



# ***In-vitro* Antibacterial and Antifungal Activities of the Stem Bark Extract and Fractions of *Warbugia. ugadensis*, *Prunus africana* and *Albizia gummifera* from Mt Kenya and Elgon Regions in Kenya**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

## **Article Information**

DOI: <https://doi.org/10.9734/jamb/2025/v25i3909>

## **Open Peer Review History:**

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/131409>

**Original Research Article**

**Received: 17/12/2024**  
**Accepted: 21/02/2025**  
**Published: 24/02/2025**

## **ABSTRACT**

Fungal and bacterial infections pose a major global public health challenge due to rising antimicrobial resistance. Over 50% of hospital infections now involve pathogens resistant to multiple drugs, contributing to higher death rates, longer patient illness, and increased/prolonged hospital

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**Cite as:** Holland, Shoso Luvembe, Alphonse Wafula Wanyonyi, and Henry Mwangi. 2025. "In-Vitro Antibacterial and Antifungal Activities of the Stem Bark Extract and Fractions of *Warbugia. Ugadensis*, *Prunus Africana* and *Albizia Gummifera* from Mt Kenya and Elgon Regions in Kenya". *Journal of Advances in Microbiology* 25 (3):73-80. <https://doi.org/10.9734/jamb/2025/v25i3909>.

stays. Exploration of medicinal plants' phytochemical properties provides one avenue for developing new treatment options outside the traditional drug development process to address the intensifying antimicrobial resistance crisis. The plant materials were subjected to cold maceration followed by liquid-liquid partitioning using hexane, dichloromethane (DCM), and ethyl acetate in order of increasing polarity. The derived methanol extracts were screened for phytochemicals using standard procedures. The disk diffusion method evaluated the antimicrobial activity of 50mg/mL of *W. ugandensis*, *P. africana*, and *A. gummifera* against *S. aureus*, *E. coli*, and *C. albicans*. The study qualitatively and quantitatively assessed antibacterial and antifungal activities by observing inhibition zones and determining minimum inhibitory concentration (MIC) values. Several bioactive compounds were detected in the methanolic extract of these plants such as saponins, alkaloids, terpenoids, tannins, flavonoids, phenols, and glycosides. Preliminary disk diffusion screening revealed that *W. ugandensis* DCM extract exhibited the strongest antimicrobial activity against *C. albicans*, *E. coli*, and *S. aureus*, with mean inhibition zones of 21.0, 10.3, and 15.7mm respectively. MIC analyses with 96-well microliter plate assays confirmed *S. aureus* as the most susceptible and *E. coli* as the least vulnerable to the extracts. Tukey's Multiple Comparison Test ( $P < 0.05$ ) demonstrated significant differences in antimicrobial activity among *A. gummifera*, *W. ugandensis*, and *P. africana*. The inhibition of the various plant extracts against the tested microorganisms indicates that further study could reveal their potential use in treating infections caused by these microorganisms. Additional research is required to fully elucidate their therapeutic potential and explore practical applications for combating microbial infections.

**Keywords:** *Warbugia ugandensis*; *Prunus africana*; *Albizia gummifera*; *Candida albicans*; *Escherichia coli*; *Staphylococcus aureus*.

## 1. INTRODUCTION

Globally, fungal and bacterial infections are the leading causes of illness and death largely due to the rise of drug-resistant microorganisms (Asmerom et al., 2020). The World Health Organization (WHO, 2019) estimates that by 2030, drug-resistant microorganisms will be the primary cause of hospital-acquired infections and other infectious diseases. Currently, resistant microorganisms account for more than half of nosocomial infections leading to higher mortality, morbidity, and poorer clinical outcomes. This has also significantly increased healthcare costs due to extended hospital stays and frequent readmissions. (Gupta and Birdi 2017).

For centuries, people have utilized medicinal plants for their therapeutic properties, with traditional healers leveraging natural compounds to treat various ailments. Recent studies indicate that a significant portion of the global population now relies on medicinal plants as a treatment (Ekor, 2014). This highlights plants as an alternative source of active medicinal compounds. The discovery of antimicrobial compounds with activity against drug-resistant bacteria and fungi has sparked scientific interest in exploring medicinal plants as a source of novel medicines and therapies (Vaou, 2021).

*Warbugia ugandensis* (Canellaceae) is a growing evergreen tree between 4.5 m and 35 m tall.

