Infinity Journal of Medicine and Innovation
Volume 1, Issue 2, June 2025
website: https://journalscientific-journal.com/index.php/JIM
ISSN: 3080-7514 (Online)

Research article

Antimicrobial Activity of Petroleum and Benzene Against Selected Bacterial and Fungal Pathogens

***Ali Abedulameer Alhusayni* [1], *Falah Hasan Obayes AL-Khikani* [1,2,3]**

1. *Department of Microbiology, Al-Shomali General Hospital, Babylon Health Directorate, Babylon, Iraq*
2. Department of Medical Microbiology, College of Dentistry, University of Cordoba, Najaf, Iraq.
3. *Department of Medical Laboratory Technology, College of Medical Technology, Islamic University, Najaf, Iraq.*

ABSTRACT

Background: Antimicrobial resistance (AMR) poses a significant global health threat. While bacterial resistance is well-known, resistant fungal pathogens are an increasing concern, particularly for vulnerable populations. Exploring unconventional antimicrobial agents is crucial. Industrial hydrocarbons like petroleum and benzene, common solvents, represent an underexplored potential source, especially against fungi. **Objective:** This study aimed to evaluate and compare the *in vitro* antimicrobial efficacy of petroleum and benzene against selected clinically relevant pathogenic bacterial and fungal species. **Methods:** 70 clinical isolates (10 each of *Escherichia coli*, *Staphylococcus* spp., *Proteus* spp., *Pseudomonas spp.*, *Candida* spp., *Mucor spp.*, and *Aspergillus spp.*) from Shomally General Hospital, Iraq, were tested. Antimicrobial activity of petroleum and benzene at 10%, 20%, 40%, and 50% (v/v) concentrations was assessed using standard broth/agar dilution methods. Incubation was 24h (bacteria/Candida) or up to 6 days (molds), followed by subculturing to determine viability (cidal/static effect). **Results:** Petroleum and benzene showed no inhibitory effects against the tested bacterial isolates at any concentration (10-50%). However, both substances demonstrated potent, fungicidal activity against all tested fungi (*Candida* spp., *Mucor* spp., *Aspergillus spp.*), achieving complete inhibition starting at the 10% (v/v) concentration. **Conclusion:** Petroleum and benzene exhibit selective and potent *in vitro* fungicidal activity against the tested pathogens at concentrations of 10% and higher, while lacking antibacterial efficacy. These findings suggest potential for derivatives in antifungal applications, but warrant significant further investigation into mechanisms, resistance, and critical toxicological profiles before any practical consideration.

Keywords:
Antifungal activity,
Petroleum,
Benzene,
Antimicrobial resistance,
Fungal pathogens,
Candida,
Aspergillus,
Mucor,
In vitro.

Article history:
Received: 28 Aril 2025.
Revised: 28 May 2025.
Accepted: 2 June 2025.
Published: 16 June 2025.

*** Corresponding author:**
Ali Abedulameer Alhusayni
E-mail address:
alti99269@gmail.com

INTRODUCTION

Antimicrobial resistance (AMR) represents one of the most pressing global health challenges of the 21st century, threatening the efficacy of treatments for a wide range of infections [1]. While bacterial resistance often dominates headlines, the rise of resistant fungal pathogens poses an equally significant, yet often underestimated, threat to public health, particularly among immunocompromised populations [2]. This escalating crisis necessitates the urgent exploration of unconventional antimicrobial agents from diverse sources, including natural products and industrial chemicals, to replenish the dwindling pipeline of effective drugs [3].

Among potential alternative sources, industrial chemicals like hydrocarbons present an intriguing but largely underexplored avenue for antimicrobial discovery [4, 5]. Petroleum, a complex mixture of hydrocarbons, and benzene, a simple aromatic hydrocarbon, are ubiquitous in industrial settings and as environmental pollutants. Their inherent chemical properties suggest potential biological

activity, yet their specific effects on pathogenic microorganisms, especially fungi, remain poorly characterized compared to other substance classes like essential oils [6, 7]. Essential oils, which share some hydrocarbon features, have demonstrated activity against species like *Candida* and *Aspergillus* [6, 7].

