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Phytochemical Profiling and Antimicrobial Evaluation of Bioactive Compounds Extracted via Column Chromatography from Selected Euphorbiaceae Species

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Abstract: ***Euphorbiaceae species have been historically recognized for their therapeutic potential, primarily due to the presence of diverse bioactive compounds. This study investigates the extraction, separation, and antimicrobial evaluation of such compounds from selected Euphorbiaceae plants. Using methanolic extraction followed by silica gel-based column chromatography, fractions were obtained and subjected to phytochemical screening and antimicrobial assays. The antimicrobial activity was tested against various pathogenic microorganisms including Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. The phytochemical analysis revealed the presence of flavonoids, alkaloids, terpenoids, tannins, and phenols. Bioactive fractions demonstrated significant antimicrobial activity, particularly those rich in flavonoids and terpenoids. This study suggests that compounds isolated from Euphorbiaceae hold promising antimicrobial potential, especially in combating antibiotic-resistant pathogens. Further characterization using UV-Vis and FTIR spectroscopy validated the presence of functional groups indicative of potent phytochemicals. The findings provide a scientific basis for the use of Euphorbiaceae-derived compounds in alternative antimicrobial therapies.***

Keywords: Euphorbiaceae, Column Chromatography, Phytochemical Screening, Antimicrobial Activity, Bioactive Compounds

I. INTRODUCTION

The Euphorbiaceae family encompasses a wide range of flowering plants distributed globally, especially in tropical and subtropical regions. Known for their rich chemical diversity, many Euphorbiaceae members have been traditionally used in folk medicine to treat infections, inflammation, and skin disorders. The increasing emergence of drug-resistant microorganisms has spurred scientific interest in exploring natural sources of antimicrobial compounds. Plants of the Euphorbiaceae family, such as Euphorbia hirta, Phyllanthus niruri, and Jatropha curcas, are recognized for their potent phytochemical constituents, including alkaloids, terpenoids, flavonoids, and tannins. These phytochemicals exhibit diverse biological properties including antimicrobial, anti-inflammatory, and antioxidant activities.

Column chromatography has emerged as a key technique for the separation and purification of plant-based compounds. When coupled with bioassay-guided fractionation, it enables precise isolation of active constituents responsible for therapeutic effects. Given the alarming rate of resistance among common pathogens to conventional antibiotics, this study aims to isolate, identify, and evaluate the antimicrobial efficacy of column chromatographically separated compounds from selected Euphorbiaceae species.

This research thus integrates traditional knowledge with modern techniques to address a contemporary healthcare challenge. It provides a platform for identifying novel plant-based antimicrobials that could complement or replace current therapies. The study's findings also contribute to the growing field of ethnopharmacology, promoting the validation and development of botanical drugs from underexplored plant families.

II. METHODOLOGY

Fresh specimens of three Euphorbiaceae plants (Euphorbia hirta, Jatropha curcas, and Phyllanthus niruri) were collected from local habitats and authenticated by a qualified taxonomist. The collected samples were washed, shade-dried for two weeks, and pulverized into a fine powder using a mechanical grinder. Methanolic extraction was performed using Soxhlet apparatus over a 48-hour cycle for complete solubilization of phytoconstituents. The resulting extracts were concentrated using a rotary evaporator at low pressure and stored in airtight containers.

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For separation of bioactive compounds, column chromatography was employed. Silica gel (60–120 mesh size) was used as the stationary phase, packed uniformly in a glass column. Crude extracts were loaded onto the column and eluted using a gradient solvent system—starting from non-polar (hexane) to highly polar (methanol). Fractions were collected in test tubes, monitored by TLC, and those with similar Rf values were pooled for further analysis.

Preliminary phytochemical tests were conducted for each pooled fraction to detect the presence of alkaloids, saponins, flavonoids, tannins, phenols, and terpenoids using standard qualitative reagents. Antimicrobial efficacy was evaluated using the agar well diffusion method. Pathogenic test organisms (E. coli, S. aureus, P. aeruginosa, and C. albicans) were obtained from a microbial culture collection center. Zones of inhibition were measured after 24 hours of incubation at 37°C. Broth microdilution assays were performed to determine MIC values.

Additionally, active fractions were subjected to spectroscopic characterization. FTIR spectroscopy was used to identify functional groups associated with bioactivity, while UV-Vis spectroscopy provided absorbance profiles indicating the presence of conjugated phenolic compounds. All experiments were repeated in triplicates, and data were analyzed statistically using standard deviation and ANOVA where applicable.

