than the tested plant-derived agents. The combination of sweet basil and hemp oils did not produce an apparent enhancement in antibacterial activity. These findings support further investigation of sweet basil-derived antibacterial compounds, including studies involving standardized formulations, broader concentration ranges, additional bacterial strains, and mechanistic evaluation.

## 1. Introduction

*Pseudomonas aeruginosa* is an important Gram-negative opportunistic pathogen associated with a wide range of infections, particularly in individuals with compromised host defenses. Its clinical significance is largely attributed to its remarkable adaptability, diverse virulence-associated properties, biofilm-forming capacity, and ability to withstand antimicrobial treatment. The increasing prevalence of antimicrobial resistance in *P. aeruginosa* has created substantial challenges for effective infection management and has stimulated the search for alternative or complementary antimicrobial strategies (Breidenstein et al., 2011).

The antimicrobial resistance of *P. aeruginosa* is multifactorial and involves intrinsic and acquired mechanisms. Low outer-membrane permeability, active efflux systems, enzymatic resistance mechanisms, target modification, and biofilm-associated tolerance can collectively reduce the effectiveness of conventional antimicrobial agents. Biofilm formation is particularly important because bacteria embedded within biofilms may exhibit substantially reduced susceptibility to antimicrobial treatment. These characteristics have increased interest in identifying novel antimicrobial compounds capable of acting through mechanisms distinct from conventional antibiotics (Breidenstein et al., 2011).

Plant-derived products have attracted considerable attention as potential sources of bioactive antimicrobial compounds. Essential oils and plant extracts contain diverse secondary metabolites that may exert antibacterial effects through several mechanisms, including disruption of bacterial membrane integrity, alteration of membrane permeability, leakage of intracellular components, inhibition of essential cellular processes, and interference with bacterial communication and biofilm-associated functions (Bakkali et al., 2008; Burt, 2004). However, the antibacterial activity of plant-derived products is influenced by several factors, including plant species, chemical composition, extraction method, concentration, bacterial strain, and experimental conditions. Therefore, experimental evaluation is necessary to establish their activity against specific bacterial pathogens.

Sweet basil (*Ocimum* spp.) is an aromatic medicinal plant that has received considerable scientific interest because of its biologically active constituents and reported antimicrobial properties. Basil essential oils may contain compounds such as linalool, eugenol, and methyl chavicol, which have been associated with antibacterial activity through effects on bacterial membranes and other cellular processes (Burt, 2004; Bakkali et al., 2008). Previous investigations have therefore supported the potential of basil-derived preparations as sources of natural antibacterial agents.

Hemp (*Cannabis sativa*)-derived preparations have also attracted interest because of their diverse biological properties and phytochemical composition. Hemp-derived oils and extracts have been investigated for antioxidant, anti-inflammatory, and other biological activities; however, their antibacterial activity may vary depending on the composition and preparation of the material tested. In comparison with essential oils possessing established antibacterial constituents, the direct antibacterial effect of hemp oil against *P. aeruginosa* requires further experimental evaluation.

An additional area of interest is the use of combinations of plant-derived products. Combining different natural products may theoretically produce additive, synergistic, or antagonistic interactions because their chemical constituents can act through different or overlapping mechanisms. Nevertheless, enhanced activity should not be described as synergy without appropriate experimental evidence. Factors such as concentration, ratio of individual components, chemical composition, and bacterial susceptibility can substantially influence the outcome of combination studies (Bakkali et al., 2008; Langeveld et al., 2014).

Considering the increasing need for alternative antimicrobial approaches and the reported biological activities of plant-derived products, systematic evaluation of their activity against *P. aeruginosa* is warranted. The present study was therefore undertaken to evaluate the in vitro antibacterial activity of sweet basil oil and hemp oil against *P. aeruginosa*, both individually and in combination. Agar well diffusion and broth microdilution-based minimum inhibitory concentration (MIC) assays were employed to assess antibacterial activity. Streptomycin was included as a positive antibacterial reference, while dimethyl sulfoxide (DMSO) served as the negative control. The study further aimed to determine whether combining sweet basil and hemp oils produced an enhanced antibacterial response under the experimental conditions employed.

