



Antimicrobial potential of *Bursera penicillata* (Sessé & Moc. ex DC.) Engl. essential oil in combating bacterial and fungal pathogens

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Abstract

Essential oils have been recognized for their antimicrobial properties, particularly as alternatives to combat antimicrobial resistance (AMR). *Bursera penicillata* (Sessé & Moc. ex DC.) Engl. has traditional medicinal uses, but its antimicrobial potential remains underexplored. This study the antimicrobial activity of *B. penicillata* essential oil against clinically relevant microorganisms and compares its efficacy to standard antibiotics and antifungals. The essential oil was extracted via hydrodistillation from bark resin. The agar well diffusion method was used against *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Enterobacter aerogenes*, *Candida albicans*, and *Aspergillus niger*. Nutrient agar and sabouraud dextrose agar were used for bacterial and fungal growth. Zones of inhibition were measured at two concentrations (1:0.5 and 1:1 dilution in DMSO), with amoxicillin and fluconazole as positive controls. The oil exhibited dose-dependent antimicrobial activity. Gram-positive bacteria (*S. aureus*, *B. cereus*, *S. epidermidis*) were more susceptible than Gram-negative bacteria (*E. coli*, *Enterobacter*). The highest inhibition was observed for *S. aureus* (1.9 cm), while *E. coli* showed the lowest (1.13 cm). Moderate antifungal activity was noted against *C. albicans* (1.3 cm) and *A. niger* (1.33 cm), but lower than fluconazole. *Bursera penicillata* essential oil exhibits significant antimicrobial activity, particularly against Gram-positive bacteria. While less potent than standard drugs, it presents a natural alternative requiring further research for clinical applications.

Keywords: *Bursera penicillata* (sessé & moc. ex dc.) engl., essential oil, antimicrobial activity, bacterial infections, fungal infections, antibiotic resistance, herbal medicine

Introduction

Essential oils have been used for centuries in traditional medicine due to their diverse bioactive compounds, including terpenes and phenolics, which exhibit strong antimicrobial properties (Lahmar *et al.*, 2017) [11]. Their ability to target multiple microbial pathways makes them promising alternatives to conventional antibiotics, particularly in the wake of antimicrobial resistance (AMR) (Chávez-González *et al.*, 2016) [6]. Unlike synthetic antibiotics, which often lead to resistance, essential oils contain complex mixtures of bioactive molecules that act synergistically, making it difficult for pathogens to develop resistance (Mittal *et al.*, 2019) [13].

Infectious diseases caused by bacterial and fungal pathogens remain a major global health concern, contributing significantly to morbidity and mortality. Bacterial infections, including pneumonia, tuberculosis, and bloodstream infections, claim over 7.7 million lives annually (Thakur *et al.*, 2019) [19]. India bears a high burden, with tuberculosis alone responsible for over 500,000 deaths each year. Hospital-acquired infections caused by multidrug-resistant bacteria, such as *S. aureus* and *E. coli*, have further complicated treatment options. Similarly, fungal infections, often underestimated, have surged in recent years, especially in immunocompromised patients. *C. albicans* and *A. niger* are among the most common fungal pathogens, with rising cases of antifungal resistance leading to treatment failures (Gnat *et al.*, 2021; Qadri *et al.*, 2023) [8, 17].

Current therapies for bacterial infections rely on antibiotics, while antifungal infections are managed using azoles, echinocandins, and polyenes. However, overuse and misuse

of these drugs have fueled resistance, rendering many standard treatments ineffective (Chandra *et al.*, 2017) [5]. The lack of new antibiotics in the pharmaceutical pipeline further exacerbates the crisis. In the case of antifungal drugs, biofilm formation by fungal pathogens makes treatment even more challenging. Given these limitations, alternative solutions, including plant-derived antimicrobial agents, have gained significant attention (Sharma *et al.*, 2020) [18].

