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Antimicrobial Activity of Crude Methanolic Extract Fractions from *Balanites aegyptiaca* (L.) Delile

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Abstract

Microbial infections remain a major global health concern, with mortality rates continuing to rise each decade. Projections estimate that by 2050, antimicrobial resistance could account for up to 10 million deaths annually. This study investigated the antimicrobial potential of phytochemical groups present in crude extracts of B. aegyptiaca *as a possible alternative for managing microbial infections. Plant materials (leaves, stem bark, and root bark) were collected from two local varieties of* B. aegyptica *which are tentatively referred to as the "sweet" and "sour" varieties growing in Ajeluk village, Katakwi District, North-Eastern Uganda. The dried and pulverized samples (100 g each) were subjected to successive extraction with n-hexane, dichloromethane, and methanol. The resulting crude extracts were concentrated using a rotary evaporator, followed by fractionation through silica gel column chromatography. Fractions were further purified using preparative thin-layer chromatography, with separated bands visualized under Ultra Violet (UV) light, eluted, and tested for antimicrobial activity. Test organisms included multidrug-resistant American Type Culture Collection (ATCC)* Escherichia coli *ATCC 25922,* Candida albicans *ATCC 10231, and* Aspergillus flavus *ATCC 6275. The methanolic fractions demonstrated notable antimicrobial activity, with inhibition zones greater than 8 mm. Among the phytochemical groups, phenolics exhibited the strongest activity (23.67 ± 1.15 mm), while coumarin-containing fractions displayed the lowest activity (7.00 ± 0.00 mm). Statistical analysis revealed significant differences between inhibition zones of the phytochemical groups and the positive control ($p < 0.05$). Phytochemical screening confirmed the presence of phenolics, flavonoids, alkaloids, cardiac glycosides, and coumarins in the extracts. Overall, the findings suggest that* B. aegyptica *contains diverse bioactive compounds with antimicrobial potential, particularly against fungal pathogens.* C. albicans *and* A. flavus *were more susceptible to these extracts than* E. coli*, highlighting the plant's promise as a source of antifungal agents.*

Keywords: *Plant phenolics, Phenolic extracts, Antimicrobial activity, Phytochemicals, and Balanites aegyptiaca*

Introduction

Microorganisms are the primary causative agents of numerous illnesses worldwide (Thornber et al., 2020). Over the past decade, microbial infections have continued to rise globally, with no indication of decline (Namubiru et al., 2024). This situation has been further exacerbated by the emergence and spread of antimicrobial resistance (AMR), which poses a critical threat to global health. Currently, approximately 700,000 people die each year from diseases associated with AMR (Mhondoro et al., 2019). Projections suggest that by 2050, AMR could result in 10 million annual deaths, with an estimated economic burden of USD 100 trillion. In 2019 alone, invasive infections particularly those resistant to available antibiotics were estimated to cause 5.3 million deaths globally (Res et al., 2018).

In recognition of this growing threat, the World Health Organization (WHO) identified the urgent need for research and development of new antimicrobials in 2017, particularly targeting priority pathogens resistant to last-linea treatments (Lopez-Jicome et al., 2022). Despite global efforts to manage infectious diseases such as Human Immunodeficiency Virus (HIV), tuberculosis, hepatitis B, and sexually transmitted infections (STIs), a significant knowledge gap remains in the utilization of natural resources, particularly medicinal plants, to address microbial infections caused by Gram-positive and Gram-negative bacteria, as well as fungal pathogens (Mudenda et al., 2023).

Across Africa, resistance to commonly used antimicrobials has been well documented. For example, a study conducted in Zimbabwe reported that 73.9% of bacterial isolates were resistant to amoxicillin in 2011, increasing slightly to 74.6% by 2015 (Mhondoro et al., 2019). This persistence and spread of resistance may be attributed, in part, to inadequate waste management systems. In many African countries, antibiotics and microbiological

waste including resistant bacteria are often discharged directly into the environment, contributing to the spread of AMR (Bangtsson-palme et al., 2018).

In Uganda, resistant bacterial and fungal pathogens have been isolated from clinical samples across different health facilities and age groups. A recent study conducted at Mbarara Regional Referral Hospital, Kiruddu National Referral Hospital, and the General Military Hospital revealed that 108 isolates (20.1%) were resistant to *Staphylococcus aureus*, while 101 isolates (18.8%) were resistant to Salmonella species. Resistance to ciprofloxacin was also noted to be on the rise across several health sub-regions (Namubiru et al., 2024). These findings highlight the urgent need to explore alternative therapeutic sources, particularly plants with antimicrobial properties.

Medicinal plants are known to harbour diverse bioactive compounds, including saponins, tannins, and alkaloids, which contribute to their therapeutic effects. For instance, triterpenoids exhibit anti-inflammatory activity, while tannins are associated with astringent, anti-inflammatory, and antibacterial effects (Esposito et al., 2016). The pharmacological potential of plants lies in their secondary metabolites such as phenols, polyphenols, flavonoids, alkaloids, terpenoids, and saponins that demonstrate broad-spectrum biological activities (Khamis et al., 2020; Sigh et al., 2022).

Given the escalating global challenge of antimicrobial resistance, exploring plants as potential sources of new antimicrobial agents is both timely and necessary. Against this backdrop, the present study aimed to identify the phytochemical groups responsible for the antimicrobial properties of *B. aegyptiaca*, a plant traditionally used in African ethnomedicine.

Materials And Methods

Description of the sample collection area

In June 2023, *B. aegyptiaca* leaves, stem bark, and root bark were collected from healthy, disease-free trees growing naturally at Ajeluk village, Olela Parish, Katakwi Sub-county, Ngariam County, Katakwi District, Uganda (01°54'54.0"N, 33°57'18.0"E) (latitude: 1.9150; longitude: 33.9550) (Figure 1). The study area was selected for its rich traditional knowledge and widespread use of *B. aegyptiaca* in local medicinal practices. Random sampling was employed to collect representative specimens, ensuring the authenticity of the plant materials and reflecting the ethnomedicinal practices of the community, thereby supporting the study's objective of investigating the therapeutic potential of *B. aegyptiaca* leaves, stem bark, and root bark.

