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Antibacterial Activity of Piliostigma Thonningii Leaf Extract Against Gram-Negative Bacteria Isolated from Urine Samples

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Abstract

The rise of antibiotic-resistant uropathogens has intensified the search for alternative antimicrobial agents, particularly from medicinal plants. Piliostigma thonningii, traditionally used to treat infections in African ethnomedicine, was investigated for its antibacterial efficacy against Gram-negative bacteria isolated from urine samples. Urine samples were collected and cultured on MacConkey agar. Pure isolates were subjected to Gram staining and a series of biochemical tests for identification following standard protocols. Crude leaf extracts of P. thonningii were prepared and subjected to phytochemical screening. Antibacterial activity was assessed using the agar well diffusion method at 100–400 mg/mL concentrations, with ciprofloxacin (10 µg) serving as a reference standard. Escherichia coli was the most prevalent isolate (51.43%), followed by Klebsiella pneumoniae (28.57%) and Enterobacter cloacae (20.00%). Phytochemical analysis revealed the presence of flavonoids, tannins, saponins, alkaloids, and terpenoids. The extract demonstrated dose-dependent antibacterial activity, with the highest inhibition against E. coli (23.0 ± 4.79 mm at 400 mg/mL). Although ciprofloxacin showed superior activity, the plant extract exhibited considerable zones of inhibition, particularly at higher concentrations. The study supports the traditional use of P. thonningii for treating bacterial infections and highlights its potential as a source of bioactive compounds. Further studies are needed to isolate active constituents, evaluate toxicity, and validate efficacy through in vivo models.

Keywords: *Piliostigma thonningii, antibacterial activity, Gram-negative bacteria, urinary tract infection, phytochemicals, medicinal plants*

1. INTRODUCTION

Plants have been employed for centuries in the prevention, diagnosis, and treatment of both physical and mental health conditions. [1]. Notably, approximately 50% of modern pharmaceutical drugs are derived either directly or indirectly from plant sources, with nearly 80% of the population in developing countries relying exclusively on plants for medicinal purposes. [2]. The rising challenge of microbial infections, due to the emergence of bacterial pathogens resistant to virtually all available antimicrobial agents, has necessitated the search for alternative therapeutic options with enhanced efficacy.[3]. In this context, traditional herbal medicine continues to play a vital role as an alternative to conventional antibiotics in many developing regions. Rich in bioactive phytochemicals, medicinal plants have long been utilized for their antiviral, antifungal, and antibacterial properties.[4]. The widespread application of plant extracts in disease management has driven a growing scientific interest in the identification and characterization of the active compounds responsible for their therapeutic potential, thereby providing promising leads for the development of new drugs.[5].

Piliostigma thonningii belongs to the family Fabaceae (Leguminosae), subfamily Caesalpinioideae, which consists of trees, shrubs, and occasionally climbers. [6]. This species is commonly found in open woodlands and wooded grasslands across sub-humid regions of Africa at medium to low altitudes. It is widely distributed throughout tropical Africa, including countries such as Botswana, Kenya, Namibia, Senegal, South Africa, Sudan, Tanzania, Uganda, and Zambia, except Somalia. [7]. The species is often found growing in association with *Annona senegalensis*, *Grewia mollis*, and various *Combretum* species. [8]. In many parts of Africa, *P. thonningii* is recognized for its extensive ethnomedicinal and economic importance. In Nigeria, it grows abundantly in the wild as an uncultivated small tree in regions such as Minna, Zaria, and Bauchi. [9]. As a leguminous plant, it has drawn considerable interest due to numerous ethnopharmacological claims regarding its therapeutic applications. Traditionally, it has been used to treat a wide range of ailments, including malaria, dysentery, fever, respiratory infections, snake bites, hookworm infestations, hepatobiliary disorders, edema (hydropsy), sterility, rickets (rachitis), and various skin diseases. [10]. Phytochemical investigations of *Piliostigma thonningii* have revealed the presence of several bioactive constituents, including flavonoids, tannins, kaurane diterpenes, alkaloids, carbohydrates, saponins, terpenes, and volatile oils. [11], [12], [13], [14]. These compounds are known to contribute to the plant's diverse pharmacological activities. Previous studies have demonstrated that extracts from different parts of the plant and isolated compounds from *P. thonningii* exhibit a broad spectrum of biological effects, including stem bark.[12], [15], leaves [11], [16], [17], Pods [14], and seed [18]**.**