Herbal medicine has been a cornerstone of traditional healing systems worldwide. Various plant extracts and essential oils have demonstrated potent antimicrobial activity against drug-resistant pathogens (AlSheikh *et al.*, 2020) [2]. Phytochemicals such as flavonoids, alkaloids, and tannins exert their effects by disrupting microbial cell walls, inhibiting biofilm formation, and interfering with metabolic pathways. Essential oils, in particular, have shown promising results in combating bacterial and fungal infections, offering a natural, multi-targeted approach to microbial control (Angelini, 2024; Kokoska *et al.*, 2019) [3, 10].

B. penicillata, a resilient member of the Burseraceae family, is a deciduous tree valued for its essential oil rich in fatty acids, monoterpenes, and sesquiterpenes, contributing to its antioxidant, antimicrobial, and anti-inflammatory properties (Piña-Torres *et al.*, 2018; Prabhakar Tirumani *et al.*, 2016) [14, 16]. Native to Mexico and Central America, it has adapted well to arid regions like Bangalore, India, where it follows a distinct growth cycle, producing fruit within 3-4 years and reaching peak oil yield at 13 years. Traditionally used for wound healing and treating respiratory and gastrointestinal ailments, its bioactive compounds, including α -pinene,

limonene, and β -caryophyllene, hold significant pharmacological promise (Tirumani *et al.*, 2017) [21]. Beyond its medicinal potential, *B. penicillata* supports biodiversity and serves as a sustainable resource for essential oil industries, particularly in soap and fragrance manufacturing, highlighting its ecological and commercial importance (Jayaveera *et al.*, 2008) [9].

Phytochemical studies have identified major compounds such as α -pinene, limonene, and β -caryophyllene, which are known for their antimicrobial efficacy. The essential oil has demonstrated activity against Gram-positive bacteria like *S. aureus* and *B. cereus*, as well as fungal pathogens like *C. albicans* and *A. niger*. The oil's ability to disrupt microbial membranes and inhibit biofilm formation makes it a strong candidate for alternative antimicrobial therapies (Theagarajan Prabhu, 1983) [20].

The increasing prevalence of antimicrobial resistance and the limitations of conventional antibiotics and antifungal drugs highlight the urgent need for alternative therapeutic strategies. While essential oils have shown promising antimicrobial properties, *B. penicillata* remains underexplored for its potential in combating resistant pathogens. This study hypothesizes that *B. penicillata* essential oil exhibits significant antimicrobial activity against bacterial and fungal pathogens due to its bioactive compounds. The rationale lies in evaluating its efficacy through scientific validation, addressing the research gap in its pharmacological potential, and exploring its application as a natural antimicrobial agent to mitigate the growing threat of drug resistance.

Materials and methods

1. Plant material and collection of essential oil

Bursera penicillata (Sessé & Moc. ex DC.) Engl. tree was located in the landscape garden of Osmania University and authenticated by Dr. L. Rasingam, Scientist E & HoO, Botanical Survey of India, Deccan Regional Center, Hyderabad (BSI/DRC/2022-23/Identification/730. Dated 30.01.2023). The essential oil of *B. penicillata* (BPE) was obtained through hydro-distillation from fresh bark resin using a Clevenger-type apparatus for 2 h. The obtained essential oil was separated, dried over anhydrous sodium sulfate, and stored in sterile amber glass vials at 4°C until further use (Allakonda *et al.*, 2024) [1].

2. Antimicrobial activity using the agar well diffusion method

2.1. Microbial strains and culture conditions

The antimicrobial activity of *B. penicillata* essential oil was tested against both bacterial and fungal pathogens. The bacterial strains included *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Enterobacter aerogenes*, while the fungal strains comprised *Candida albicans* and *Aspergillus niger*. All microbial strains were obtained from a culture collection and maintained at -80°C in glycerol stocks. Prior to experimentation, bacteria were subcultured in Nutrient Broth (NB) and incubated at 37°C for 18-24 h. For fungal strains, Sabouraud Dextrose Broth (SDB) was used, with incubation at 28°C for 48 h (Madhavi *et al.*, 2025) [12].