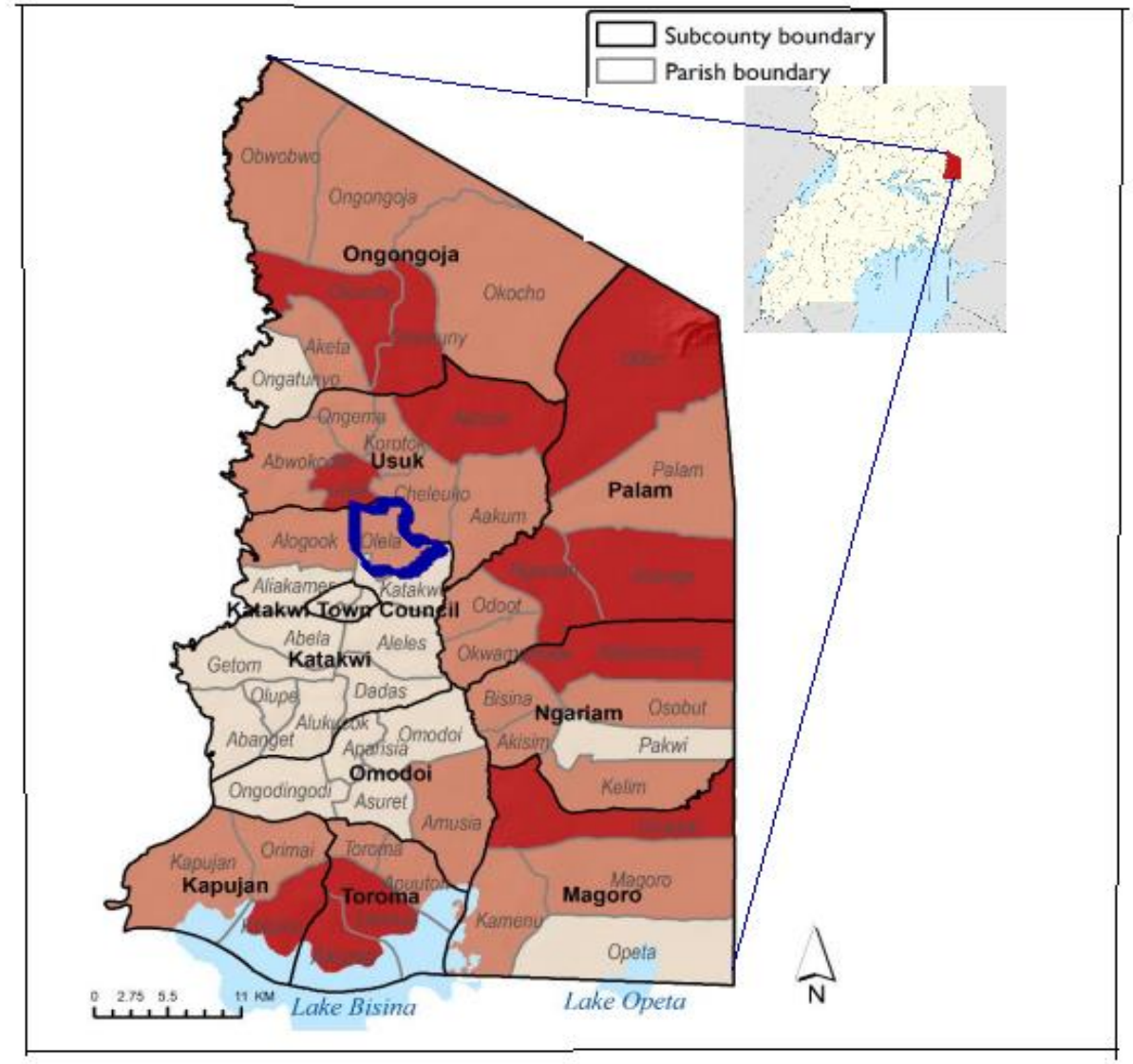

Figure 1. Sample collection site at Olela parish, Katakwi district

Study design

An experimental study was done to determine the antimicrobial activities of the crude organic extracts of the selected parts of *B. aegyptiaca* against selected microbes in the laboratory using the agar-well diffusion method (Umar et al., 2023; Usman et al., 2020).

Collection of plant samples, authentication and preparation

The samples were being identified as authentic by a botanist at Makerere University in Kampala, Uganda, fresh leaves, stem barks, and root barks of *B. aegyptiaca* were collected from Katakwi District. The voucher specimen was deposited under voucher number 220079. The collected plant materials were taken to the Bioscience Research Laboratory at Kyambogo University. The various plant parts were washed with distilled water to eliminate any dust, the samples were allowed to air dry at room temperature (25± 5°C) in the laboratory under room temperature. The plant pieces were dried thoroughly in the laboratory for one month and then pulverized into a coarse powder. The plant components were broken down into fine particles to expose internal tissues and cells to the solvents. The powdered materials were kept in sterile, closed glass containers prior to extraction of the secondary metabolites in the plant samples.

Test organisms

Resistant *E. coli* ATCC 25922, *C. albicans* (ATCC 10231) and *A. flavus* (ATCC 6275) were collected from the Microbiology Laboratory of the College of Veterinary Medicine and Biosecurity of Makerere University, Kampala, Uganda, and then transported to the Bioscience Research Laboratory of Kyambogo University under the cold chain.