## 2. Materials and Methods

# 2.1 Study Design and Experimental Setting

The present study was designed as an experimental, laboratory-based in vitro investigation to evaluate the antibacterial activity of sweet basil oil and hemp oil, individually and in combination, against *Pseudomonas aeruginosa*. The experimental work was conducted at the Taqgene Training and Research Laboratory, Dehradun, Uttarakhand, India, using standard microbiological procedures and aseptic laboratory conditions.

# 2.2 Bacterial Strain and Maintenance

A culture of *Pseudomonas aeruginosa* was obtained from the Taqgene Training and Research Laboratory, Dehradun, Uttarakhand. The bacterial culture was revived on nutrient agar and subsequently subjected to cultural, microscopic, and biochemical characterization before antibacterial testing. The confirmed isolate was used for agar well diffusion and minimum inhibitory concentration (MIC) assays.

## 2.3 Preparation and Source of Test Oils

Sweet basil and hemp preparations were selected as the plant-derived test agents. The preparations were obtained from a reliable source and stored under the recommended conditions until use. Where required, stock preparations were made using dimethyl sulfoxide (DMSO) as the solvent. The study evaluated the antibacterial activity of the individual oils as well as their combination.

## 2.4 Standardization of Bacterial Inoculum

Fresh bacterial colonies were inoculated into Mueller–Hinton broth and incubated at 37°C for 18–24 h. The resulting bacterial suspension was adjusted to a 0.5 McFarland standard, corresponding to approximately $1.5 \times 10^8$ CFU/mL. The standardized suspension was subsequently used for antibacterial assays.

## 2.5 Biochemical Identification of *Pseudomonas aeruginosa*

The identity of the test organism was confirmed using Gram staining and standard biochemical tests. The biochemical characterization included catalase, indole, methyl red (MR), Voges–Proskauer (VP), citrate utilization, and triple sugar iron (TSI) tests. The isolate demonstrated a Gram-negative, rod-shaped morphology, catalase positivity, indole negativity, MR negativity, VP negativity, citrate utilization, and a K/K TSI reaction without hydrogen sulfide production, consistent with the characteristic profile of *P. aeruginosa*.

## 2.6 Agar Well Diffusion Assay

Antibacterial activity was evaluated by the agar well diffusion method using Mueller–Hinton agar (MHA). An 18–24 h culture of *P. aeruginosa* was used to prepare a 0.5 McFarland-standardized inoculum. The bacterial suspension was uniformly spread over the MHA surface to obtain a confluent bacterial lawn.

Sterile wells of 6 mm diameter were prepared in the agar using a sterile cork borer. The respective test preparations were introduced into the wells using sterile micropipette tips. Plates were allowed to stand at room temperature to facilitate diffusion and were subsequently incubated under the experimental conditions described in the study. Following incubation, the diameter of each zone of inhibition was measured in millimetres. Each treatment was evaluated in triplicate and the mean inhibition-zone diameter was recorded.

The test groups included sweet basil oil, hemp oil, their combination, and streptomycin as the positive antibacterial reference. DMSO was used as the negative control.

## 2.7 Minimum Inhibitory Concentration Assay

The MIC of the test preparations was evaluated using a broth microdilution assay in sterile 96-well microtitre plates containing Mueller–Hinton broth. Standardized *P. aeruginosa* inoculum was added to the wells along with the respective test preparations. Sweet basil oil and hemp oil were prepared in DMSO before testing.

Following incubation, resazurin dye was used as a colorimetric indicator of bacterial growth. A change toward pink indicated bacterial growth, whereas a blue colour was interpreted as inhibition of bacterial growth under the assay conditions. The MIC assay was used to compare the concentration-dependent antibacterial responses of sweet basil oil, hemp oil, their combination, and streptomycin.

## 2.8 Controls and Replication

Streptomycin was included as the positive antibacterial reference, while DMSO served as the negative control. The agar well diffusion experiments were performed in triplicate, and inhibition zones were expressed as mean diameters in millimetres.

## 2.9 Assessment of Combination Effects

The antibacterial response of the combined sweet basil and hemp oils was compared with the activity observed for each oil individually. The purpose of this comparison was to determine whether the combination produced an enhanced antibacterial response relative to the individual preparations. Because the experimental study did not establish a formal quantitative synergy index, combination effects were interpreted descriptively rather than being classified as confirmed synergistic interactions.