2.2. Preparation of culture media

The required quantities of peptone, beef extract, NaCl, and agar were weighed and dissolved in 1 liter of distilled water. The mixture was heated with continuous stirring until complete dissolution of all components. The pH of the

medium was adjusted to 7.2 ± 0.2 using 1N NaOH or 1N HCl, if necessary. The medium was then sterilized by autoclaving at 121°C and 15 psi pressure for 15 min. After autoclaving, the medium was allowed to cool to approximately $45\text{--}50^{\circ}\text{C}$, and then 25 ml was poured into each sterile Petri dish under aseptic conditions inside a laminar airflow chamber. The plates were left to solidify before use. Sabouraud Dextrose Agar was used for fungal strains, particularly *C. albicans* and *A. niger*.

The required components were dissolved in 1 liter of distilled water and stirred thoroughly. The pH was adjusted to 5.6 ± 0.2 using 1N HCl or 1N NaOH if required. The medium was sterilized by autoclaving at 121°C and 15 psi pressure for 15 min. After sterilization, the medium was cooled to $45\text{--}50^{\circ}\text{C}$, and 25 ml was poured into sterile Petri dishes inside a laminar airflow chamber. The plates were left to solidify before being used for fungal cultures (Tripathy *et al.*, 2023) [22].

2.3. Preparation of inoculum

For bacterial strains, a standardized inoculum was prepared by adjusting the optical density of actively growing cultures to match the 0.5 McFarland standard, corresponding to 1×10^8 CFU/ml. The turbidity was adjusted using a spectrophotometer at 600 nm. Fungal suspensions were prepared by harvesting spores from mature cultures of *A. niger* grown on sabouraud dextrose agar (SDA). The spores were suspended in sterile 0.85% NaCl containing 0.01% Tween 80, and the suspension was adjusted to 1×10^6 spores/ml for standardization (Dash *et al.*, 2023) [7].

2.4. Agar well diffusion assay

The antimicrobial activity of BPE was determined using the agar well diffusion method. Sterile mueller-hinton agar (MHA) plates were used for bacterial strains, while sabouraud dextrose agar (SDA) plates were utilized for fungal strains. Each plate was uniformly inoculated with 100 μl of the standardized microbial suspension, ensuring even distribution using a sterile cotton swab.

A sterile cork borer (6 mm diameter) was used to punch wells into the agar. Each well was carefully filled with 100 μl of the test samples. The experimental groups included a low-dose essential oil preparation (1:0.5 dilution in DMSO) and a high-dose essential oil preparation (1:1 dilution in DMSO). A negative control (DMSO alone) was included to assess the solvent's potential effect, and a positive control (amoxicillin for bacteria and fluconazole for fungi) was incorporated for comparison (Aswany *et al.*, 2023) [4].

2.5. Incubation and measurement of inhibition zones

The inoculated plates were left at room temperature for 30 min to allow the essential oil to diffuse into the agar before incubation. The bacterial plates were incubated at 37°C for 24 h, while fungal plates were incubated at 28°C for 48 h. After incubation, the antimicrobial activity was assessed by measuring the diameter of the inhibition zones around the wells using a calibrated digital Vernier calliper. The diameters were recorded in centimetres (cm), and all tests were performed in triplicate to ensure reproducibility (Poorniammal and Prabhu, 2022) [15].

Results

The antimicrobial activity of *B. penicillata* essential oil (BPE) was evaluated against a panel of bacterial and fungal pathogens using the agar well diffusion method. The results, expressed as the diameter of the inhibition zones (in cm),

demonstrate a dose-dependent response where the inhibition zones increased with a higher concentration of the essential oil (Table 1). However, when compared to the positive

control, the essential oil exhibited moderate activity, suggesting its potential but limited efficacy against the tested microorganisms (Figure 1-3).