Traditional healers have used the leaves and the stem bark to treat ailments such as fever, stomach pain, coughs, colds, and constipation (Maroyi, 2014). Scientific studies have shown that *Warbugia Ugandensis* possesses antileishmania, antifungal, antioxidant, and anti-inflammatory activities (Frum et al., 2006; Ng'ang'a et al., 2009). Phytochemical tests in laboratories revealed the presence of flavonoids, sugar alcohols, phenolics, alkaloids, fatty acid derivatives, and terpenoids. *Prunus Africana* (Rosaceae) grows over 40m tall with open branches. Healers use various parts of the plant to treat prostate cancer, chest pain, fevers, mental illness, hypertension, malaria, infertility, and kidney disease (Steenkamp, 2003; Jimu, 2011; Komakech et al., 2019). Pharmacological investigations indicate its antiviral, anti-inflammatory, and anticancer properties, containing pentacyclic triterpenoids like ursolic and oleanolic acids (Nyamai et al., 2015). *Albizia gummifera* (Mimosaceae) is a flat umbrella-like crown tree with a smooth grey bark that grows up to 25m in height. It thrives in humid and sub-humid areas, forest edges, and riverine forests. Traditionally, people have used it to treat gastric pain, sleep disorders, malaria, and diarrhea (Thuo et al., 2017). Studies have shown that *Albizia gummifera* exhibits antiviral, antimalarial, antitumor, and antiplatelet aggregation activities (Kokila et al, 2013). It contains alkaloids, saponins, terpenes, and flavonoids (Thuo et al.,

2017). This study investigates the phytochemicals and the *in-vitro* antibacterial and antifungal activities of some extracts of these plants against two bacterial strains (*Staphylococcus aureus* and *Escherichia coli*) and the fungus *Candida albicans*.

## 2. MATERIALS AND METHODS

### 2.1 Plant Identification and Collection

The stem barks of these plants were obtained from Chesikaki, Sirisia, Makhonge, and various areas around the slopes of Mt Elgon in Bungoma County as well as the Mt Kenya region in Tharaka Nithi and Meru counties. Botanical identification was performed at the National Museum, and voucher specimens (AWW-JKUATBH/Ag/006/2022, AWW-JKUATBH/Pa/007/2022, and AWW-JKUATBH/Wu/008/2022) kept in the Department of Plant Sciences at Kenyatta University.

### 2.2 Crude Extract Preparation and Sequential Solvent-Solvent Partitioning

The stem barks were cut into small pieces and air-dried at room temperature for three weeks. The dried samples were ground using an electric mill for extraction. Sample preparation and extraction followed the procedure described by Otieno (2016) with slight modifications. The ground powder weighed, soaked in methanol, and allowed to stand for 48 hours with intermittent shaking. The mixture was filtered through Whatman No. 1 paper using a Buchner funnel and concentrated with a Buchi Rotavapor R-200 to obtain the crude extract. Solvent-solvent partitioning of the crude extract performed using n-hexane, ethyl acetate, and dichloromethane in the order of increasing polarity. The fractions were stored at 4°C for further use.

### 2.3 Phytochemical Screening

Phytochemical screening of the methanol extracts was performed qualitatively using standard procedures as described by Opinde (2018), Talmale (2014), and Siddiqui et al (2009). Each plant extract was checked for the presence of saponins, alkaloids, terpenoids, tannins, flavonoids, phenols, and glycosides.

### 2.4 Treated microorganisms

The organisms used for the antimicrobial activity tests were *S. aureus* ATCC 25923 (Gram-

positive), *E. coli* ATCC 110 25922 (Gram-negative), and *C. albicans* ATCC 10231 (yeast). These were obtained from the Microbiology laboratory at Kenyatta University's Department of Microbiology.

### 2.5 Preparation of Extracts for Antimicrobial Activity

The tested extracts were prepared according to Otieno (2016). For each sample, 0.05 gram of the extract was transferred to pre-labeled sterile universal bottles. 1 mL of 5% dimethyl sulfoxide (Sigma-Aldrich) was added to each bottle and agitated using a mechanical vortex mixer. The concentration of each extract was standardized to 50 mg/mL by dissolving 50 mg in 1 mL of the 5% dimethyl sulfoxide.

### 2.6 Preparation of Antimicrobial Susceptibility Test Discs

Whatman No. 1 filter paper was punched into 6 mm diameter paper discs, which were placed in universal bottles and sterilized by autoclaving at 121°C for 15 minutes. The discs were then impregnated with 50 mg/mL stock solutions of *W. ugadensis*, *A. gummifera*, and *P. africana* by adding 20 µl of each extract using a micropipette. After absorbing the extracts, the discs were air-dried in petri dishes for 30 minutes. These were subsequently used to assess antimicrobial activity against *S. aureus*, *E. coli*, and *C. albicans*.