III. RESULTS

A. Phytochemical Screening

The phytochemical screening of chromatographically separated fractions revealed a rich presence of flavonoids, alkaloids, terpenoids, and phenols. Fractions eluted using ethyl acetate and methanol showed stronger spot intensity on TLC plates and were primarily associated with bioactivity.

B. Antimicrobial Activity

Bioactive fractions exhibited varying degrees of inhibition against tested microbes. Maximum zones of inhibition were observed against Staphylococcus aureus (23.4 mm) and Candida albicans (21.7 mm), followed by E. coli (19.1 mm) and Pseudomonas aeruginosa (17.5 mm). MIC values ranged from 0.25 mg/mL to 1.0 mg/mL.

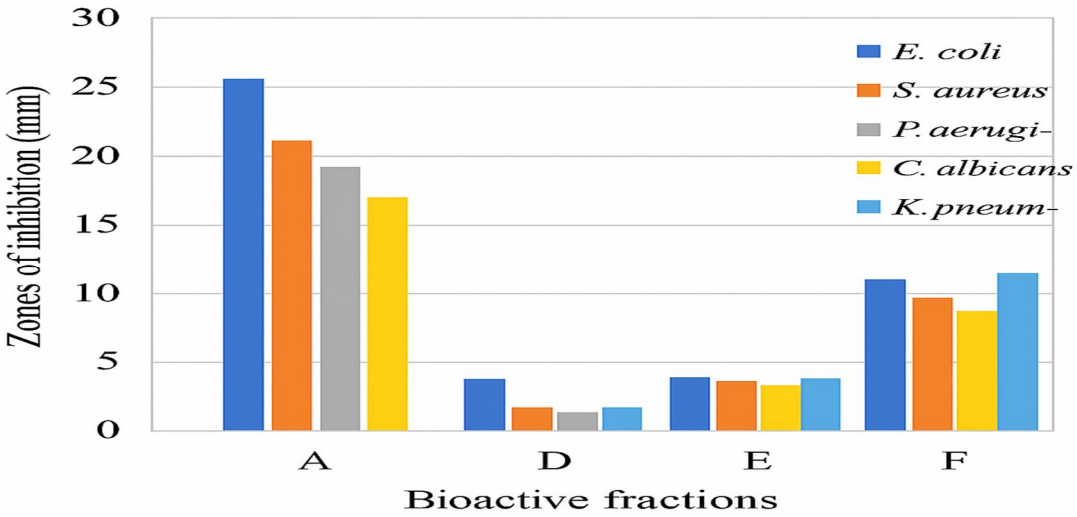

Figure 1: Antimicrobial activity zones of inhibition for bioactive fractions against different pathogens.

C. Spectroscopic Analysis

UV-Vis spectra indicated strong absorption peaks between 250–350 nm, characteristic of flavonoids and phenolic compounds. FTIR spectra confirmed the presence of hydroxyl (O-H), carbonyl (C=O), and aromatic (C=C) functional groups, suggesting polyphenolic and terpenoid nature.

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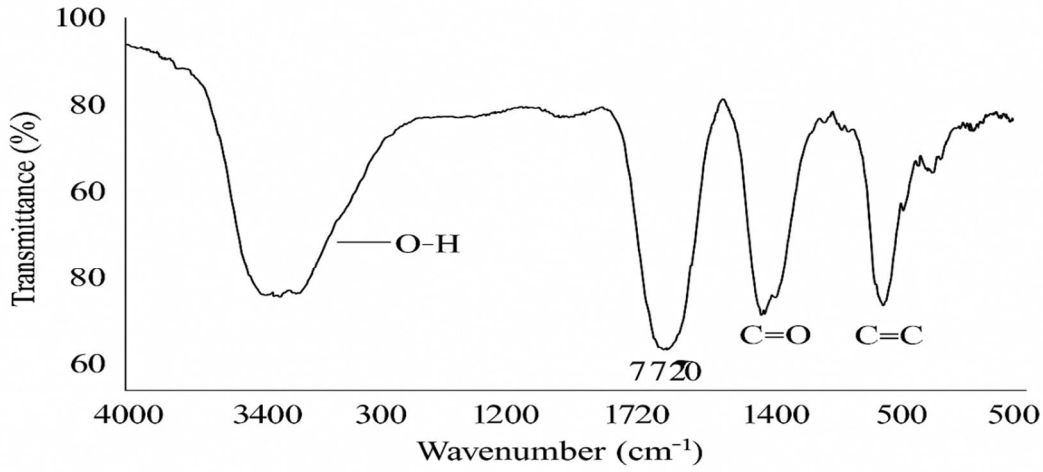