Table 1: Antimicrobial activity of *B. penicillata* essential oil

Treatment	Antibacterial					Antifungal	
	<i>S. aureus</i>	<i>B. cereus</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>E. aerogenes</i>	<i>C. albicans</i>	<i>A. niger</i>
Low dose (1:0.5 of DMSO and BPE)	1.27 ± 0.12	1.1 ± 0	1.13 ± 0.06	1.03 ± 0.0	1.03 ± 0.06	1.2 ± 0.17	1.17 ± 0.15
High dose (1:1ml of DMSO and BPE)	1.9 ± 0.36	1.67 ± 0.5	1.77 ± 0.49	1.13 ± 0.06	1.17 ± 0.06	1.3 ± 0.17	1.33 ± 0.12
Positive control	3.33 ± 0.12	3.17 ± 0.46	3.27 ± 0.47	4.27 ± 0.64	4.23 ± 0.15	3.57 ± 0.38	3.7 ± 0.36

Results are expressed as Mean±SD of triplicates

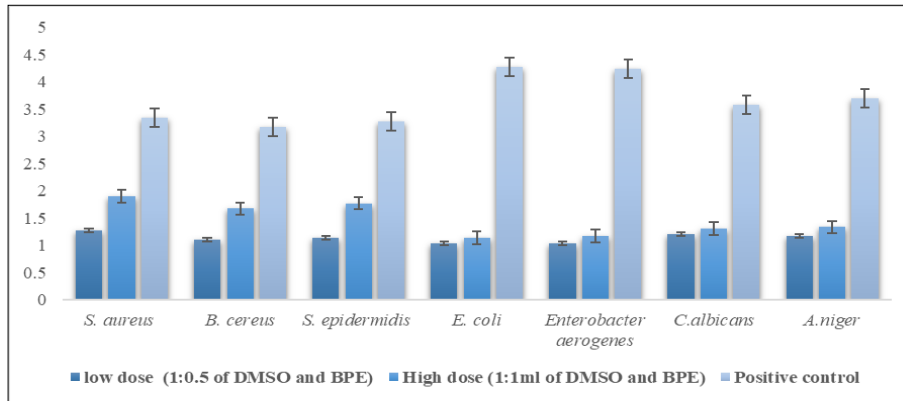
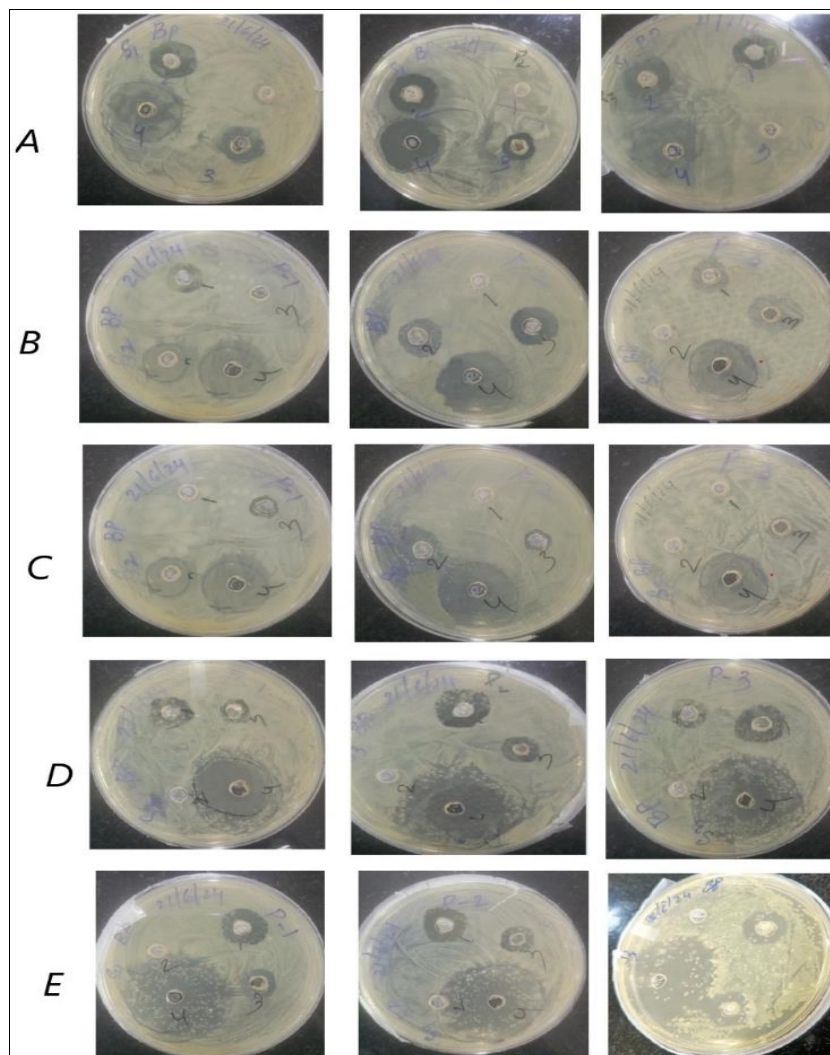
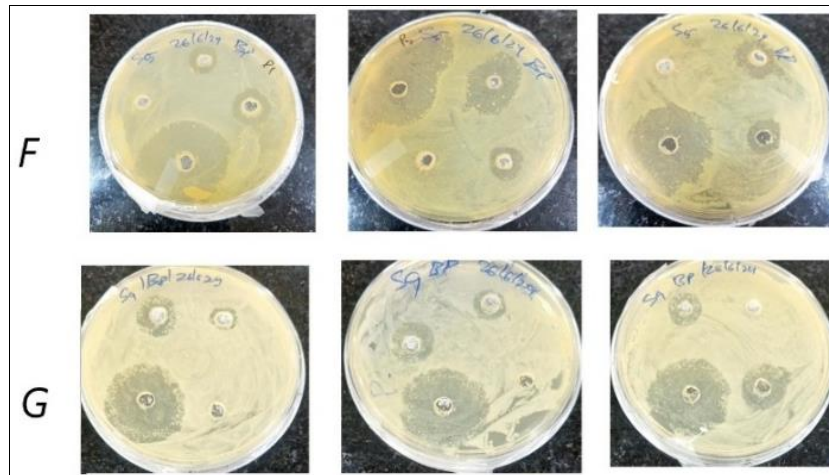