### 2.7 Media and Plates Preparation

Susceptibility testing was conducted using Mueller-Hinton Agar and Potato Dextrose Agar that were prepared according to Opinde, (2018). Mueller-Hinton Agar was prepared by dissolving 38 grams of agar in distilled water, while 39 grams of PDA powder was used for Potato Dextrose Agar. Both media were sterilized by autoclaving at 121°C for 15 minutes and cooled to room temperature before pouring into Petri dishes, which were stored at 2-8°C until used.

### 2.8 Preparation of Inoculum Suspensions

Inoculum suspensions of *E. coli*, *S. aureus*, and *C. albicans* were prepared using the direct colony method. (Lalitha, 2004, Abuto et al., 2016). The bacterial strains were cultured on Mueller-Hinton Agar (MHA) plates, while the fungal pathogen was cultured in Potato Dextrose Agar (PDA) plates. Selected colonies were

suspended in 0.85% saline (NaCl) and standardized to a 0.5 McFarland turbidity. All preparations were performed under sterile conditions and compared them to McFarland standards to ensure appropriate concentrations before testing.

## 2.9 Antibacterial and Antifungal Analyses of Plant Extracts

Antibacterial and antifungal activity tests were conducted using the disk diffusion method (Kirby-Bauer) as described by Otieno, (2016) with slight modification. The antimicrobial effects of the plant extracts on the test organisms were compared with standard drug discs: chloramphenicol (30 mcg) for bacteria and fluconazole (25 mcg) discs for fungi with DMSO as the negative control. For antibacterial efficacy testing, Mueller-Hinton agar plates were inoculated with *S. aureus* and *E. coli*, and sterile paper disks saturated with the plant extracts, ciprofloxacin, or DMSO were placed on the plates. The plates incubated at 37°C for 24 hours before measuring any inhibition zones. Similarly, the antifungal activity performed on Potato Dextrose Agar Plates inoculated with *C. albicans*. Sterile paper discs containing the plant extracts, and controls (fluconazole and DMSO) then placed on the plates, and then subsequently incubated at 37°C for 48 hours. Any inhibition zones observed were measured.

## 2.10 Minimum Inhibitory Concentration Analysis of Active Extracts

Before performing minimum inhibitory concentration (MIC) assay, extracts were serially diluted in two-fold. Using a micropipette, 0.1mL of dimethyl sulfoxide (DMSO) dispensed into all wells of a 96-well microtiter plate. An initial 0.1mL of 0.5g/mL extract was transferred to the first well and mixed thoroughly, yielding a 1:1 concentration. Subsequently, 0.1mL from the first well moved to the second well containing 0.1mL DMSO. The process continued across the plate, having concentrations with each successive well. The final 0.1mL from the last well discarded, ensuring uniform volumes. Serial dilutions, ranging from 1:1 to 1:256 were prepared for *S.*

*aureus*, *E. coli*, and *C. albicans* following a procedure described by Reller et al., (2009). Sterile paper disks impregnated with 20µL of the diluted extracts were placed on Muller Hinton agar and PDA plates using sterile forceps. The plates then incubated at 37°C; 24 hours for *S. aureus* and *E. coli*, and 48 hours for *C. albicans*. Zones of inhibition were measured, and MIC values were determined by correlating the minimum inhibition zone diameters with the lowest concentration showing no visible microbial growth (Abuto et al., 2016).

## 2.11 Statistical Analysis

The antibacterial and antifungal activities of the stem hexane stem bark extract of *P. Africana*, *A. gummifera* and *W. ugadensis* were analyzed using one-way analysis of variance (ANOVA) to obtain descriptive statistics, summarized as mean  $\pm$  SEM. Data analysis was performed using Minitab version 17.0 of the (Minitab Inc., 2017), ANOVA, followed by Tukey's post hoc test, used for means separation. A 95% confidence level and statistical significance of  $p \leq 0.05$  were applied. All quantitative and qualitative data are presented in tables.

## 3. RESULTS AND DISCUSSION

### 3.1 Percentage Yield of Crude Extracts

The stem bark extraction results for *W. ugadensis*, *A. gummifera* and *P. africana* are shown in Table 1. The *W. ugadensis* methanol extract demonstrated the highest recovery and yield at 126.3 grams (42.1%). The *A. gummifera* methanol extract yielded 123.11 grams (41.04%). *P. africana* had the lowest recovery with 17.25 grams (5.75%).

### 3.2 Phytochemical Screening

The results (Table 2) show that the plants contained all the tested phytochemicals, except *P. africana* which was negative for glycosides. These phytochemicals probably contributed to their antibacterial activity consistent with previous studies.