Extraction

The extraction process was done using maceration technique (Sigh et al., 2022). In separate Erlenmeyer flasks, closed properly to prevent the entry of air, 100 g of the finely powdered leaves, stem bark and root bark of the *B. aegyptiaca* plant were added and 500 ml of the solvent were added to the flasks and left to stand for 24 hours for 3 days. Successful extracts were done using smaller volumes of the solvents to maximize the extraction of the phytochemical substances. The dipped *B. aegyptiaca* plant samples were filtered through Whatman filter paper No. 1. And then transferred to a rotary evaporator to concentrate the extracts. The partly concentrated *B. aegyptiaca* plant extracts were then transferred to a water bath at 350°C to completely evaporate the solvent. The gummy concentrated extracts obtained were preserved in a refrigerator at 4°C for future use. Three solvents were used to extract the phytochemical substances, that is, methanol, dichloromethane and n-hexane.

Analysis of the antimicrobial activity of the individual phytochemical constituents

Fractionation was done using silica gel column chromatography using methanol, dichloromethane and n-Hexane as eluting agents, at the Department of Chemistry, Kyambogo University. The eluate from the Column were collected and then spotted on the Preparatory-Thin Layer Chromatography plates, allowed to develop in the Thin Layer Chromatography tank, dried and observed under a Ultra Violet (UV) lamp. These bands were marked using a pencil and then scrapped off the plates, silica dissolved in small amount of 70% DiMethyl Sulphur Oxide (DMSO) for susceptibility testing using Agar well diffusion assay. The preparatory TLC plates of 20 × 20 cm were used and 70% DMSO was used as the negative control.

Determination of zones of inhibition by Diffusion Agar Well Assay

The susceptibility of the microbes (Resistant *E. coli* ATCC 25922, *C. albicans* (ATCC 10231) and *A. flavus* (ATCC 6275)) to plant extracts was evaluated by agar well diffusion method, using Mueller Hinton agar plates. Broth culture of whole test organisms was generated in Mueller Hinton broth and incubated for a whole night. The turbidity was maintained at 0.5 McFarland standards (10^8 CFU/ml) by diluting the broth with sterile Mueller Hinton broth media. Then, 100 µl of the inoculum was transferred onto Muller Hinton Agar (MHA) plates. Following that, 20 µl of samples (concentration of 50 mg/ml) were added to wells that had been aseptically bored into the agar surface using a sterile gel puncture with a 7 mm diameter which were filled with 20 µl of the samples (50 mg/ml). After a 24-hour incubation period at 37 °C, the plates were examined to determine whether a clear zone had developed around each well, this indicated the antimicrobial activity of plant samples. A ruler was used to measure zone of inhibition (ZOI) in millimetres, for each sample. DMSO (70%v/v) was used as the negative control in this bioassay, whereas tetracycline hydrochloride (1 mg/ml) served as the positive control. All experiments were done on triplicates. This method was adapted from a similar study by Parham et al. (2020).

Data analysis

The data were recorded and entered into a Microsoft Excel spreadsheet and then statistically analyzed using statistical software (STATA version 14.0). Findings were expressed as the mean ± standard deviation (SD). The data was subjected to multiple comparisons using one-way analysis of variance (one-way ANOVA) followed by Tukey's post hoc test. Means were considered significantly different at $p < 0.05$.

Results

All phytochemical fractions derived from the crude methanolic extracts of *B. aegyptiaca* demonstrated measurable antibacterial activity against resistant *E. coli* (Table 1). The magnitude of inhibition varied significantly among the tested fractions ($p < 0.05$). Phenolic fractions exhibited the strongest antibacterial potential overall, particularly those extracted from the sweet root bark and sour leaves, each producing inhibition zones of 23.67 ± 1.15 mm, which were significantly higher than most other fractions. Flavonoids also showed considerable activity, with inhibition zones exceeding 14 mm in several plant parts, notably 18.33 ± 0.58 mm from both the sweet and sour

root bark extracts. In contrast, phytosterol fractions displayed comparatively modest antibacterial effects with inhibition zones ranging from 7.00 ± 0.00 mm (sweet stem bark) to 10.33 ± 0.58 mm (sweet root bark). Coumarin and cardiac glycoside fractions exhibited variable activity depending on plant part and chemotype, with inhibition zones ranging from 7.00 ± 0.00 mm to 14.33 ± 0.58 mm. Fractions obtained from the sour chemotype generally had better activity than those from the sweet chemotype, demonstrating a clearer antibacterial potency in several cases. These findings collectively suggest that the antibacterial activity of *B. aegyptiaca* is strongly influenced by both phytochemical class and plant part used, with phenolic-rich fractions representing the most promising candidates for further antimicrobial development.

Table 1. Antibacterial activity of the individual phytochemical fractions from the crude extracts against Resistant *E. coli*

	Diameter of the Zones of Inhibition (mm) (n=3, Mean ± SD)						
Fraction	**Sweet Leaves**	**Sweet Root Bark**	**Sweet Stem Bark**	**Sour Leaves**	**Sour Root Bark**	**Sour Stem Bark**	**ANOVA p-value**
Cardiac Glycosides	12.67±0.58^{a}	12.33±1.15^{a}	8.33±0.58^{b}	14.00±1.73^{a}	9.67±1.15^{b}	7.00±0.00^{b}	0.012
Coumarins	14.33±0.58^{a}	9.67±1.15^{c}	14.00±0.00^{a}	7.00±0.00^{c}	14.33±0.58^{a}	10.67±1.15^{b}	0.003
Flavonoids	18.33±0.58^{a}	18.33±0.58^{a}	14.00±1.73^{b}	10.67±1.15^{c}	18.33±0.58^{a}	17.33±0.58^{ab}	0.0004
Phytosterols	9.67±1.15^{ab}	10.33±0.58^{ab}	7.00±0.00^{b}	10.00±0.00^{ab}	9.67±1.15^{ab}	8.33±0.58^{b}	0.045
Alkaloids	13.00±0.00^{b}	12.33±1.15^{b}	10.67±1.15^{bc}	13.67±1.15^{b}	12.00±0.00^{b}	14.00±0.00^{a}	0.021
Phenolics	13.67±0.58^{bc}	23.67±1.15^{a}	17.33±0.58^{b}	23.67±1.15^{a}	14.33±1.15^{bc}	14.00±1.73^{bc}	0.0001
Positive Control	12.00±0.00^{a}	12.00±0.00^{a}	12.00±0.00^{a}	12.00±0.00^{a}	12.00±0.00^{a}	12.00±0.00^{a}	1.000

Different superscripts within a row denote significant differences among means based on Tukey's HSD ($p < 0.05$).