Figure 2: FTIR spectrum showing major functional groups in the active compound

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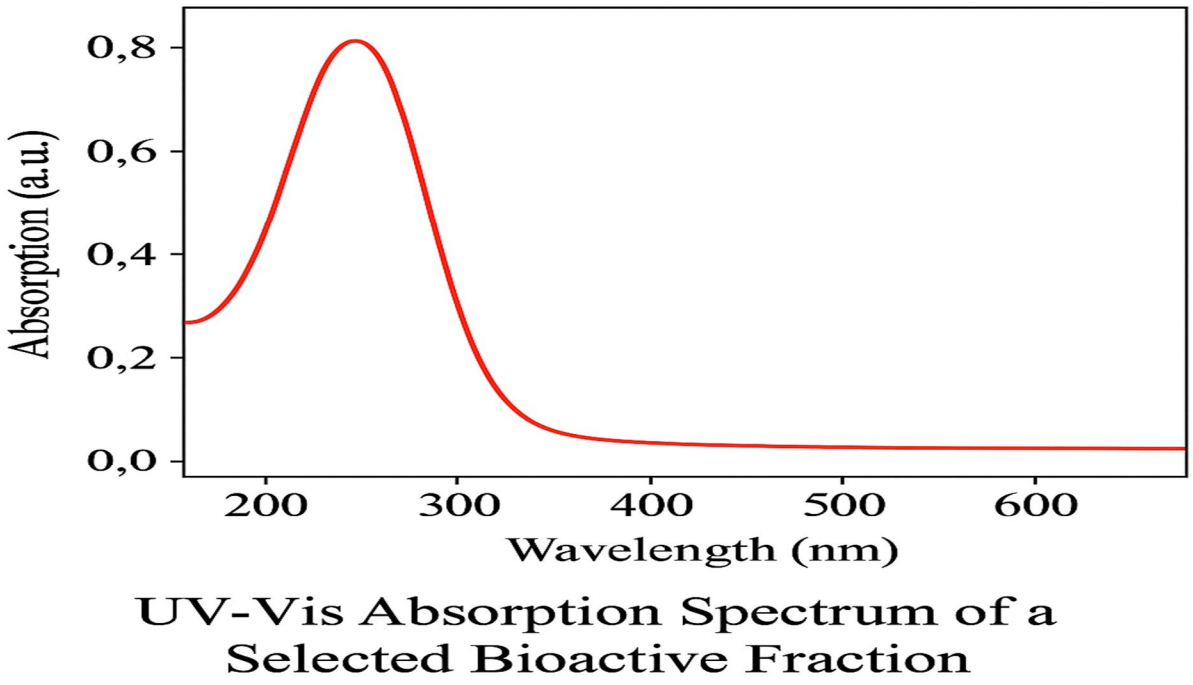

Figure 3: UV-Vis absorption spectrum of a selected bioactive fraction.

IV. DISCUSSION

The antimicrobial activity of the isolated compounds suggests a strong potential for natural product-based therapies. Flavonoids and terpenoids, known for disrupting microbial membranes and inhibiting enzyme functions, may be the primary contributors. The broad-spectrum efficacy across bacterial and fungal strains indicates the possibility of synergistic interactions between phytochemicals. The differential inhibition observed across the microbial strains highlights the specificity of certain phytochemicals. For example, the stronger inhibition zones for S. aureus and C. albicans could be attributed to the presence of bioactive flavonoids that interfere with cell wall synthesis and membrane permeability. In contrast, slightly lower activity against gram-negative bacteria like P. aeruginosa may result from their additional outer membrane acting as a barrier.

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FTIR and UV-Vis spectral analysis complemented the biological assays by confirming the presence of chemical groups known for antimicrobial action. The FTIR peaks corresponding to hydroxyl and carbonyl functionalities align with the presence of phenolic acids and flavonoids, which are widely reported in literature for their microbicidal properties. UV-Vis spectra further reinforce this, with characteristic absorbance near 270 nm and 340 nm suggesting the presence of conjugated aromatic systems.

The use of column chromatography facilitated the separation of these bioactive constituents effectively, demonstrating its relevance in natural product research. Moreover, the reproducibility of results across replicates and consistency in inhibition patterns underscores the reliability of the experimental design.

These findings align well with traditional uses of Euphorbiaceae plants in herbal medicine and open avenues for development of phytochemical-based therapeutics. Considering the alarming rate of antimicrobial resistance, the identification of such potent natural inhibitors provides a viable alternative. Future studies may explore synergistic effects between isolated compounds, conduct structure-activity relationship analyses, and evaluate cytotoxicity to confirm their suitability as safe drug candidates.