# 3. Results

## 3.1 Confirmation of the Test Organism

The bacterial isolate used in the present study was identified as *Pseudomonas aeruginosa* based on cultural characteristics, Gram staining, and biochemical characterization. The isolate showed a Gram-negative rod-shaped morphology and the biochemical profile was consistent with the characteristic features of *P. aeruginosa*. The confirmed isolate was subsequently used for evaluation of the antibacterial activity of the selected plant-derived oils and the reference antibiotic.

## 3.2 Antibacterial Activity by Agar Well Diffusion

The antibacterial activity of hemp oil, sweet basil oil, streptomycin, and DMSO was evaluated against *P. aeruginosa* using the agar well diffusion assay. Each treatment was tested on three plates and the inhibition-zone diameter was recorded in millimetres.

Sweet basil oil produced a measurable inhibition zone with a mean diameter of **14 mm**, indicating antibacterial activity against the test organism. In contrast, **no inhibition zone (0 mm)** was observed for hemp oil. Streptomycin produced the largest inhibition zone, with a mean diameter of **33 mm**, demonstrating substantially greater antibacterial activity than either of the tested plant oils. DMSO produced no inhibition zone (0 mm), confirming the absence of detectable antibacterial activity attributable to the solvent under the experimental conditions.

Table 1
Antibacterial activity of test preparations against *Pseudomonas aeruginosa* determined by agar well diffusion.

| Treatment | Number of plates | Mean inhibition zone (mm) |
|---|---|---|
| Hemp oil | 3 | 0 |
| Sweet basil oil | 3 | 14 |
| Streptomycin | 3 | 33 |
| DMSO | 3 | 0 |

The results demonstrated clear differences in antibacterial activity among the tested preparations. Streptomycin exhibited the strongest activity, while sweet basil oil showed moderate measurable inhibition. Hemp oil did not produce a detectable inhibition zone in the assay.

## 3.3 Minimum Inhibitory Concentration Assay

The antibacterial activity of sweet basil oil, hemp oil, their combination, and streptomycin was further investigated using broth microdilution with resazurin as a growth indicator. The colour response was used to distinguish bacterial growth from growth inhibition.

Sweet basil oil showed inhibition at higher concentrations, as indicated by the blue colour observed in the initial wells, whereas subsequent wells following serial dilution showed a change toward pink, indicating bacterial growth. Hemp oil-treated wells remained pink, suggesting limited antibacterial activity under the tested conditions. The combination of sweet basil oil and

hemp oil did not demonstrate a marked enhancement in growth inhibition compared with the individual treatment. Streptomycin demonstrated effective growth inhibition and served as the positive antibacterial reference.

Table 2
Concentration-dependent response of test preparations against *Pseudomonas aeruginosa* determined by broth microdilution assay.

| Treatment | Initial volume (µL) | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sweet basil oil | 70 | 41.18% | 20.59% | 10.29% | 5.15% | 2.57% | 1.29% | 0.64% | 0.32% | 0.16% | 0.08% |
| Hemp oil | 70 | 41.18% | 20.59% | 10.29% | 5.15% | 2.57% | 1.29% | 0.64% | 0.32% | 0.16% | 0.08% |
| Sweet basil + hemp oil | 50 | 33.33% | 16.67% | 8.33% | 4.17% | 2.08% | 1.04% | 0.52% | 0.26% | 0.13% | 0.065% |
| Streptomycin (positive control) | **100** | — | — | — | — | — | — | — | — | — | — |
| DMSO (negative control) | — | — | — | — | — | — | — | — | — | — | — |

## 3.4 Comparative Antibacterial Response

Overall, the antibacterial response varied considerably among the tested agents. Sweet basil oil demonstrated measurable activity against *P. aeruginosa*, whereas hemp oil showed little or no detectable activity in the agar diffusion assay. Streptomycin exhibited the greatest antibacterial effect in both the agar diffusion and MIC assessments. The combination of sweet basil and hemp oils did not demonstrate a marked enhancement under the experimental conditions used.

These findings indicate that the antibacterial effect observed in the present study was primarily associated with sweet basil oil rather than hemp oil, while the conventional antibiotic reference remained substantially more active against the test organism.

# 4. Discussion

The present study evaluated the antibacterial activity of sweet basil oil and hemp oil, individually and in combination, against *Pseudomonas aeruginosa*. The findings demonstrated a clear difference in antibacterial activity among the tested preparations. Sweet basil oil produced a measurable mean inhibition zone of 14 mm, whereas hemp oil produced no detectable inhibition zone. Streptomycin showed the greatest activity, with a mean inhibition zone of 33 mm. The absence of inhibition with DMSO supported the interpretation that the observed activity of sweet basil oil was attributable to the test preparation rather than the solvent.