Fig 1: Antimicrobial activity of *B. penicillata* essential oil



A: *S. aureus*, B: *B. cereus*, C: *S. epidermidis*, D: *E. coli*, E: *E. aerogenes*

Fig 2: Zone of inhibition of the antibacterial studies



F: *C. albicans*, G: *A. niger*

Fig 3: Zone of inhibition of the antifungal studies.

Discussion

A clear dose-dependent antimicrobial effect of BPE was observed. At the low-dose concentration (1:0.5 dilution in DMSO), inhibition zones were relatively small across all tested microorganisms, ranging between 1.03 cm and 1.27 cm. However, at the higher dose (1:1 dilution in DMSO), the inhibition zones increased, ranging from 1.13 cm to 1.9 cm, indicating a stronger antimicrobial response. This suggests that the essential oil exhibits a concentration-dependent activity, where a higher concentration results in greater microbial inhibition.

Among the tested bacterial species, Gram-positive bacteria (*S. aureus*, *B. cereus*, and *S. epidermidis*) exhibited greater susceptibility to BPE compared to Gram-negative bacteria (*E. coli* and *Enterobacter*). At the high dose, *S. aureus* showed the largest inhibition zone (1.9 cm), followed by *S. epidermidis* (1.77 cm) and *B. cereus* (1.67 cm). This trend is consistent with the general antimicrobial susceptibility patterns, where essential oils tend to be more effective against Gram-positive bacteria due to their simpler cell wall structure, which allows for easier penetration of lipophilic compounds. In contrast, the Gram-negative bacteria, *E. coli* and *Enterobacter*, displayed the smallest inhibition zones, 1.13 cm and 1.17 cm, respectively, even at the higher dose. The outer membrane of Gram-negative bacteria, rich in lipopolysaccharides, acts as a permeability barrier, restricting the penetration of hydrophobic essential oil components. This suggests that BPE might be more effective against Gram-positive bacterial infections rather than Gram-negative pathogens.