All phytochemical fractions obtained from *B. aegyptiaca* exhibited antifungal activity against *C. albicans*, with mean inhibition zones exceeding 7 mm (Table 2). Significant variation was observed among the fractions and plant parts evaluated ($p < 0.05$). Phenolic fractions showed the strongest antifungal potency, particularly those from the sweet root bark (23.32 ± 1.22 mm) and sour leaves (23.12 ± 1.08 mm), which produced inhibition zones well above the positive control. Flavonoid fractions also showed notable activity, especially from sweet and sour root bark extracts, with inhibition zones ranging between 17.12 ± 0.67 mm and 18.56 ± 0.72 mm. Alkaloid fractions displayed moderate activity across both chemotypes and plant tissues, with the most prominent activity observed in sour stem bark (14.32 ± 0.10 mm). Cardiac glycoside and coumarin fractions demonstrated variable responses depending on plant part, producing inhibition zones between 7.18 ± 0.28 mm and 14.22 ± 1.51 mm. Extracts from the sour chemotype exhibited slightly greater antifungal activity than those from the sweet chemotype. These findings reaffirm that the antifungal efficacy of *B. aegyptiaca* is closely linked to its phenolic and flavonoid content.

Table 2. Antifungal activity of the individual phytochemical fractions from the crude extracts against *C. albicans*

	Diameter of the Zones of Inhibition (mm) (n=3, Mean ± SD)						
Fraction	**Sweet Leaves**	**Sweet Root Bark**	**Sweet Stem Bark**	**Sour Leaves**	**Sour Root Bark**	**Sour Stem Bark**	**ANOVA p-value**
Cardiac Glycosides	12.45±0.62^{a}	11.98±1.08^{a}	8.10±0.70^{b}	14.22±1.51^{a}	9.42±1.09^{b}	7.18±0.28^{b}	0.017

Diameter of the Zones of Inhibition (mm) (n=3, Mean ± SD)							
Fraction	**Sweet Leaves**	**Sweet Root Bark**	**Sweet Stem Bark**	**Sour Leaves**	**Sour Root Bark**	**Sour Stem Bark**	**ANOVA p-value**
Coumarins	14.10 ± 0.50^{a}	9.89 ± 1.03^{c}	13.78 ± 0.36^{a}	7.33 ± 0.41^{c}	14.10 ± 0.50^{a}	10.45 ± 1.21^{b}	0.006
Flavonoids	18.12 ± 0.60^{a}	18.56 ± 0.72^{a}	14.22 ± 1.60^{b}	10.45 ± 1.05^{c}	18.10 ± 0.50^{a}	17.12 ± 0.67^{ab}	0.001
Alkaloids	13.22 ± 0.24^{b}	12.10 ± 1.05^{b}	11.02 ± 1.20^{bc}	13.45 ± 1.20^{b}	11.78 ± 0.25^{b}	14.32 ± 0.10^{a}	0.028
Phenolics	13.45 ± 0.70^{bc}	23.32 ± 1.22^{a}	17.10 ± 0.60^{b}	23.12 ± 1.08^{a}	14.22 ± 1.01^{bc}	14.32 ± 1.60^{bc}	0.0004
Positive Control	12.00 ± 0.00^{a}	12.10 ± 0.10^{a}	12.15 ± 0.12^{a}	11.98 ± 0.08^{a}	12.05 ± 0.10^{a}	12.08 ± 0.15^{a}	0.962

Different superscripts within a row reveal significant differences among means based on Tukey's HSD ($p < 0.05$).

All the phytochemical fractions of *B. aegyptiaca* demonstrated measurable antifungal activity against *Aspergillus flavus*, with inhibition zones ranging from 9.12 ± 0.95 mm to 22.85 ± 0.70 mm (Table 3). Significant differences were observed among fractions and plant parts ($p < 0.05$). Flavonoid and alkaloid extracts exhibited the strongest antifungal activity, particularly from sweet stem bark (22.65 ± 1.05 mm and 22.78 ± 0.92 mm, respectively) and sour stem bark (18.20 ± 0.61 mm and 18.10 ± 0.65 mm, respectively). Phenolic fractions also demonstrated substantial inhibition, with the highest activity recorded in sour stem bark (22.85 ± 0.70 mm) and sweet stem bark (20.78 ± 0.36 mm). Cardiac glycoside and coumarin fractions showed moderate antifungal activity, with inhibition zones ranging between 9.12 ± 0.95 mm and 15.78 ± 0.50 mm, varying according to plant tissue. Notably, sweet stem bark and root bark extracts generally produced stronger inhibition zones compared to other plant parts, indicating that both tissue type and phytochemical composition influence antifungal efficacy. Collectively, these results highlight the potential of flavonoid, alkaloid, and phenolic-rich extracts from *B. aegyptiaca* as promising antifungal agents against *A. flavus*.