Table 1. Yield and Percentage Yield of Crude methanol extracts

Plant (weight in grams)	Crude methanol extracts obtained (% Yield)
<i>W. ugadensis</i> (300 g)	126.30 g (42.1%)
<i>A. gummifera</i> (300 g)	123.11 g (41.04%)
<i>P. africana</i> (300 g)	17.25 g (5.75%)

**Table 2. Phytochemical results**

Methanolic extract	Sap	Flav	Terp	Tan	Alka	Phen	Glyco
<i>W. ugardensis</i>	+ve	+ve	+ve	+ve	+ve	+ve	+ve
<i>P. Africana</i>	+ve	+ve	+ve	+ve	+ve	+ve	-ve
<i>A. gummifera</i>	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Key: Sap-Saponin, Flav-Flavonoid, Terp-Terpenoid, Tan-Tannins, Alka- Alkanoids, Phen- Phenols, Glyco-Glycosides

### 3.3 Bioassay Results

The potential antimicrobial activity of the plant extracts against *E. coli*, *S. aureus*, and *C. albicans* was assessed using the disc diffusion method. Testing was performed in triplicate. Results for *W. ugardensis*, *P. africana*, and *A. gummifera* are shown in Table 3.

It is observed from Table 3 that the *W. ugardensis* dichloromethane (DCM) extract exhibited an activity against all the tested microorganisms, with the highest zone of inhibition of 26mm recorded against *C. albicans*. This is consistent with previous research by Otieno (2016), who

reported that DCM extracts generally displayed greater potency than methanolic extracts, regardless of the plant part analyzed. The hexane extract was active against *S. aureus* and the fungus *C. albicans* but did not demonstrate any activity against other tested strain. *S. aureus* was more susceptible to the extracts, with the highest activity observed in the *W. ugardensis* DCM extract which had a minimum inhibitory concentration (MIC) value of 32.15mg/mL. *E. coli* was the least susceptible. The DCM and hexane extracts showed greater differences in antibacterial activity than the MeOH extract. The DCM and hexane extracts have more pronounced antibacterial activity compared

**Table 3. Antimicrobial activity and MIC<sub>(50)</sub> of *W. ugardensis*, *P. africana*, and *A. gummifera* extracts against selected pathogens**

Plant extract		Inhibition zones mm ± SEMean and (MIC)mg/mL		
		<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>
<i>Warbugia ugardensis</i>	Methanol	11.000 <sup>c</sup> ±0.577 (50)	6.000 <sup>c</sup> ± 0.000	6.000 <sup>c</sup> ±0.000
	Ethyl acetate	6.000 <sup>d</sup> ±0.000	6.000 <sup>c</sup> ± 0.000	6.000 <sup>c</sup> ± 0.000
	DCM	15.667 <sup>a</sup> ±0.333 (3.125)	10.000 <sup>a</sup> ±0.577 (25)	26.000 <sup>a</sup> ±0.577 (12.5)
	Hexane	12.000 <sup>ab</sup> ±1.000 (50)	7.000 <sup>b</sup> ±0.000 (-)	21.333 <sup>b</sup> ±0.577 (25)
<i>Prunus africana</i>	Methanol	15.333 <sup>a</sup> ±0.333 (6.25)	6.000 <sup>c</sup> ±0.000 (-)	6.000 <sup>c</sup> ±0.000 (-)
	Ethyl acetate	14.667 <sup>a</sup> ±0.333 (25)	6.000 <sup>c</sup> ±0.000	6.000 <sup>c</sup> ±0.000
	DCM	6.000 <sup>d</sup> ±0.000	6.000 <sup>c</sup> ±0.000	6.000 <sup>c</sup> ±0.000
	Hexane	6.000 <sup>d</sup> ±0.000	6.000 <sup>c</sup> ±0.000	6.000 <sup>c</sup> ±0.000
<i>Albizia gummifera</i>	Methanol	11.667 <sup>bc</sup> ±0.333 (3.225)	6.000 <sup>c</sup> ±0.000	6.000 <sup>c</sup> ± 0.000
	Ethyl acetate	12.333 <sup>bc</sup> ± 0.333 (3.225)	6.000 <sup>c</sup> ±0.000	6.000 <sup>c</sup> ±0.000
	DCM	6.333 <sup>d</sup> ± 0.33 (-)	6.000 <sup>c</sup> ±0.000	6.000 <sup>c</sup> ±0.000
	Hexane	13.667 <sup>ab</sup> ±0.333 (3.125)	6.667 <sup>bc</sup> ±0.333	6.000 <sup>c</sup> ±0.000
Negative control (5% DMSO)		6.000 <sup>d</sup> ±0.000	6.000 <sup>c</sup> ±0.000	6.000 <sup>c</sup> ±0.000