The essential oil also exhibited antifungal activity against *C. albicans* (yeast) and *A. niger* (filamentous fungus). At the low-dose concentration, the inhibition zones for *C. albicans* (1.2 cm) and *A. niger* (1.17 cm) were comparable to those observed for bacterial strains. However, at the higher dose, *C. albicans* showed slightly increased susceptibility (1.3 cm), while *A. niger* exhibited a zone of inhibition of 1.33 cm. Although the antifungal activity of BPE was lower than the positive control, it indicates that the essential oil possesses some degree of antifungal potential, which may be attributed to bioactive terpenoid and phenolic compounds present in the oil. The large difference in inhibition zones suggests that either the concentration of active compounds in BPE is relatively low, or the bioavailability and diffusion properties of its constituents are limited in the agar medium. However, this does not necessarily negate its therapeutic

potential, as essential oils may act synergistically with other antimicrobials or exert effects via mechanisms not solely dependent on direct growth inhibition.

Conclusion

The antimicrobial evaluation of *B. penicillata* essential oil demonstrated its potential as a natural alternative against bacterial and fungal pathogens. The study revealed a dose-dependent inhibition of microbial growth, with greater efficacy observed against Gram-positive bacteria and moderate activity against fungal strains. Although the essential oil exhibited lower potency compared to standard antibiotics, its broad-spectrum activity suggests the presence of bioactive compounds capable of disrupting microbial cell structures. Given the increasing threat of antimicrobial resistance, *B. penicillata* essential oil presents a promising candidate for further development as a complementary or alternative antimicrobial agent. Future studies should focus on its mechanism of action, toxicity profile, and formulation strategies to enhance its therapeutic potential in clinical applications.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

- Allakonda L, Potnuri AG, Kumar Dokuparthi S, Danaboina GB, Kurra S, Ranjan R. Commelina benghalensis attenuates cyclophosphamide-induced hepatotoxicity by preserving hepatic mitochondrial activity through upregulating pro-mitochondrial proteins. *Tradit Kampu Med*,2024;11(3):230–41. doi:10.1002/tkm2.1432.
- AlSheikh HM, Sultan I, Kumar V, Rather IA, Al-Sheikh H, Tasleem Jan A, Haq QMR. Plant-based phytochemicals as possible alternative to antibiotics in combating bacterial drug resistance. *Antibiotics*,2020;9(8):480. doi:10.3390/antibiotics9080480.
- Angelini P. Plant-derived antimicrobials and their crucial role in combating antimicrobial resistance.

