**Screening and identification of phytochemicals from selected medicinal plants against *Escherichia coli* and *Candida albicans* using *in silico* approach/computational methods**

In Silico Screening and Identification of Phytochemicals from Selected Medicinal Plants (Bidens pilosa and Rosmarinus officinalis) for Potential Activity Against Escherichia coli and Candida albicans

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# DECLARATION

I declare that this proposal is my own work and has been submitted with the approval of my supervisors

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I confirm that this proposal was written by the above named student and has been submitted with our approval as supervisor.

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**Acknowledgements**

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**Abstract**

**Chapter 1: Introduction**

**1.1 Background to the study**

- provide a background on the research topic that includes known and controversial findings, challenging issues, and the hypothesis of the research.

*The increasing prevalence of antimicrobial resistance (AMR) has become a significant global health threat, reducing the efficacy of conventional antibiotics and antifungal agents. AMR has led to a rise in persistent infections, prolonged illness, and increased mortality rates, making it one of the most pressing medical challenges worldwide 1. Some Escherichia coli strains are the primary cause of bacterial infections2,3, including urinary tract infections, septicaemia, and foodborne illnesses, while Candida albicans is an opportunistic fungal pathogen4 responsible for infections ranging from superficial candidiasis to life-threatening systemic candidemia. The emergence of drug-resistant strains of these pathogens necessitates the urgent search for new antimicrobial compounds with novel mechanisms of action 5.*

### Throughout the centuries, medicinal plants have been utilized for treating infectious diseases due to their rich phytochemical composition. Plants such as *Bidens pilosa* (blackjack) and *Rosmarinus officinalis* (rosemary) have been extensively studied for their antimicrobial, anti-inflammatory, and antioxidant properties 6–9.These plants contain bioactive compounds such as flavonoids, alkaloids, terpenes, and polyphenols, which have demonstrated inhibitory effects on microbial growth. However, while some studies support their antimicrobial potential, the specific mechanisms of action and molecular targets of these phytochemicals remain largely unexplored (Omojate et al., 2022).

### The antimicrobial potential of *B. pilosa* and *R. officinalis* has been well-documented, with several studies demonstrating their effectiveness against bacterial and fungal pathogens. Research findings indicate that extracts from these plants can inhibit the growth of *E. coli* and *C. albicans*, suggesting their potential as alternative antimicrobial agents (Abdissa et al., 2019). However, variations in experimental methods, such as differences in extraction techniques, solvent types, and microbial strains tested, have led to inconsistent results (Elisha et al., 2017). Additionally, the exact molecular interactions through which flavonoids and terpenes disrupt microbial cell walls and enzymatic activity remain unclear (Nazzaro et al., 2020).

### In silico screening has emerged as a valuable tool in drug discovery, allowing researchers to predict potential drug candidates and their interactions with microbial proteins. Computational studies have suggested that certain compounds found in *B. pilosa* and *R. officinalis* exhibit strong binding affinities to bacterial and fungal targets (Shao et al., 2023). However, while in silico methods provide important preliminary insights, they require further in vitro and in vivo validation. Some researchers argue that computational predictions alone are insufficient for confirming antimicrobial efficacy, whereas others emphasize their efficiency in narrowing down promising candidates for further study (Gupta & Kaur, 2021).

### Despite the potential benefits of plant-derived antimicrobial agents, several challenges remain. One of the primary difficulties is the complexity of phytochemical interactions. Medicinal plants contain a diverse array of bioactive compounds, making it difficult to isolate and determine which specific phytochemicals are responsible for their antimicrobial effects (Tariq et al., 2019). Additionally, microbial resistance mechanisms continue to evolve, with pathogens such as *E. coli* and *C. albicans* developing sophisticated strategies to neutralize antimicrobial compounds, potentially limiting the efficacy of plant-based treatments (Li et al., 2021). Another significant challenge is the reliability of computational approaches. While molecular docking and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analyses provide valuable insights into drug-likeness and potential toxicity, their accuracy depends on the quality of molecular databases and software algorithms (Rasool et al., 2022).