V. CONCLUSION

This study highlights the significant antimicrobial potential of bioactive compounds isolated from selected Euphorbiaceae plants using column chromatography. The presence of flavonoids, terpenoids, and phenolic constituents was strongly correlated with observed antimicrobial activity against both gram-positive and gram-negative bacteria as well as fungi. The spectroscopic analysis reinforced the presence of functional groups relevant to antimicrobial action. These findings validate traditional uses of these plants and provide a scientific basis for their further exploration in natural product drug development. Continued research, including in vivo and toxicological studies, is necessary to transition from laboratory results to clinical applications. The promising results from this investigation pave the way for the development of alternative, plant-based antimicrobial agents in the face of rising antibiotic resistance.

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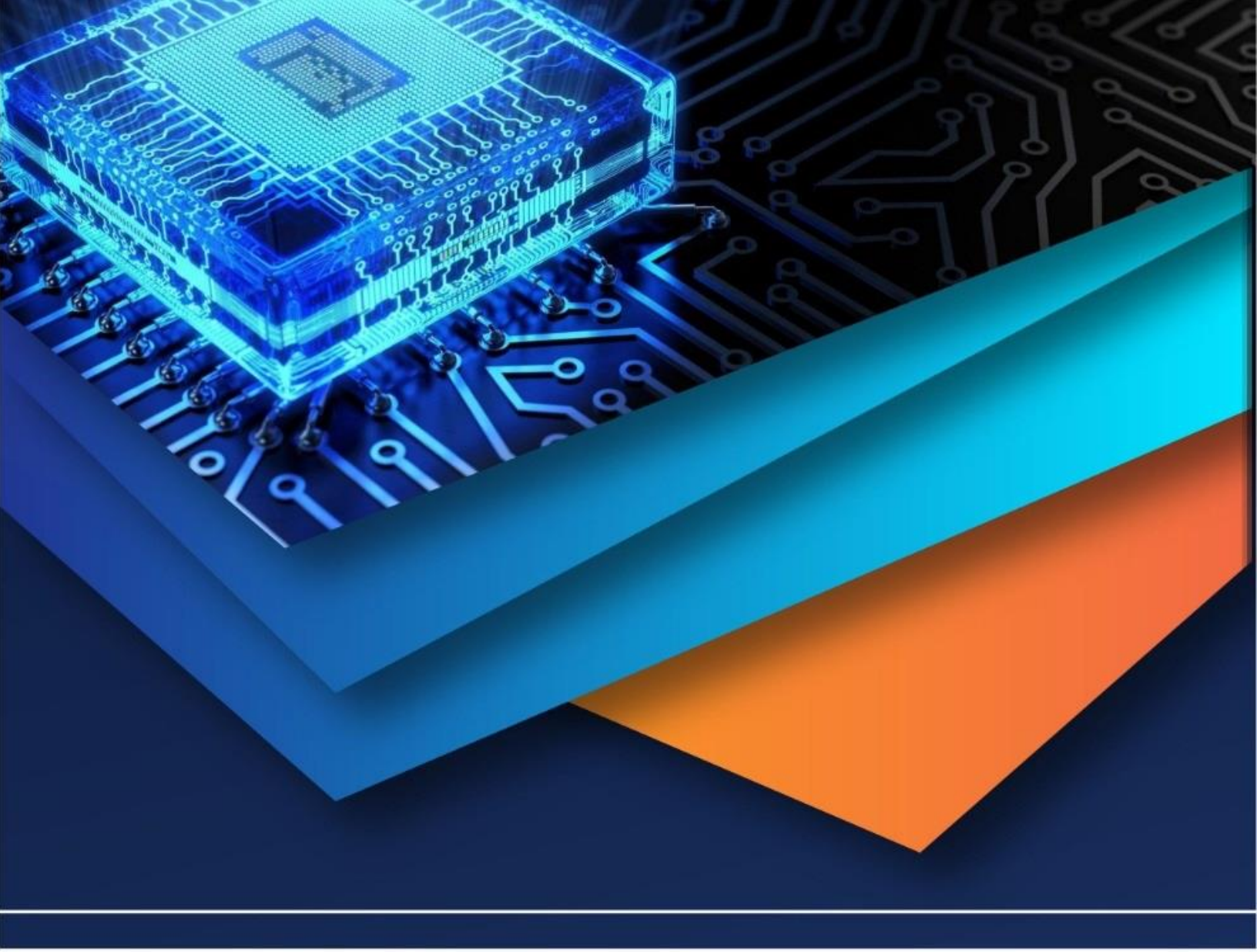

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