The antibacterial activity observed for sweet basil oil is consistent with previous investigations reporting antimicrobial properties of *Ocimum basilicum* essential oil. Freire et al. reported antibacterial activity of *O. basilicum* oil against clinically important bacteria and also demonstrated interactions between basil oil and conventional antibiotics against *P. aeruginosa*. Their findings suggested that the activity of basil oil may be associated, at least in part, with constituents such as linalool (Freire et al., 2015).

Other studies, however, have reported considerable variation in the susceptibility of *P. aeruginosa* to basil-derived oils. Stojanović-Radić et al. demonstrated that basil essential oil could affect biofilm formation, motility, and pyocyanin production in *P. aeruginosa* isolates, suggesting that the biological effects of basil oil may extend beyond direct inhibition of planktonic bacterial growth (Stojanović-Radić et al., 2020). Conversely, earlier studies have reported limited activity of some basil essential oils against *Pseudomonas* species, highlighting the influence of oil composition and bacterial strain on the observed antibacterial response (Lachowicz et al., 1998; Yadav et al., 2010).

The observed 14-mm inhibition zone produced by sweet basil oil in the present study therefore indicates measurable antibacterial activity but should be interpreted in the context of the experimental conditions. The composition of basil oil can vary according to geographical origin, plant variety, cultivation conditions, harvesting stage, and extraction procedure. Joshi reported substantial variation in the chemical composition of *O. basilicum* essential oil and identified methyl eugenol and methyl chavicol as major constituents in an Indian basil oil chemotype (Joshi, 2014). Such chemical variation may contribute to differences in antibacterial activity between studies.

In contrast, hemp oil did not produce a measurable inhibition zone against *P. aeruginosa* in the present agar diffusion assay. This finding indicates that, under the conditions employed, hemp oil did not demonstrate detectable antibacterial activity by this method. Importantly, absence of an inhibition zone should not necessarily be interpreted as complete absence of biological activity because agar diffusion depends not only on antimicrobial potency but also on diffusion characteristics, solubility, viscosity, and interaction of the test material with the agar matrix. Therefore, the negative diffusion result should be considered specifically as a lack of detectable activity in the assay used.

The comparatively greater activity of streptomycin was expected because it is a conventional antibacterial agent with established activity against susceptible bacterial strains. The substantially larger inhibition zone observed with streptomycin compared with sweet basil oil demonstrates that the plant-derived preparation did not approach the antibacterial activity of the reference antibiotic under the experimental conditions. Accordingly, the present findings support consideration of sweet basil oil as a potential source of antibacterial compounds rather than as a replacement for established antimicrobial therapy.

The combination experiment did not demonstrate a marked enhancement of antibacterial activity compared with sweet basil oil alone. This finding is important because combination of two natural products should not automatically be interpreted as synergistic. Demonstration of synergy generally requires an appropriate quantitative interaction method, such as checkerboard analysis with calculation of the fractional inhibitory concentration index. In a previous study, Freire et al. demonstrated that *O. basilicum* essential oil could produce additive or synergistic interactions when combined with certain conventional antibiotics against *P. aeruginosa*, but the interaction depended on the antibiotic and bacterial strain (Freire et al., 2015). Therefore, the absence of a marked enhancement in the present hemp–sweet basil combination suggests that the two oils did not produce an obvious beneficial interaction under the concentrations and conditions tested.

The broader literature supports the potential of plant-derived essential oils against *P. aeruginosa*, but also emphasizes substantial variability in activity and important limitations concerning standardization and clinical translation. Recent evidence indicates that essential oils may exert antibacterial, antibiofilm, and anti-virulence effects through multiple mechanisms, while their chemical variability, toxicity, formulation, and lack of clinical evidence remain important barriers to therapeutic application (Álvarez-Martínez et al., 2021; current review literature).