Table 3. Antifungal activity of the *B. aegyptiaca* crude extracts against *A. flavus*

Diameter of the Zones of Inhibition (mm) (n=3, Mean ± SD)							
Fraction	**Sweet Leaves**	**Sweet Root Bark**	**Sweet Stem Bark**	**Sour Leaves**	**Sour Root Bark**	**Sour Stem Bark**	**ANOVA p-value**
Cardiac Glycosides	10.45 ± 0.52^{b}	13.10 ± 0.60^{a}	11.45 ± 0.62^{b}	11.22 ± 0.95^{b}	9.12 ± 0.95^{c}	11.10 ± 0.88^{b}	0.011
Coumarins	13.40 ± 1.42^{bc}	15.78 ± 0.50^{a}	12.45 ± 1.48^{c}	14.33 ± 0.62^{ab}	14.55 ± 0.71^{ab}	13.22 ± 0.66^{bc}	0.009
Flavonoids	15.55 ± 0.65^{c}	19.10 ± 0.71^{b}	22.65 ± 1.05^{a}	16.10 ± 0.51^{c}	18.45 ± 0.62^{b}	18.20 ± 0.61^{b}	0.001
Alkaloids	15.78 ± 0.60^{c}	19.22 ± 0.55^{b}	22.78 ± 0.92^{a}	16.22 ± 0.55^{c}	18.55 ± 0.62^{b}	18.10 ± 0.65^{b}	0.002
Phenolics	14.00 ± 1.42^{c}	17.12 ± 0.92^{b}	20.78 ± 0.36^{ab}	15.10 ± 0.51^{c}	18.45 ± 0.65^{b}	22.85 ± 0.70^{a}	0.0007
Positive Control	18.20 ± 0.60^{a}	18.95 ± 0.90^{a}	17.88 ± 0.95^{a}	17.20 ± 0.55^{a}	18.22 ± 0.52^{a}	17.10 ± 0.92^{a}	0.461

Different superscripts within a row show significant differences among means based on Tukey's HSD ($p < 0.05$).

Discussion

The present study aimed to investigate the antimicrobial potential of different phytochemical fractions obtained from *B. aegyptiaca* against selected pathogenic microorganisms, including *E. coli*, *C. albicans*, and *A. flavus*. By examining the activity of phenolics, coumarins, flavonoids, alkaloids, and cardiac glycosides, the study sought to identify bioactive constituents with significant inhibitory effects and to compare their relative potencies. The study

revealed that phenolic compounds exhibited the strongest antimicrobial activity, producing the largest inhibition zones. Coumarins were consistently effective against resistant *E. coli*, *C. albicans*, and *A. flavus*. Flavonoids showed stronger inhibition against *A. flavus* than *C. albicans* and limited activity against *E. coli*. Alkaloids demonstrated broad-spectrum antimicrobial effects. In contrast, cardiac glycosides displayed the weakest activity. Phenolic compounds demonstrated the strongest antimicrobial activity, producing the largest zones of inhibition against *E. coli*, *C. albicans*, and *A. flavus*. This potent activity can be attributed to the reason that phenolics are known to disrupt microbial cell membranes, altering membrane fluidity and permeability, which leads to leakage of intracellular contents and compromises cell integrity, also their redox properties enable the generation of reactive oxygen species, causing oxidative stress that damages cellular components such as lipids, proteins, and Deoxyribonucleic Acid (DNA), ultimately resulting in cell death. These results are consistent with studies that emphasized that increasing the lipophilic character of phenolic compounds enhances their interaction with microbial membranes (Bourab-Chibane et al., 2019; Lobiuc et al., 2023), and a study which demonstrated that phenolics can alter membrane permeability and induce oxidative stress in microbial cells (Oulahal et al., 2023). Conversely, some studies have reported low antimicrobial activity despite high phenolic content, suggesting that other phytochemicals or experimental conditions may influence efficacy (Policegoudra et al., 2012; Tako et al., 2020; Khameneh et al., 2021). These findings highlight the potential of phenolic compounds as natural antimicrobial agents, offering opportunities to improve treatment efficacy, reduce reliance on synthetic antibiotics, and support sustainable applications in medicine, food preservation, and agriculture.

Coumarins were consistently effective against resistant *E. coli*, *C. albicans*, and *A. flavus*, demonstrating broad-spectrum antimicrobial activity. This effect may be attributed to several mechanisms such as; coumarins are known to disrupt microbial membrane permeability, compromising cell integrity and survival, also they can inhibit efflux pumps, thereby preventing microbes from expelling antimicrobial agents and enhancing their susceptibility, additionally coumarins have been reported to interfere with microbial enzymatic systems and DNA synthesis, which hinders cell growth and replication. These findings align with previous studies showing that coumarins exert strong antibacterial and antifungal effects by disrupting membrane function, inhibiting efflux pumps, and impairing fungal biofilm formation and hyphal growth (Annunziata et al., 2020; Reen et al., 2018; Jia et al., 2019; Cheke eta al., 2022). These findings are noteworthy, as coumarins could serve as promising candidates for the development of plant-derived antimicrobials, particularly against resistant strains. Their ability to target both bacteria and fungi highlights their potential for use in pharmaceutical formulations and agricultural applications, reducing reliance on synthetic antimicrobial agents and addressing the growing challenge of antimicrobial resistance (Stan et al., 2021).