Urinary tract infections (UTIs) are among the most prevalent bacterial infections affecting individuals globally and are a leading cause of both community- and hospital-acquired infections. [19]. These infections are primarily caused by Gram-negative bacteria, including *Escherichia coli*, *Klebsiella* spp., *Proteus mirabilis*, and *Pseudomonas aeruginosa*, as well as Gram-positive organisms such as *Staphylococcus saprophyticus* [20]. It is estimated that approximately 150 million cases of UTIs occur worldwide each year, resulting in healthcare expenditures exceeding six billion U.S. dollars. The increasing prevalence of antibiotic-resistant uropathogens, particularly multidrug-resistant (MDR) Gram-negative bacteria, has significantly complicated the management of UTIs [21]. Resistance to commonly prescribed antibiotics has led to reduced treatment efficacy, prolonged illness, increased hospitalization rates, and elevated healthcare costs. [22]. In response to the global antimicrobial resistance (AMR) crisis, there is growing interest in the exploration of alternative therapeutic agents, particularly from natural sources. *Piliostigma thonningii* has a well-established history of use in traditional medicine for the treatment of numerous ailments, including infections. While preliminary reports suggest that this plant may possess antimicrobial properties, scientific evidence supporting its effectiveness against uropathogenic Gram-negative bacteria remains scarce. This study aims to investigate its antibacterial activity against Gram-negative bacteria isolated from urine samples. The findings could contribute to the development of novel plant-based therapies for managing drug-resistant UTIs.

2. MATERIALS AND METHODS

2.1. Study Area and Sample Collection

The study was conducted in the Kebbi State University of Science and Technology, Aliero, where urine samples were collected from female students. A total of 35 midstream urine samples were aseptically collected using sterile universal containers, labelled appropriately, and transported in ice packs to the Microbiology Laboratory for immediate processing.[23].

2.2. Isolation and Identification of Gram-negative Bacteria

Urine samples were cultured on MacConkey agar using a sterile loop. Plates were incubated aerobically at 37 °C for 24 hours. Significant bacteriuria was defined as bacterial growth $\geq 10^5$ CFU/mL. Pure isolates were subjected to Gram staining and a series of biochemical tests (oxidase, indole, citrate utilization, urease, triple sugar iron test, and motility) for identification following standard protocols. [24], [25].

2.3. Preparation of Piliostigma thonningii Crude Extract

Fresh leaves of *Piliostigma thonningii* were collected from Aliero, Kebbi state, Nigeria, and authenticated at the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero (voucher references: KSUSTA/PSB/H/119L). The leaves were air-dried in the shade for 10 days and pulverized into fine powder using an electric grinder. About 200 g of powdered material was macerated in 1 L of 70% ethanol for 72 hours with intermittent shaking. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C, then dried to obtain a semi-solid crude extract. [26]. The extract was stored at 4 °C in airtight containers until further use.

2.4. Phytochemical Screening of Crude Extract

Preliminary phytochemical screening was conducted to detect the presence of alkaloids, tannins, flavonoids, saponins, phenols, glycosides, terpenoids, and steroids using standard qualitative procedures as described by [27] and [28].

2.5. Preparation of Extract Concentrations

Stock solutions were prepared by dissolving the crude extract in 5% dimethyl sulfoxide (DMSO). Working concentrations of 100, 200, 300, and 400 mg/mL were prepared for antibacterial testing.

2.6. Antibacterial Susceptibility Testing

The antibacterial activity of *P. thonningii* extract was assessed using the agar well diffusion method (CLSI, 2023). Mueller-Hinton agar (MHA) plates were inoculated with standardized bacterial suspensions adjusted to a 0.5 McFarland turbidity standard (~1.5×10^8 CFU/mL). Wells of 6 mm diameter were bored into the agar, and 100 µL of each extract concentration was introduced into the respective wells. Plates were incubated at 37 °C for 24 hours. The diameter of inhibition zones was measured in millimetres using a transparent ruler. Ciprofloxacin (10 µg) discs served as the positive control, while 5% DMSO served as the negative control. Each test was performed in triplicate, and mean inhibition zones were calculated.

2.7. Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined using the broth tube dilution method. Two-fold serial dilutions of the extract were made in the nutrient broth to obtain concentrations ranging from 12.5 to 200 mg/mL. Each tube was inoculated with 0.1 mL of standardized bacterial suspension and incubated at 37 °C for 24 hours. MIC was recorded as the lowest concentration of extract that showed no visible turbidity [29].

2.8. Determination of Minimum Bactericidal Concentration (MBC)

Tubes that showed no visible growth in the MIC test were subcultured on fresh nutrient agar and incubated at 37 °C for 24 hours. MBC was defined as the lowest concentration at which no bacterial growth occurred, indicating bactericidal activity [30].