Overall, the present findings indicate that sweet basil oil exhibited measurable in vitro antibacterial activity against *P. aeruginosa*, whereas hemp oil showed no detectable activity in the agar diffusion assay. The combination of the two oils did not produce an apparent enhancement, and streptomycin remained substantially more active than the plant-derived preparations. Further investigations incorporating chemical profiling of the oils, multiple *P. aeruginosa* strains, standardized quantitative combination assays, and mechanistic studies would be necessary to determine the reproducibility and biological basis of the observed activity.

## 5. Conclusion

The present study demonstrated differential antibacterial activity of sweet basil oil and hemp oil against *Pseudomonas aeruginosa*. Sweet basil oil produced a measurable inhibition zone of 14 mm, whereas hemp oil showed no detectable inhibition in the agar well diffusion assay. Streptomycin demonstrated substantially greater antibacterial activity than both plant-derived preparations. The MIC assessment further supported the antibacterial activity of sweet basil oil at higher concentrations, while hemp oil showed limited activity under the experimental conditions.

The combination of sweet basil and hemp oils did not produce a marked enhancement of antibacterial activity compared with sweet basil oil alone. Therefore, the present findings do not provide sufficient evidence to characterize the combination as synergistic. Overall, sweet basil oil demonstrated greater in vitro antibacterial potential against the tested *P. aeruginosa* isolate than hemp oil. These findings support further investigation of sweet basil-derived bioactive compounds, particularly through chemical characterization, standardized quantitative combination assays, evaluation against multiple clinical isolates, and mechanistic studies. Essential oils should currently be considered investigational or potentially complementary antimicrobial approaches rather than substitutes for established antibacterial therapy. 

## 6. Limitations

The findings of this study should be interpreted in light of several limitations. First, the antibacterial evaluation was performed using a single *P. aeruginosa* isolate, which limits the generalizability of the findings to other clinical or environmental strains. Second, the study evaluated antibacterial activity primarily under in vitro laboratory conditions and therefore does not establish clinical efficacy or safety. Third, the chemical composition of the tested oils was not characterized by analytical techniques such as gas chromatography–mass spectrometry (GC–MS), preventing direct correlation between individual phytochemical constituents and antibacterial activity. Fourth, the combination experiment was evaluated by comparative antibacterial response rather than a formal checkerboard assay with fractional inhibitory concentration (FIC) index calculation; consequently, no definitive claim of synergism can be made. Future studies should address these limitations through standardized oil characterization, larger panels of *P. aeruginosa* isolates, quantitative combination assays, cytotoxicity evaluation, and appropriate in vivo investigations.

## Declarations

**Ethics Approval**

This study involved in vitro microbiological evaluation and did not involve human participants or experimental animals. Therefore, formal human or animal ethics approval was not applicable.

**Consent for Publication**

Not applicable.

**Funding**

No external funding was reported for this study.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

**Author Contributions**

**Vaishali Negi:** Investigation, laboratory experimentation, data collection,

**Anuj Gaur:** Conceptualization, data interpretation, manuscript preparation, critical revision, initial manuscript preparation and correspondence with the journal.

Both authors reviewed and approved the final manuscript.

**Data Availability**

The data generated during the present study are available from the corresponding author upon reasonable request.

## References

1. Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils—a review. Food Chem Toxicol 46(2):446–475. 10.1016/j.fct.2007.09.106
2. Breidenstein EBM, de la Fuente-Núñez C, Hancock REW (2011) Pseudomonas aeruginosa: all roads lead to resistance. Trends Microbiol 19(8):419–426. 10.1016/j.tim.2011.04.005
3. Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94(3):223–253. 10.1016/j.ijfoodmicro.2004.03.022
4. Joshi RK (2014) Chemical composition and antimicrobial activity of the essential oil of Ocimum basilicum L. (sweet basil) from Western Ghats of North West Karnataka, India. Anc Sci Life 33(3):151–156. 10.4103/0257-7941.144618
5. Langeveld WT, Veldhuizen EJA, Burt SA (2014) Synergy between essential oil components and antibiotics: a review. Crit Rev Microbiol 40(1):76–94. 10.3109/1040841X.2013.763219
6. Pejčić M, Stojanović-Radić Z, Genčić M, Dimitrijević M, Radulović N (2020) Anti-virulence potential of basil and sage essential oils: inhibition of biofilm formation, motility and pyocyanin production of Pseudomonas aeruginosa isolates. Food Chem Toxicol 141:111431. 10.1016/j.fct.2020.111431
7. Silva VA, Sousa JP, Pessôa HLF, Freitas AFR, Coutinho HDM, Alves LBN, Lima EO (2016) Ocimum basilicum: antibacterial activity and association study with antibiotics against bacteria of clinical importance. Pharm Biol 54(5):863–871. 10.3109/13880209.2015.1088551
8. World Health Organization (2024) WHO bacterial priority pathogens list, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. World Health Organization, Geneva