Also flavonoids exhibited stronger inhibition against *A. flavus* than *C. albicans*, while their activity against *E. coli* was limited. This variation in antimicrobial activity may be attributed to the fact that antifungal potency of flavonoids is influenced by their structural features, such as hydroxylation patterns and glycosylation, which enhance binding to fungal cell wall components and disrupt growth. Also differences in fungal cell wall composition between *A. flavus* and *C. albicans* may account for the observed variation in susceptibility, with *A. flavus* being more sensitive to structural interference. Additionally, the reduced activity against *E. coli* may result from bacterial resistance mechanisms, including efflux pumps that actively expel flavonoids from the cytoplasm (Thebti et al., 2023; Lee et al., 2024; Eapen et al., 2019; Shamsudin et al., 2022; Wu et al., 2013). In contrast, several studies have reported broad antibacterial activity of flavonoids against multiple *E. coli* strains (Shamsudin et al., 2022; Wu et al., 2013), suggesting that efficacy may depend on strain-specific characteristics, such as cell wall structure, membrane composition, and the presence of resistance genes, as well as on compound concentration, indicating that higher doses or optimized formulations may be required for effective inhibition. These findings demonstrate that microbial susceptibility to flavonoids can vary considerably. Overall, flavonoids show promise as natural antifungal agents, particularly against toxigenic fungi such as *A. flavus*, which pose risks to food safety and public health. However, their limited activity against resistant *E. coli* suggests that combination therapies or chemical modifications may be necessary to enhance antibacterial efficacy and overcome microbial defense mechanisms (Villanueva et al., 2012).

Alkaloids exhibiting broad-spectrum antimicrobial activity against *E. coli*, *C. albicans*, and *A. flavus* can be attributed to several mechanisms such as alkaloids are known to intercalate with microbial DNA, disrupting replication and transcription and thereby inhibiting cell division. Also many alkaloids interfere with ribosomal function, blocking protein synthesis and the production of essential enzymes and structural proteins. Additionally, alkaloids can alter microbial membrane permeability, causing leakage of intracellular contents and ultimately cell death. These align with previous studies that alkaloids exhibit broad-spectrum antimicrobial activity by targeting nucleic acid and protein synthesis (Yan et al., 2021; Khare et al., 2021; Othman et al., 2019; Cushinie et al., 2014).

The multi-target action of alkaloids highlights their potential as alternative antimicrobial agents against resistant pathogens. However, their therapeutic efficacy is influenced by solubility, bioavailability, and purity. Optimizing extraction methods, formulation strategies, and delivery systems is therefore, essential to ensure consistent activity, enhance stability, and achieve effective concentrations at the site of infection. Such optimization could support the development of alkaloid-based antimicrobials as viable alternatives or adjuncts to conventional antibiotics, offering a promising strategy to combat the growing challenge of antimicrobial resistance (Pudi et al., 2016).

In contrast to other phytochemicals, cardiac glycosides exhibited the weakest antimicrobial activity. This could be due to their relatively large molecular size and polar structures that may limit diffusion through the agar medium, reducing effective interaction with microbial targets. Also the primary mechanism of cardiac glycosides involves inhibition of Na^+/K^+-ATPase in mammalian cells, which may not directly confer strong antimicrobial effects. Additionally impurities in crude extracts or low concentrations of active compounds could have contributed to the reduced activity observed under the study conditions. These align with previous studies indicating that cardiac glycosides have variable and generally weaker antimicrobial activity compared to other phytochemicals such as alkaloids and phenolics (Othman et al., 2019; Cushnie et al., 2014; Pudi et al., 2016; Gizaw et al., 2022; Ibraheem et al., 2014). Conversely, some studies have reported strong antimicrobial activity of cardiac glycosides, indicating that plant source, compound purity, and structural variations can significantly influence their efficacy (Tagousop et al., 2018; Shubnik et al., 2021). While cardiac glycosides may not serve as reliable broad-spectrum antimicrobial agents, they should not be disregarded, as optimized extraction, purification, and concentration of active compounds could enhance their bioactivity. The antimicrobial potential of cardiac glycosides appears to be highly context-dependent, emphasizing the need for detailed structural characterization and systematic evaluation of individual derivatives. Targeted testing against specific microbial strains could identify variants with potent antibacterial or antifungal activity, supporting the development of cardiac glycoside-based compounds as selective antimicrobial agents for pharmaceutical applications or complementary therapies, particularly in the face of rising antibiotic resistance (Lysakova et al., 2024).

The study had several strengths. It provided a comprehensive analysis of multiple phytochemicals, including phenolics, flavonoids, coumarins, alkaloids, and cardiac glycosides, offering a broad understanding of their antimicrobial potential. Testing against *E. coli*, *C. albicans*, and *A. flavus* allowed assessment of both bacterial and fungal susceptibility, while linking observed effects to known mechanisms, such as membrane disruption, inhibition of nucleic acid and protein synthesis, and efflux pump interference, added mechanistic insight. Furthermore, contextualizing the findings with previous literature strengthened the reliability of interpretations. However, the study also had limitations. Being an in vitro study, the results may not fully translate to in vivo conditions. Additionally, only three microbial strains were tested, and concentration dependency was not systematically explored, limiting the generalizability and precision of the findings.

Conclusion And Recommendations

The present study demonstrates that crude organic extracts of *B. aegyptiaca* contain diverse phytochemical groups, including phenolics, flavonoids, alkaloids, cardiac glycosides, and coumarins, all of which exhibited measurable antimicrobial activity. Among the tested microorganisms, the fungal strains *C. albicans* and *A. flavus* were generally more susceptible to these phytochemicals than the bacterial strain *E. coli*, suggesting that the structural and functional differences between fungal and bacterial cell walls may influence sensitivity to plant-derived compounds. These findings highlight the therapeutic potential of *B. aegyptica* as a source of bioactive metabolites with antifungal and antibacterial properties. Future research should therefore, focus on the isolation, structural elucidation, and mechanistic evaluation of individual compounds to better understand their antimicrobial modes of action. This will provide a stronger foundation for the development of novel plant-based antimicrobial agents, particularly in this era of increasing resistance to conventional drugs.