- Antibiotics,2024;13(8):746.
doi:10.3390/antibiotics13080746.
4. Aswany T, Helen PAM, Ijnu TP, Sasidharan SP, Akhilesh VP, George V, Pushpangadan P. Antioxidant and antimicrobial potential of *Areca catechu* L. (Arecaceae) inflorescence extracts. *Ann Phytomed*,2023;12(2):730–44.
doi:10.54085/ap.2023.12.2.86.
 5. Chandra H, Bishnoi P, Yadav A, Patni B, Mishra A, Nautiyal A. Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials—A review. *Plants*,2017;6(2):16.
doi:10.3390/plants6020016.
 6. Chávez-González ML, Rodríguez-Herrera R, Aguilar CN. Essential oils. In: *Antibiotic Resistance*, 2016, 227–37. Elsevier. doi:10.1016/B978-0-12-803642-6.00011-3.
 7. Dash PP, Kumar S, Mishra A, Srivastava S. Antimicrobial activity of *Haldina cordifolia* (Roxb.) Ridsdale and *Thevetia peruviana* (Pers.) Schum. leaf extract against multidrug-resistant microbes. *Ann Phytomed*,2023;12(1):431–9.
doi:10.54085/ap.2023.12.1.14.
 8. Gnat S, Łagowski D, Nowakiewicz A, Dyląg M. A global view on fungal infections in humans and animals: Opportunistic infections and microsporidiosis. *J Appl Microbiol*,2021;131(5):2095–113.
doi:10.1111/jam.15032.
 9. Jayaveera KN, VenkataRaju RR, Venkat Ratnam K, Bhakshu LMD, Rajendra Prasad A, Jayasankar Reddy V. Chemical characterization and *in vitro* antimicrobial activity of essential oil from the husk of *Bursera penicillata* (Sesse & Moc. ex DC.) Engl. *J Pharm Chem*,2008;2(3):149–52.
 10. Kokoska L, Kloucek P, Leuner O, Novy P. Plant-derived products as antibacterial and antifungal agents in human health care. *Curr Med Chem*,2019;26(29):5501–41.
doi:10.2174/0929867325666180831144344.
 11. Lahmar A, Bedoui A, Mokdad-Bzeouich I, Dhaouifi Z, Kalboussi Z, Cheraif I, Ghedira K, Chekir-Ghedira L. Reversal of resistance in bacteria underlies synergistic effect of essential oils with conventional antibiotics. *Microb Pathog*,2017;106:50–9.
doi:10.1016/j.micpath.2016.10.018.
 12. Madhavi SV, Rames P, Sudheer Kumar D, Kiran Kuma B. Evaluation of antimicrobial efficacy of ethanolic fruit extracts of *Terminalia pallida* Brandis. *Int J Biosci*,2025;26(1):22–9. doi:10.12692/ijb/26.1.22-29.
 13. Mittal RP, Rana A, Jaitak V. Essential oils: An impending substitute of synthetic antimicrobial agents to overcome antimicrobial resistance. *Curr Drug Targets*,2019;20(6):605–24.
doi:10.2174/1389450119666181031122917.
 14. Piña-Torres C, Lucero-Gómez P, Nieto S, Vázquez A, Bucio L, Belio I, Vega R, Mathe C, Vieillescazes C. An analytical strategy based on Fourier transform infrared spectroscopy, principal component analysis and linear discriminant analysis to suggest the botanical origin of resins from *Bursera*. Application to archaeological Aztec samples. *J Cult Herit*,2018;33:48–59.
doi:10.1016/j.culher.2018.02.006.
 15. Poorniammal R, Prabhu S. Antimicrobial and wound healing potential of fungal pigments from *Thermomyces* sp. and *Penicillium purpurogenum* in Wistar rats. *Ann Phytomed*,2022;11(1):376–82.
doi:10.54085/ap.2022.11.1.42.
 16. Prabhakar Tirumani A, Rajashekhar AV, Naga Raju Turlapati. Determination of phenolic content and *in vitro* antioxidant activity of leaves of Indian lavender plant *Bursera penicillata* ENGL. *Int J Pharm Sci Rev Res*,2016;37(1):125–9.
 17. Qadri H, Shah AH, Alkhanani M, Almilaibary A, Mir MA. Immunotherapies against human bacterial and fungal infectious diseases: A review. *Front Med*,2023;10:1135541.
doi:10.3389/fmed.2023.1135541.
 18. Sharma M, Kumar N. Use of plant-derived antimicrobials as an alternative to antibiotics. *J Pharmacogn Phytochem*,2020;9(2):1524–32.
doi:10.22271/phyto.2020.v9.i2y.11069.
 19. Thakur A, Mikkelsen H, Jungersen G. Intracellular pathogens: Host immunity and microbial persistence strategies. *J Immunol Res*,2019;2019:1356540.
doi:10.1155/2019/1356540.
 20. Theagarajan P, Prabhu VV. Chemical investigation of fatty oil of *Bursera penicillata* seed. *Indian For*,1983;109(1):41–4.
 21. Tirumani P, Sridhar G, Rajashekhar AV. Study on *in vitro* assessment of antioxidant activity of *Bursera penicillata* ENGL (Burseraceae) stem. *Int J Pharm Technol*,2017;9(1):29003–15.
 22. Tripathy A, Dash D, Rath CC. Evaluation of antimicrobial efficacy of mycoendophytic isolates from rice (*Oryza sativa* L.) crop. *Ann Phytomed*,2023;12(1):423–30.
doi:10.54085/ap.2023.12.1.9.