## Figures

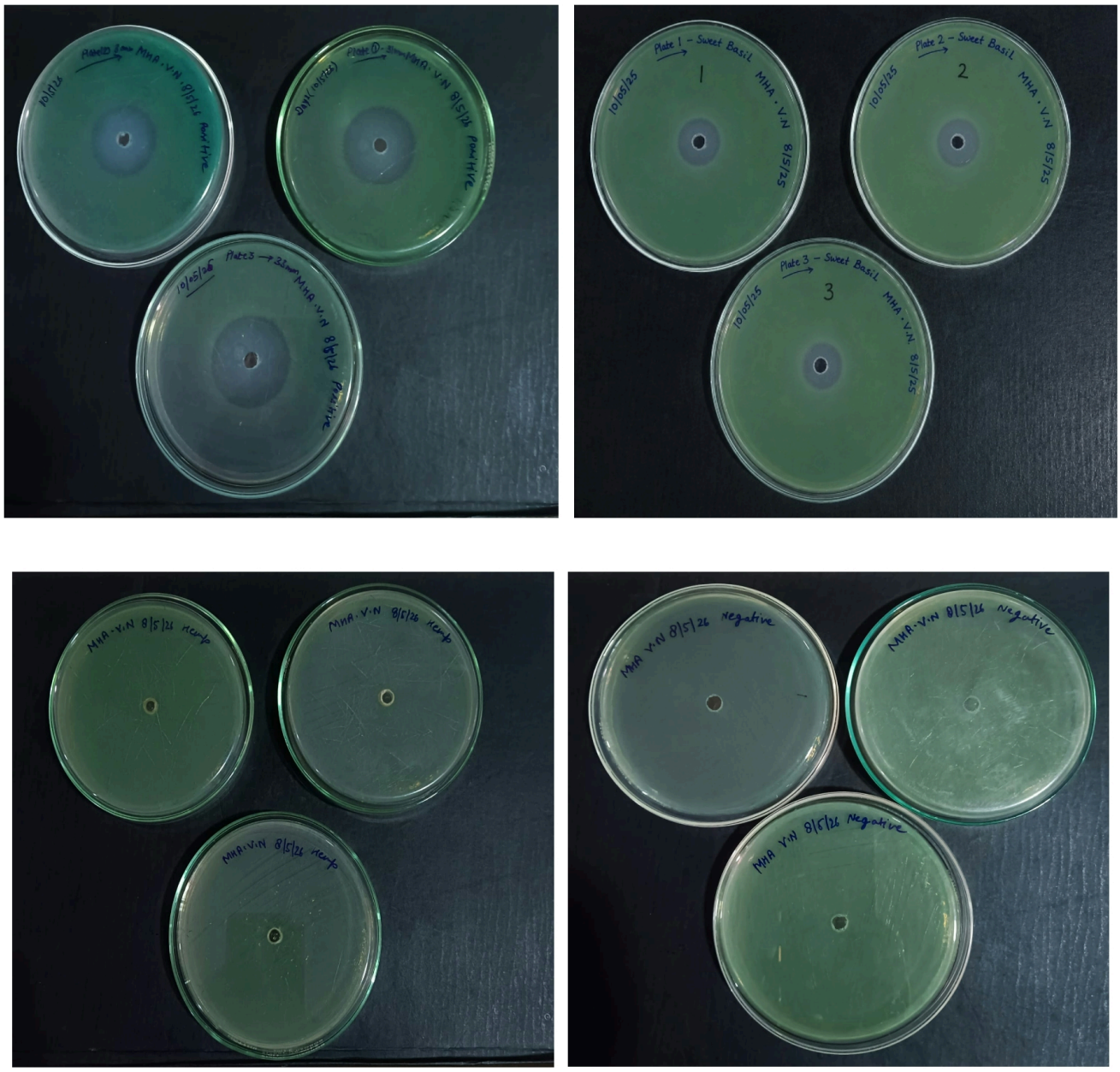


**Figure 1**

Agar well diffusion assay against *Pseudomonas aeruginosa*: (A) Streptomycin, (B) Hemp oil, (C) Sweet basil oil, and (D) DMSO.