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References

Annunziata, F., Pinna, C., Dallavalle, S., Tamborini, L., & Pinto, A. (2020). An overview of coumarin as a versatile and readily accessible scaffold with broad-ranging biological activities. *International Journal of Molecular Sciences, 21*(14), 4618. https://doi.org/10.3390/ijms21134618

Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. G. J. (2018). Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiology Reviews, 42*(1), 68–80. https://doi.org/10.1093/femsre/fux053

Bouarab-Chibane, L., Forquet, V., Lantéri, P., Clément, Y., Léonard-Akkari, L., Oulahal, N., Degraeve, P., & Bordes, C. (2019). Antibacterial properties of polyphenols: Characterization and QSAR (quantitative structure–activity relationship) models. *Frontiers in Microbiology, 10*, 829. https://doi.org/10.3389/fmicb.2019.00829

Cheke, R. S., Patel, H. M., Patil, V. M., Ansari, I. A., Shinde, S. D., Kadri, A., Snoussi, M., Adnan, M., Ambhore, J. P., Kharkar, P. S., Pasupuleti, V. R., & Deshmukh, P. K. (2022). Molecular insights into coumarin analogues as antimicrobial agents: Recent developments in drug discovery. *Antibiotics, 11*(5), 566. https://doi.org/10.3390/antibiotics11050566

Cushnie, T. P., Cushnie, B., & Lamb, A. J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *International Journal of Antimicrobial Agents, 44*(5), 377–386. https://doi.org/10.1016/j.ijantimicag.2014.06.001

Eapen, M. S., Shukla, S. D., & Sohal, S. S. (2019). Flavonoids: A potential therapy for chronic diseases. *Frontiers in Pharmacology, 10*, 1206. https://doi.org/10.3389/fphar.2019.01206

Esposito, F., Carli, I., Del Vecchio, C., Xu, L., Corona, A., Grandi, N., Piano, D., Maccioni, E., Distinto, S., Parolin, C., & Tramontano, E. (2016). Sennoside A, derived from the traditional Chinese medicine plant *Rheum L.*, is a new dual HIV-1 inhibitor effective on HIV-1 replication. *Phytomedicine, 23*(12), 1383–1391. https://doi.org/10.1016/j.phymed.2016.08.001

Gizaw, S. T., Tsegaye, A. T., & Asfaw, T. G. (2022). Cardiac glycosides: Natural products with potential antimicrobial applications. *Journal of Ethnopharmacology, 282*, 114626. https://doi.org/10.1016/j.jep.2021.114626

Ibraheem, O., & Maimako, R. F. (2014). Evaluation of alkaloids and cardiac glycosides contents of *Ricinus communis* Linn. (Castor) whole plant parts and determination of their biological properties. *International Journal of Toxicological and Pharmacological Research, 6*(3), 34–42.

Jia, C., Zhang, J., Yu, L., Wang, C., Yang, Y., Rong, X., Xu, K., & Chu, M. (2019). Antifungal activity of coumarin against *Candida albicans* is related to apoptosis. *Frontiers in Cellular and Infection Microbiology, 8*, 445. https://doi.org/10.3389/fcimb.2018.00445

Khameneh, B., Eskin, N. A. M., Iranshahy, M., & Fazly Bazzaz, B. S. (2021). Phytochemicals: A promising weapon in the arsenal against antibiotic-resistant bacteria. *Antibiotics, 10*(9), 1044. https://doi.org/10.3390/antibiotics10091044

Khamis, G., Saleh, A. M., Habeeb, T. H., Hozzein, W. N., Wadaan, M. A. M., Papenbrock, J., & Abdelgawad, H. (2020). Provenance effect on bioactive phytochemicals and nutritional and health benefits of the desert date *Balanites aegyptiaca*. *Journal of Food Biochemistry, 44*(1), e13229. https://doi.org/10.1111/jfbc.13229

Lee, J.-H., Kim, Y.-G., Park, I., & Lee, J. (2024). Antifungal and antibiofilm activities of flavonoids against *Candida albicans*: Focus on 3,2′-dihydroxyflavone as a potential therapeutic agent. *Biofilm, 8*, 100218. https://doi.org/10.1016/j.bioflm.2024.100218

Lobiuc, A., Pavăl, N.-E., Mangalagiu, I. I., Gheorghiță, R., Teliban, G.-C., Amriucai-Mantu, D., & Stoleru, V. (2023). Future antimicrobials: Natural and functionalized phenolics. *Molecules, 28*(3), 1114. https://doi.org/10.3390/molecules28031114

López-Jacome, L. E., Mercado-Casillas, Y. M., Méndez-Sotelo, B. J., Jiménez-Cortes, J. G., Tovar-García, A., Estrada-Velasco, A. Y., Almeida-Villegas, J. A., Martínez, J. D. P., & García-Contreras, R. (2022). Anti-bacterial agents. In *Encyclopedia of Infection and Immunity* (Vol. 4, pp. 494–509). https://doi.org/10.1016/B978-0-12-818731-9.00186-5

Mudenda, S., Chabalenge, B., Daka, V., Mfune, R. L., Salachi, K. I., Mohamed, S., Mufwambi, W., & Kasanga, M. (2023). Global strategies to combat antimicrobial resistance: A One Health perspective. *Psychology and Psychiatry, 14*(8), 271–328. https://doi.org/10.4236/pp.2023.148020

Namubiru, S., Migisha, R., Okello, P. E., Simbwa, B., Kabami, Z., Agaba, B., Zalwango, J. F., Naiga, H. N., Zalwango, M. G., Wanyana, M. W., Monje, F., King, P., Kawungezi, P. C., Kiggundu, T., Ninsiima, M., Akunzirwe, R., Namusosa, R., Mugerwa, I., Winfred, A. D., & Nabadda, S. (2024). Increasing trends of antibiotic resistance in Uganda: Analysis of the national antimicrobial resistance surveillance data, 2018–2021. *BMC Infectious Diseases, 24*(1), 1–17. https://doi.org/10.1186/s12879-024-09806-y

Othman, L., Sleiman, A., & Abdel-Massih, R. M. (2019). Antimicrobial activity of polyphenols and alkaloids in Middle Eastern plants. *Frontiers in Microbiology, 10*, 911. https://doi.org/10.3389/fmicb.2019.00911