### In conclusion, medicinal plants such as *B. pilosa* and *R. officinalis* offer promising potential in the search for new antimicrobial compounds, particularly in response to the growing threat of AMR. While their bioactive compounds exhibit antimicrobial properties, further research is needed to elucidate their precise mechanisms of action. In silico approaches provide a valuable starting point in identifying potential drug candidates, but they must be complemented by experimental validation to ensure reliability and efficacy. Overcoming the challenges of phytochemical complexity, microbial resistance, and computational accuracy will be essential in advancing plant-based antimicrobial therapies. Continued research and interdisciplinary collaboration are crucial in harnessing the full potential of medicinal plants for combating drug-resistant infections.

This study hypothesizes that bioactive compounds present in *Bidens pilosa* and *Rosmarinus officinalis* exhibit inhibitory effects against key molecular targets in *Escherichia coli* and *Candida albicans*. Using in silico approaches, it is expected that specific phytochemicals from these plants will demonstrate high binding affinity to microbial enzymes and proteins involved in pathogenicity, suggesting their potential as alternative antimicrobial agents.

**1.2 Problem statement**

The increasing prevalence of antimicrobial-resistant bacterial and fungal infections poses a significant threat to our economic future and global public health.10,11 *Escherichia coli* and *Candida albicans* remain among the most common pathogens responsible for a wide range of infections, including urinary tract infections, bloodstream infections, and opportunistic fungal diseases. 12,13 Conventional antimicrobial treatments are becoming less effective due to rising resistance, necessitating the urgent search for novel therapeutic alternatives.14,15

Medicinal plants, such as *Bidens pilosa* 16(blackjack) and *Rosmarinus officinalis* 17(rosemary), have been traditionally used for their antimicrobial properties. However, the specific bioactive compounds responsible for their therapeutic effects remain underexplored. Advances in computational methods, including molecular docking and pharmacokinetic modeling, offer a cost-effective and efficient approach to screening phytochemicals for potential antimicrobial activity.18,19

The aim of this study is to evaluate the bioactive compounds present in *Bidens pilosa* and *Rosmarinus officinalis* using in silico techniques to determine their potential interactions with molecular targets in *E. coli* and *C. albicans*. While leveraging computational drug discovery tools, this research seeks to provide valuable insights into alternative antimicrobial agents that could contribute to the development of novel treatments against resistant microbial strains.

**1.3 Justification**

The rise of antimicrobial resistance (AMR) in pathogens such as *Escherichia coli20* and *Candida albicans21* has become a major global health concern, limiting the effectiveness of existing antibiotics and antifungal treatments. There is an urgent need to explore novel therapeutic options, particularly from natural sources, to address this growing challenge. Medicinal plants have long been recognized for their pharmacological potential, with compounds that exhibit antimicrobial properties (Cowan, 1999). However, the identification and characterization of these bioactive compounds require systematic scientific investigation.

*Bidens pilosa* (blackjack) and *Rosmarinus officinalis* (rosemary) have been traditionally used for their medicinal properties, yet their antimicrobial potential against *E. coli* and *C. albicans* remains inadequately studied at the molecular level. 22In silico approaches, such as molecular docking, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis, and molecular dynamics simulations, provide a rapid and cost-effective method for screening and predicting the efficacy of plant-derived compounds against microbial targets (Kitchen et al., 2004).

This study is justified by the need to discover novel, plant-based antimicrobial agents that could contribute to the development of new therapeutic alternatives. By utilizing computational methods to identify potential bioactive compounds from *B. pilosa* and *R. officinalis*, this research aims to bridge the gap between traditional herbal medicine and modern drug discovery, ultimately supporting efforts to combat antimicrobial resistance.

**1.4 Objectives**

**1.4.1 Main objective**

To evaluate the antibacterial and antifungal potential of bioactive compounds fromBlackjack and Rosemary plants using computational methods.

**1.4.2 Specific objectives**

1. To identify novel DNA gyraseand Secreted Aspartyl Proteinase 3 – 6inhibitors from blackjack (*Bidens pilosa*) rosemary(*Rosmarinus officinalis* L.) through molecular docking and molecular dynamics simulation (MDS)
2. To determine binding modes and binding affinities of top hits on DNA gyrase **and** Secreted Aspartyl Proteinase 3 – 6
3. To examine the druglikeliness, pharmacokinetics and toxicological profile of hit molecules of each target upon ADMET analysis

**Chapter 2: Literature review**

2.1 Microbial infections

* Overview of bacterial and fungal infections, with a focus on *Escherichia coli* and *Candida albicans*.
* Pathogenic mechanisms and clinical manifestations.