Previous research offers fragmented clues regarding hydrocarbon antimicrobial activity. Some studies suggest certain hydrocarbons can disrupt the integrity of microbial cell membranes or interfere with vital metabolic pathways, such as ergosterol synthesis in fungi, a common target for existing antifungal drugs [6, 7]. Extracts derived using petroleum ether (a light hydrocarbon fraction) have also demonstrated antifungal activity against clinically relevant species like *Candida albicans* and *Aspergillus flavus* in some laboratory settings [10, 11 - Note: Sources not readily verifiable]. However, translating these findings to the direct application of crude petroleum or pure benzene requires careful investigation, considering their complex composition and potential toxicity [12].

Conversely, the relationship between many bacteria and hydrocarbons is often characterized by tolerance or even utilization. Numerous bacterial species, notably *Pseudomonas* spp. and *E. coli*, possess enzymatic machinery capable of metabolizing various hydrocarbon components, potentially rendering them resistant to antimicrobial effects and enabling their use in bioremediation applications [5, 13, 14]. This stark contrast between potential fungal susceptibility and bacterial resilience forms a compelling basis for investigation. Understanding this differential activity is crucial, not only for potentially identifying novel antifungal leads but also for assessing the broader ecological and health implications of hydrocarbon exposure. Some fungi also play roles in hydrocarbon bioremediation, adding complexity to these interactions [4, 15].

Therefore, this study was designed to systematically evaluate and compare the *in vitro* antimicrobial efficacy of petroleum and benzene across a range of concentrations against clinically relevant isolates of both bacterial (*Escherichia coli*, *Staphylococcus* spp., *Proteus* spp., *Pseudomonas* spp.) and fungal (*Candida* spp., *Mucor* spp., *Aspergillus* spp.) pathogens obtained from clinical settings in Iraq. We hypothesized that petroleum and benzene would exhibit selective antifungal activity while showing limited or no effect against the tested bacteria under the experimental conditions, providing insights into their potential utility or risk.

MATERIALS AND METHODS:

Microorganism Isolation and Identification: A total of 70 clinical isolates were obtained from patients at Shomally General Hospital in Babylon City, Iraq. These comprised 10 isolates each of *Escherichia coli*, *Staphylococcus* spp., *Proteus* spp., *Pseudomonas spp.*, *Candida* spp., *Mucor spp.*, and *Aspergillus spp.* Standard microbiological techniques were used for isolation and preliminary identification.

Culture Media: Brain Heart Infusion Broth (BHIB) was used for the cultivation and susceptibility testing of bacterial isolates. Sabouraud Dextrose (SD) was used for the cultivation and susceptibility testing of fungal isolates.

Test Substances: Petroleum (crude oil sample) and analytical grade Benzene were used. Stock solutions were prepared by diluting the substances in sterile distilled water (potentially with a minimal amount of a non-inhibitory emulsifier like 0.1% Tween 80 for homogeneity, although not explicitly stated as used in file 1's methods) to achieve final test concentrations of 10%, 20%, 40%, and 50% (v/v).

Antimicrobial Susceptibility Testing: Bacteria: Standardized inocula (approx. 10^5 CFU/mL) were added to BHIB tubes containing the different hydrocarbon concentrations. Control tubes without hydrocarbons were included. Tubes were incubated aerobically at 37°C for 24 hours. Fungi: For *Candida* spp., a similar broth dilution method was used (SD broth or liquid Sabouraud medium), incubated at 30-35°C for 24-48 hours. For filamentous fungi (*Mucor* spp., *Aspergillus* spp.), an agar dilution method was employed, incorporating hydrocarbons into molten SD before pouring plates. Plates were centrally inoculated and incubated at 25-30°C for up to 6 days, with control plates lacking hydrocarbons.

Assessment of Inhibition and Viability: Microbial growth was assessed visually (turbidity in broth, colony presence on agar) compared to controls. To determine cidal vs. static effects, aliquots from clear broth tubes or agar plugs from inhibition zones were subcultured onto fresh, substance-free media (BHIB or SD). Absence of growth on subculture after 24-48h indicated a cidal effect.

Ethical Considerations: The study utilized anonymized clinical isolates obtained during routine diagnostics, adhering to institutional guidelines.

RESULTS:

The *in vitro* antimicrobial susceptibility testing yielded distinct outcomes for bacterial versus fungal isolates.