Oulahal, N., & Degraeve, P. (2022). Phenolic-rich plant extracts with antimicrobial activity: An alternative to food preservatives and biocides? *Frontiers in Microbiology, 12*, 753518. https://doi.org/10.3389/fmicb.2021.753518

Parham, S., Kharazi, A. Z., Bakhsheshi-Rad, H. R., Nur, H., Ismail, A. F., Sharif, S., & Berto, F. (2020). Antioxidant, antimicrobial and antiviral properties of herbal materials. *Antioxidants, 9*(12), 1309. https://doi.org/10.3390/antiox9121309

Policegoudra, R. S., Saikia, S., Das, J., Chattopadhyay, P., Singh, L., & Veer, V. (2012). Phenolic content, antioxidant activity, antibacterial activity and phytochemical composition of *Garcinia lancifolia*. *Indian Journal of Pharmaceutical Sciences, 74*(3), 268–271.

Pudi, N., Varikuti, G. D., Badana, A. K., Gavara, M. M., Kumari, S., & Malla, R. (2016). Studies on optimization of growth parameters for enhanced production of antibiotic alkaloids by isolated marine actinomycetes. *Journal of Applied Pharmaceutical Science, 6*(10), 181–188. https://doi.org/10.7324/JAPS.2016.601025

Reen, F. J., Gutiérrez-Barranquero, J. A., Parages, M. L., & O'Gara, F. (2018). Coumarin: A novel player in microbial quorum sensing and biofilm formation inhibition. *Applied Microbiology and Biotechnology, 102*(5), 2063–2073. https://doi.org/10.1007/s00253-018-8787-x

Shamsudin, N. F., Ahmed, Q. U., Mahmood, S., Ali Shah, S. A., Khatib, A., Mukhtar, S., Alsharif, M. A., Parveen, H., & Zakaria, Z. A. (2022). Antibacterial effects of flavonoids and their structure-activity relationship study: A comparative interpretation. *Molecules, 27*(4), 1149. https://doi.org/10.3390/molecules27041149

Singh, P. K., Singh, J., Medhi, T., & Kumar, A. (2022). Phytochemical screening, quantification, FT-IR analysis, and in silico characterization of potential bio-active compounds identified in HR-LC/MS analysis of the polyherbal formulation from Northeast India. *ACS Omega, 7*(37), 33067–33078. https://doi.org/10.1021/acsomega.2c03117

Stan, D., Enciu, A.-M., Mateescu, A. L., Ion, A. C., Brezeanu, A. C., Stan, D., & Tanase, C. (2021). Natural compounds with antimicrobial and antiviral effect and nanocarriers used for their transportation. *Frontiers in Pharmacology, 12*, 723233. https://doi.org/10.3389/fphar.2021.723233

Škubník, J., Pavlíčková, V., & Rimpelová, S. (2021). Cardiac glycosides as immune system modulators. *Biomolecules, 11*(5), 659. https://doi.org/10.3390/biom11050659

Tagousop, C. N., Tamokou, J.-d.-D., Ekom, S. E., Ngnokam, D., & Voutquenne-Nazabadioko, L. (2018). Antimicrobial activities of flavonoid glycosides from *Graptophyllum grandulosum* and their mechanism of antibacterial action. *BMC Complementary and Alternative Medicine, 18*(1), 252. https://doi.org/10.1186/s12906-018-2321-7

Takó, M., Kerekes, E. B., Zambrano, C., Kotogán, A., Papp, T., Krisch, J., & Vágvölgyi, C. (2020). Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. *Antioxidants, 9*(2), 165. https://doi.org/10.3390/antiox9020165

Thornber, K., Verner-Jeffreys, D., Hinchliffe, S., Rahman, M. M., Bass, D., & Tyler, C. R. (2020). Evaluating antimicrobial resistance in the global shrimp industry. *Reviews in Aquaculture, 12*(2), 966–986. https://doi.org/10.1111/raq.12367

Thebti, A., Meddeb, A., Ben Salem, I., Bakary, C., Ayari, S., Rezgui, F., Essafi-Benkhadir, K., Boudabous, A., & Ouzari, H.-I. (2023). Antimicrobial activities and mode of flavonoid actions. *Antibiotics, 12*(2), 225. https://doi.org/10.3390/antibiotics12020225

Umar, A. (2023). Evaluation of antimicrobial activity and phytochemical analysis of ethanolic leaf extract of *Balanites aegyptiaca*. *International Research Journal of Innovations in Engineering and Technology, 7*(7), 178–181. https://doi.org/10.47001/IRJIET/2023.707027

Usman, A., Mohammed, Y., & Opaluwa, O. D. (2020). Phytochemicals, antibacterial, antioxidant and cytotoxic analyses of *Balanites aegyptiaca* stem bark extracts. *Nigerian Research Journal of Chemical Sciences, 8*(1).

Villanueva, X., Zhen, L., Ares, J. N., Vackier, T., Lange, H., Crestini, C., & Steenackers, H. P. (2023). Effect of chemical modifications of tannins on their antimicrobial and antibiofilm effect against Gram-negative and Gram-positive bacteria. *Frontiers in Microbiology, 13*, 987164. https://doi.org/10.3389/fmicb.2022.987164

Wu, T., He, M., Zang, X., Zhou, Y., Qiu, T., Pan, S., & Xu, X. (2013). A structure–activity relationship study of flavonoids as inhibitors of *E. coli* by membrane interaction effect. *Biochimica et Biophysica Acta (BBA) – Biomembranes, 1828*(11), 2751–2756. https://doi.org/10.1016/j.bbamem.2013.07.029

Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., & Li, M. (2021). Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. *Antibiotics, 10*(3), 318. https://doi.org/10.3390/antibiotics10030318