2.2 Antimicrobial resistance

* Mechanisms of resistance in *E. coli* and *C. albicans*.
* The global challenge of multidrug resistance and its implications.
* The need for novel therapeutic strategies.

2.3 Targeting *Escherichia coli* and *Candida albicans*

- Drugs and targets

* Current **drugs and drug resistance** trends for these pathogens.
* Identification of **critical molecular targets**, such as DNA gyrase (in bacteria) and Secreted Aspartyl Proteinase 3–6 (in fungi).
* Molecular strategies for inhibiting microbial survival.

2.4 Management of *Escherichia coli* and *Candida albicans* using natural products

* Traditional medicinal plants with known antimicrobial properties.
* The role of phytochemicals in combatting **resistant microbial strains**.
* Molecular mechanisms of phytochemical action and their potential therapeutic applications.

2.5 Computer-aided design methods in the discovery of antibacterial and antifungal compounds

* **Molecular docking** and **molecular dynamics simulations (MDS)** for predicting phytochemical interactions.
* **ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) analysis** for evaluating pharmacokinetics and drug-likeness.
* AI-driven **virtual screening** and structure-based drug discovery.

**Chapter 3: Materials and methods**

**3.1 Preparation of structures of protein molecules**

**- *Escherichia coli* DNA gyrase B inhibitors**

**-** Secreted Aspartyl Proteinase 3 – 6inhibitors of *Candida albican*

**3.2 Construction and preparation of ligands**

- Bioactive compounds from blackjack and rosemary plants

- Also include commonly used drugs here

**3.3 Molecular docking studies**

**3.4 Molecular dynamic simulation studies**

**3.5 Binding Free Energy Calculations**

**3.6 Drug likeness and Pharmacokinetic analyses**

**3.7 Toxicology analysis**

**References**

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*(4), 564-582.  
Ekins, S., Mestres, J., & Testa, B. (2007). In silico pharmacology for drug discovery: Methods for virtual ligand screening and profiling. *British Journal of Pharmacology, 152*(1), 9-20.  
Kitchen, D. B., Decornez, H., Furr, J. R., & Bajorath, J. (2004). Docking and scoring in virtual screening for drug discovery: Methods and applications. *Nature Reviews Drug Discovery, 3*(11), 935-949.  
Köhler, J. R., Casadevall, A., & Perfect, J. (2017). The spectrum of fungi that infects humans. *Cold Spring Harbor Perspectives in Medicine, 5*(1), a019273.  
Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K., Wertheim, H. F., Sumpradit, N., ... & Cars, O. (2013). Antibiotic resistance—the need for global solutions. *The Lancet Infectious Diseases, 13*(12), 1057-1098.  
Nostro, A., Germano, M. P., D’Angelo, V., Marino, A., & Cannatelli, M. A. (2005). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in Applied Microbiology, 30*(5), 379-384.  
Salehi, B., Mishra, A. P., Shukla, I., Sharifi-Rad, M., Contreras, M. M. M., Segura-Carretero, A., ... & Sharifi-Rad, J. (2018). Thymol, thyme, and other plant sources: Health and potential uses. *Phytotherapy Research, 32*(9), 1688-1706.  
Ventola, C. L. (2015). The antibiotic resistance crisis: Part 1: Causes and threats. *Pharmacy and Therapeutics, 40*(4), 277-283.  
WHO. (2022). Antimicrobial resistance. Retrieved from <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>.

1. Global antimicrobial resistance and use surveillance system (‎GLASS)‎ report: 2022. https://www.who.int/publications/i/item/9789240062702.

2. E. coli. https://www.who.int/news-room/fact-sheets/detail/e-coli.

3. for Disease Control C. *Shiga Toxin–Producing Escherichia Coli O157:H7 Illness Outbreak Associated with Untreated, Pressurized, Municipal Irrigation Water — Utah, 2023*.; 2024. https://cfpub.epa.gov/si/si\_public\_record\_Report.cfm?dirEntryId=355726&

4. Antimicrobial-Resistant Invasive Candidiasis. https://www.cdc.gov/candidiasis/antimicrobial-resistance/index.html.

5. Sharma S, Chauhan A, Ranjan A, et al. Emerging challenges in antimicrobial resistance: implications for pathogenic microorganisms, novel antibiotics, and their impact on sustainability. *Front Microbiol*. 2024;15. doi:10.3389/fmicb.2024.1403168

6. Mtenga D V., Ripanda AS. A review on the potential of underutilized Blackjack (Biden Pilosa) naturally occurring in sub-Saharan Africa. *Heliyon*. 2022;8. doi:10.1016/j.heliyon.2022.e09586

7. What to Know About Blackjack (Bidens Pilosa). https://www.webmd.com/a-to-z-guides/what-to-know-about-blackjack-bidens-pilosa.