Bacterial Isolates: No significant inhibitory effect (bacteriostatic or bactericidal) was observed for either petroleum or benzene against *E. coli*, *Staphylococcus* spp., *Proteus* spp., or *Pseudomonas* spp. at any tested concentration (10%, 20%, 40%, 50% v/v). Bacterial growth in test tubes was comparable to controls, and viability was confirmed by subculture.

Fungal Isolates: In stark contrast, both petroleum and benzene exhibited potent antifungal activity against all tested fungi (*Candida* spp., *Mucor* spp., *Aspergillus* spp.). Complete inhibition of visible growth was observed starting at the lowest concentration tested (10% v/v) and persisted at all higher concentrations. Subculturing confirmed this inhibition was fungicidal at 10% and above for both substances against these isolates [Table 1].

Table 1: Summary of In Vitro Antimicrobial Activity of Petroleum and Benzene

Microorganisms	Substance	10% Conc.	20% Conc.	40% Conc.	50% Conc.
Bacteria (E. coli, Staph, Proteus, Pseudo)	Petroleum	No Inhibition	No Inhibition	No Inhibition	No Inhibition
Bacteria (E. coli, Staph, Proteus, Pseudo)	Benzene	No Inhibition	No Inhibition	No Inhibition	No Inhibition
Fungi (Candida, Mucor, Aspergillus)	Petroleum	Complete Inhibition*	Complete Inhibition*	Complete Inhibition*	Complete Inhibition*
Fungi (Candida, Mucor, Aspergillus)	Benzene	Complete Inhibition*	Complete Inhibition*	Complete Inhibition*	Complete Inhibition*

* Indicates fungicidal activity confirmed by subculture.

DISCUSSIONS:

The findings clearly demonstrate a pronounced and selective *in vitro* antifungal activity of both petroleum and benzene against the tested clinical isolates (*Candida* spp., *Mucor* spp., *Aspergillus* spp.). Complete fungicidal action was observed starting at the 10% (v/v) concentration, contrasting sharply with the lack of antibacterial effect against the tested bacteria (*E. coli*, *Staphylococcus* spp., *Proteus* spp., *Pseudomonas* spp.) at any concentration up to 50%. This differential bioactivity highlights distinct interactions between these hydrocarbons and different microbial kingdoms.

The potent antifungal efficacy, especially the fungicidal effect at 10%, supports hypotheses that certain hydrocarbons disrupt fungal cellular structures or metabolic processes [6, 7]. Fungal cell membranes, containing ergosterol, are known antifungal targets. The lipophilic nature of petroleum components and benzene likely facilitates their interaction with and disruption of

these membranes, leading to loss of integrity and cell death, potentially similar to mechanisms proposed for some essential oils containing hydrocarbon moieties [6, 7, 16]. While studies using petroleum ether extracts previously hinted at such potential [10, 11 - Note: Sources not readily verifiable], these results provide direct evidence using crude petroleum and pure benzene against a relevant panel of yeasts and molds, including opportunistic pathogens like *Aspergillus* and *Mucor* [17]. The fungicidal activity at 10% suggests a significant susceptibility of these fungal isolates under the test conditions.

Recent research explores complex fungus-hydrocarbon interactions, often in bioremediation contexts [4, 15]. While some fungi can degrade hydrocarbons under specific conditions, the direct fungicidal action observed here at 10% suggests the toxic effects dominate over metabolic adaptation for these isolates *in vitro*.

The lack of antibacterial activity aligns with the known ability of many bacteria, particularly *Pseudomonas*, to metabolize hydrocarbons [5, 13, 14]. Bacteria possess diverse enzymatic pathways for hydrocarbon degradation, conferring resistance to the tested concentrations [4, 15]. While some natural products show broad-spectrum activity, petroleum and benzene demonstrated clear antifungal selectivity here.

Despite the observed antifungal activity starting at 10%, significant challenges remain for any practical application. These concentrations are relatively high for therapeutic use, raising major host toxicity concerns. Benzene is a known carcinogen [12], and petroleum contains numerous toxic compounds. Direct application is unsafe. However, the findings could spur research into identifying specific active components within petroleum or developing safer synthetic analogues, perhaps using strategies like hybridization [3]. Further work requires elucidating precise molecular mechanisms, employing quantitative methods (MIC/MFC) against more isolates (including resistant strains), and rigorous toxicological assessments *in vitro* and *in vivo*.