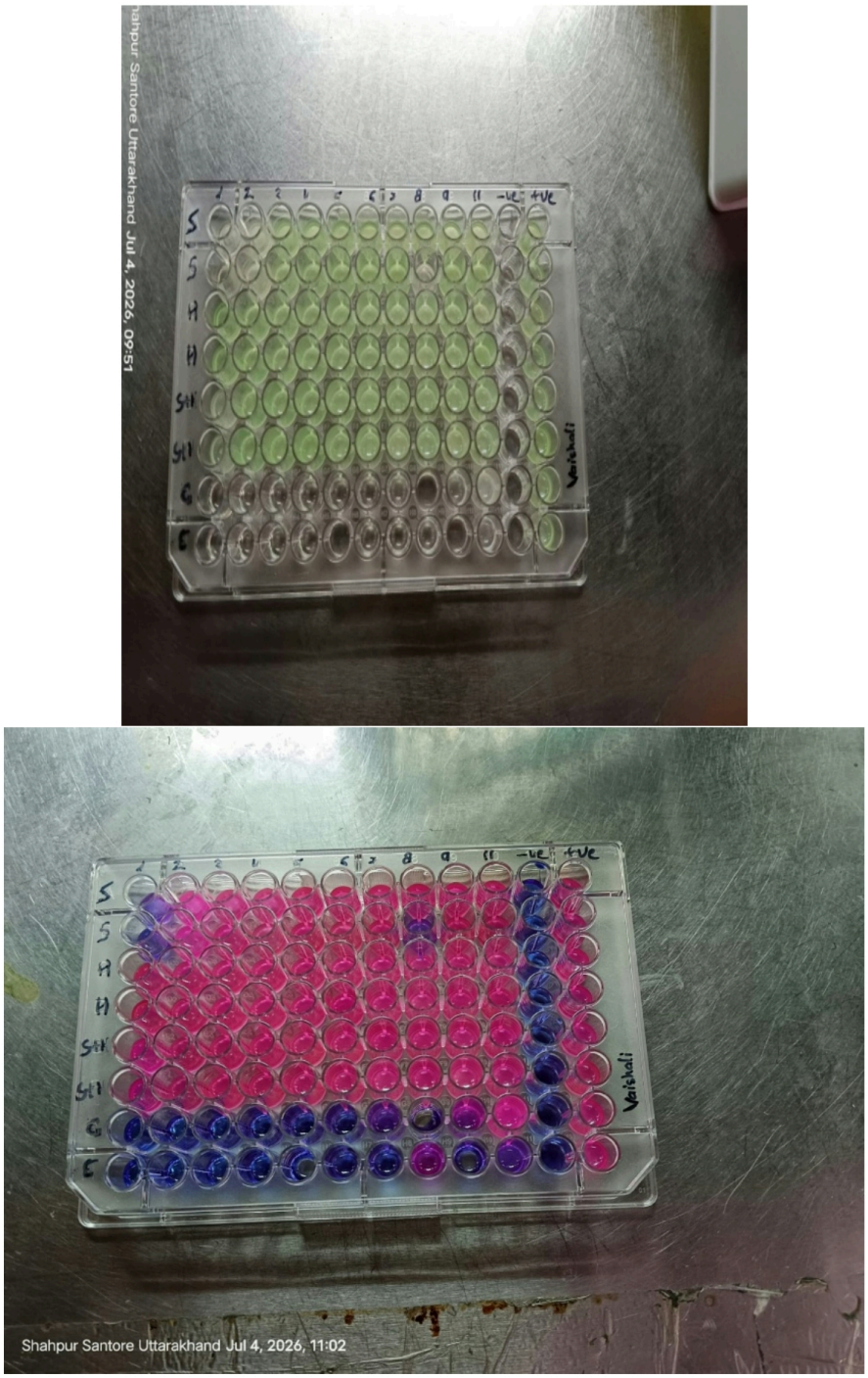


**Figure 2**

Broth microdilution assay against *Pseudomonas aeruginosa*: (A) 96-well plate before addition of resazurin dye, showing the serially diluted test preparations, and (B) 96-well plate after addition of resazurin dye, showing the concentration-dependent response of the tested preparations.