8. Nieto G, Ros G, Castillo J. Antioxidant and Antimicrobial Properties of Rosemary (Rosmarinus officinalis, L.): A Review. *Medicines*. 2018;5:98. doi:10.3390/medicines5030098

9. Medicinal Plants of East Africa. https://books.google.co.ke/books?hl=en&lr=&id=msyHLY0dhPwC&oi=fnd&pg=PR1&ots=XUUfeFIiHm&sig=KwXYZaTNvh7CwTWTx6AYrl5xz6E&redir\_esc=y#v=onepage&q&f=false.

10. *DRUG-RESISTANT INFECTIONS A Threat to Our Economic Future*.; 2017. www.worldbank.org

11. Murray CJ, Ikuta KS, Sharara F, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*. 2022;399(10325):629-655. doi:10.1016/S0140-6736(21)02724-0

12. Mueller M, Tainter CR. Escherichia coli Infection. In: *Encyclopedia of Toxicology, Fourth Edition: Volume 1-9*. Vol 4. Elsevier; 2023:V4-357-V4-367. doi:10.1016/B978-0-12-824315-2.00190-1

13. Katsipoulaki M, Stappers MHT, Malavia-Jones D, Brunke S, Hube B, Gow NAR. Candida albicans and Candida glabrata : global priority pathogens . *Microbiology and Molecular Biology Reviews*. 2024;88. doi:10.1128/mmbr.00021-23

14. Koh SWC, Ng TSM, Loh VWK, et al. Antibiotic treatment failure of uncomplicated urinary tract infections in primary care. *Antimicrob Resist Infect Control*. 2023;12. doi:10.1186/s13756-023-01282-4

15. Ben-Ami R. Treatment of Invasive Candidiasis: A Narrative Review. *J Fungi (Basel)*. 2018;4. doi:10.3390/jof4030097

16. Odongo EA, Mutai PC, Amugune BK, Mungai NN. A Systematic Review of Medicinal Plants of Kenya used in the Management of Bacterial Infections. *Evid Based Complement Alternat Med*. 2022;2022:9089360. doi:10.1155/2022/9089360

17. De Oliveira JR, Camargo SEA, De Oliveira LD. Rosmarinus officinalis L. (rosemary) as therapeutic and prophylactic agent. *J Biomed Sci*. 2019;26. doi:10.1186/s12929-019-0499-8

18. Saliu TP, Umar HI, Ogunsile OJ, Okpara MO, Yanaka N, Elekofehinti OO. Molecular docking and pharmacokinetic studies of phytocompounds from Nigerian Medicinal Plants as promising inhibitory agents against SARS-CoV-2 methyltransferase (nsp16). *Journal of Genetic Engineering and Biotechnology*. 2021;19. doi:10.1186/s43141-021-00273-5

19. Chikowe I, Bwaila KD, Ugbaja SC, Abouzied AS. GC–MS analysis, molecular docking, and pharmacokinetic studies of Multidentia crassa extracts’ compounds for analgesic and anti-inflammatory activities in dentistry. *Sci Rep*. 2024;14. doi:10.1038/s41598-023-47737-x

20. Poirel L, Madec JY, Lupo A, et al. Antimicrobial Resistance in Escherichia coli . *Microbiol Spectr*. 2018;6. doi:10.1128/microbiolspec.arba-0026-2017

21. Dhasarathan P, AlSalhi MS, Devanesan S, et al. Drug resistance in Candida albicans isolates and related changes in the structural domain of Mdr1 protein. *J Infect Public Health*. 2021;14:1848-1853. doi:10.1016/j.jiph.2021.11.002

22. Yiğitkan S, Fırat M. ESSENTIAL OIL CONTENTS AND BIOLOGICAL ACTIVITIES OF THYMUS CANOVIRIDIS JALAS AND THYMUS SIPYLEUS BOISS. *Ankara Universitesi Eczacilik Fakultesi Dergisi*. 2024;48:28-28. doi:10.33483/jfpau.1484485

**Work plan**

**Budget**