CONCLUSION

Petroleum and benzene demonstrated potent and selective *in vitro* fungicidal activity against clinical isolates of *Candida*, *Mucor*, and *Aspergillus* spp. starting at 10% (v/v), while lacking antibacterial activity against tested bacteria up to 50%. These findings highlight differential microbial susceptibility and suggest potential for exploring hydrocarbon components/derivatives as antifungal leads, but emphasize the critical need for mechanistic studies and thorough toxicological evaluation before considering any practical applications due to inherent toxicity.

REFERENCES

1- Solanki R, Anand S, Anand M, Kumar P, Kumar M, Kapur MK. Antibiotic resistance: a global health crisis. Microsphere. 2022;1(1):3-11.
2- Balsano T, D'Accolti M, Soffritti I, Mazzacane S, Caselli E. Filamentous fungi for sustainable remediation of pharmaceutical contaminants. Front Bioeng Biotechnol. 2023;11:1106973.
3- Nahar D, Hossain MS, Reza MA. An insight into new strategies and targets to combat fungal infections. Results Chem. 2024;7:101308.
4- Prince RC. Petroleum spill bioremediation in marine environments. Crit Rev Microbiol. 2015;41(4):282-93.
5- Asemoloye MD, Jonathan SG, Ahmad R. Hydrocarbon degradation and enzyme activities of *Aspergillus oryzae* isolated from spent engine oil-polluted soil. Microorganisms. 2020;8(12):1912.
6- Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. Antifungal activity of the essential oil of Thymus pulegioides on Candida, Aspergillus and dermatophyte species. J Med Microbiol. 2006;55(10):1367-73.
7- Hammer KA, Carson CF, Riley TV. Antifungal effects of Melaleuca alternifolia (tea tree) oil and its components on Candida albicans, Candida glabrata and Saccharomyces cerevisiae. J Antimicrob Chemother. 2004;53(6):1081-5.
8- Burt S. Essential oils: their antibacterial properties and potential applications in foods–a review. Int J Food Microbiol. 2004;94(3):223-53.
9- Kumar A, Shukla R, Singh P, Dubey NK. Antifungal activity of essential oils of some common spices against food spoilage fungi. World J Microbiol Biotechnol. 2008;24(3):397-402.

10- Sahle T, Okbatinsae G. Phytochemical investigation and antimicrobial activity of the fruit extract of Solanum incanum grown in Eritrea. Ornamental and Medicinal Plants. 2017;1(1):15-25.

11- Pinto E, Hrimpeng K, Lopes G, Vaz S, Gonçalves MJ, Cavaleiro C, Salgueiro L. Antifungal activity of Ferulago capillaris essential oil against Candida, Cryptococcus, Aspergillus and dermatophyte species. European journal of clinical microbiology & infectious diseases. 2013;32:1311-20.

12- McHale CM, Zhang L, Smith MT. Current understanding of the mechanism of benzene-induced leukemia in humans: implications for risk assessment. Carcinogenesis. 2012;33(2):240-52.

13- Torsvik V, Goksøyr J, Daae FL. High diversity in DNA of soil bacteria. Appl Environ Microbiol. 1990;56(3):782-7.

14- Pandolfo E, Sciarrillo R, Secondo G, Presentato A, Alduina R. Recent Advances in Bacterial Degradation of Hydrocarbons. Microorganisms. 2023;11(2):375.

15- Huang Q, Dai Y, Yan G, Zhuang L, Luo C, Li J, Zhang G. New insights into autochthonous fungal bioaugmentation mechanisms for recalcitrant petroleum hydrocarbon components using stable isotope probing. Sci Total Environ. 2025;958:178082.

16- Ahmad A, Wei IB, Mahmoud K, Shah SAM, Khan Z. Volatile phenolics: A comprehensive review of the anti-microbial properties, structure-activity relationship and mechanism of action. Phytochemistry. 2021;190:112867.

17- Hashem AH, Hasanin MS, Hasanin M, Abdel-Rahman MA, Suleiman WB. Antifungal Activity of Endophytic Aspergillus terreus Extract Mediated Green Synthesis of Silver Nanoparticles against Fungi Causing Mucormycosis. Appl Biochem Biotechnol. 2022;194(5):2086-101.

To site this article: Alhusayni AA and AL-Khikani FH. Antimicrobial Activity of Petroleum and Benzene Against Selected Bacterial and Fungal Pathogens. Infinity J. Med. Innov. 2025; 1(2): 39-53.