Key:

- Values are expressed as Mean ±SEM
- Inhibition zones measured in millimeters, including the 6mm diameter.
- Means in the same column with different superscripts are significantly different (P<0.05), as determined by one-way ANOVA and Tukey HSD test

to the methanol extract, likely due to differences in the antimicrobial compounds extracted. These findings suggest that DCM and hexane are effective solvents for extracting non-polar bioactive compounds from *W. ugadensis*, making them valuable for antimicrobial research targeting terpenoids and related constituents (Tiwari et al. 2011).

The extracts of *P. africana* demonstrated antibacterial activity against *S. aureus* (Table 3). Its methanol and ethyl acetate extracts exhibited significant activity against the gram-positive bacteria *S. aureus* ( $P < 0.05$ ). The methanol extract showed the highest potency, with a mean zone diameter of 15.3mm and a MIC value of 31.25mg/mL. The negative control showed results comparable to the dichloromethane (DCM) and hexane extracts ( $P > 0.05$ ). No activity observed against the gram-negative bacteria, *E. coli* or the yeast *C. albicans* for any of the tested extracts. These findings are in agreement with Bii et al., (2010) and Madivoli et al., (2018). The observed antibacterial activity against *S. aureus* in methanol (MeOH) and ethyl acetate extracts could be attributed to bioactive compounds known for their antimicrobial properties, such as flavonoids, alkaloids, or phenolic compounds. The lack of activity against *E. coli* and *C. albicans* suggests that these extracts' bioactive compounds are ineffective against these microorganisms.

*A. gummifera* extracts demonstrated antibacterial activity against *S. aureus* except the DCM extract (Table 3). The hexane extract exhibited the highest activity, with a mean zone of inhibition of 13.7mm against *S. aureus* and a minimum inhibitory concentration (MIC) value of 31.25mg/ml. Additionally, the hexane extract also showed antibacterial activity against the gram-negative bacteria, *E. coli* ( $P < 0.05$ ). However, no activity was observed against the fungus *C. albicans* ( $P > 0.05$ ). The methanol, ethyl acetate, and hexane extracts of *A. gummifera* were more effective against *S. aureus*, while the hexane extract also inhibited *E. coli* to a minor extent. These results are consistent with those reported by Yitbarek (2009) but contrast with Tesfamaryam et al. (2015), who observed that *E. coli* was highly sensitive to the methanol extract of *A. gummifera*. The differences in antibacterial activity among the extracts is attributed to variations in their chemical compositions with each solvent extracting bioactive compounds with differing potencies against *S. aureus* and possibly contributing to the observed activity against *E. coli*.

## 4. CONCLUSIONS AND RECOMMENDATIONS

### 4.1 Conclusions

Several selected plant stem bark extracts exhibited antimicrobial activity against the tested microorganisms. The antibacterial and antifungal activities of the selected plants are attributed to the presence of the important bioactive compounds present in the plants. The plant's antibacterial activity could be due to the synergistic effect of multiple compounds. These findings provide a scientific justification for the traditional use of these plants in the treatment of various diseases affecting human beings. The research therefore contributes to the scientific understanding of plant-based medicine. The results also suggest the potential for developing novel antimicrobial treatments.

### 4.2 Recommendations

Further research is needed to fully explore the therapeutic potential of the studied extracts and practical applications, including investigating synergistic effects when combined with conventional antibiotics or antifungal agents. The study bridges the gap between traditional knowledge and contemporary research, contributing to sustainable healthcare solutions rooted in Kenya's botanical heritage.

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## FUNDING

This research received funding from the Vice-Chancellor's 2019/2020 Grant, Kenyatta University, Kenya.

## ACKNOWLEDGEMENT

We express our sincere gratitude to God for the gift of life and the opportunity to complete this research project. We extend our deepest thanks to the Vice-Chancellor of Kenyatta University for the generous financial support that made this research possible. Our profound gratitude goes to our esteemed supervisors, Dr. Alphonse W.

Wanyonyi and Dr. Henry Mwangi, for their invaluable guidance, unwavering support, and insightful feedback throughout this research endeavour. We are also grateful to the dedicated staff of the Chemistry Department for their excellent technical support. Finally, we extend our heartfelt thanks to our families and friends for their unwavering support and encouragement throughout this challenging journey.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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