2.9. Statistical Analysis

All experiments were performed in triplicate. Results were expressed as mean ± standard deviation. Data were analyzed using SPSS Version 24 and presented using tables and charts. One-way ANOVA was used to assess differences between inhibition zones at various extract concentrations. A p-value < 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

Medicinal plants have long served as important sources of therapeutic agents, offering a diverse range of bioactive compounds known for their antimicrobial, anti-inflammatory, and antioxidant effects. *Piliostigma thonningii*, commonly referred to as camel's foot or monkey bread tree, is a leguminous species found widely across the tropical regions of Africa. Traditionally, different parts of the plant, including the leaves, bark, roots, and pods, have been employed in the treatment of infections, wounds, diarrhea, fevers, and respiratory conditions. This study aimed to evaluate the antimicrobial properties of crude leaf extracts of *P. thonningii*, to provide scientific validation for its traditional medicinal applications in combating microbial infections.

3.1. Isolation and Identification of Gram-Negative Bacteria

A total of 35 Gram-negative bacterial isolates were recovered from urine samples (Table 1). The most predominant isolate was *Escherichia coli* (51.43%), followed by *Klebsiella pneumoniae* (28.57%) and *Enterobacter cloacae* (20.00%).

Table 1: Isolation and Identification of Gram-Negative Bacteria from Urine Samples

Bacterial Species	**Number Isolated**	**Percentage (%)**
Escherichia coli	18	51.43
Klebsiella pneumonia	10	28.57
Enterobacter cloacae	7	20.00
Total	**35**	**100.00**

The high prevalence of *Escherichia coli* (51.43%) among Gram-negative uropathogens observed in this study is consistent with previous reports by Kumar & Saikumar (2021) and Ajani et al. (2020), which identified *E. coli* as the most common causative agent of urinary tract infections (UTIs), particularly in female patients. Similarly, the isolation of *Klebsiella pneumoniae* (28.57%) and *Enterobacter cloacae* (20.00%) supports findings by Whelan et al. (2023) Who highlighted the increasing involvement of these species in nosocomial UTIs, as well as their rising resistance to standard antibiotic therapies.

3.2. Phytochemical Composition of Piliostigma thonningii Extract

Phytochemical screening of the ethanolic leaf extract of *Piliostigma thonningii* revealed the presence of flavonoids, tannins, alkaloids, saponins, and terpenoids, while steroids were not detected (Table 2). Saponins and terpenoids were found in relatively higher abundance, as indicated by strong positive reactions (++), whereas flavonoids, tannins, and alkaloids were present at lower levels (+).

Table 2: Phytochemical Screening of *Piliostigma thonningii* Extract

Phytochemical	**Result**
Flavonoids	+

Saponins	++
Tannins	+
Alkaloids	+
Terpenoids	++
Steroids	–

Key: = Present (low) ++ = Present (high) – = absent

Phytochemical analysis confirmed the presence of flavonoids, alkaloids, tannins, terpenoids, and saponins in the *Piliostigma thonningii* leaf extract. These findings align with earlier studies by Usin & Daramola (2022) and Adebayo and Boualam et al. (2021), which also reported similar bioactive compounds in the leaves and bark of *P. thonningii*. Notably, the strong presence of saponins and terpenoids (indicated by ++ reactions) supports their recognized role in antimicrobial activity. These compounds are known to exert their effects by disrupting microbial cell membranes and interfering with essential metabolic processes, as described by Cowan. [34]. The detection of these secondary metabolites is consistent with previous reports on the phytochemical richness of *P. thonningii.* [35]. These compounds are known to contribute to the antimicrobial efficacy of plant extracts. Flavonoids and alkaloids have been shown to interfere with microbial cell wall synthesis and DNA replication, while saponins and terpenoids are associated with membrane disruption and cytotoxic effects. [36]. The absence of steroids in the extract suggests that they may not play a role in the plant's bioactivity against the tested Gram-negative uropathogens.

3.3. Antibacterial Activity of Piliostigma thonningii Extract

The antibacterial activity of *Piliostigma thonningii* ethanolic extract was assessed using the agar well diffusion method. Mean zones of inhibition are expressed in millimeters (mm) ± standard deviation (SD) based on triplicate measurements (Table 3). The results demonstrate that the ethanolic leaf extract of *Piliostigma thonningii* exhibited measurable antibacterial activity against all three tested Gram-negative uropathogens, *Escherichia coli*, *Enterobacter cloacae*, and *Klebsiella pneumoniae*. The antibacterial effect was concentration-dependent, with larger zones of inhibition observed at higher extract concentrations. At 100 mg/mL, the extract showed moderate activity against E. coli (17.5 ± 4.18 mm), weak activity against K. pneumoniae (9.5 ± 3.08 mm), and very low inhibition of E. cloacae (3.0 ± 1.73 mm). As the concentration increased, the zones of inhibition expanded significantly. At 400 mg/mL, the extract produced inhibition zones of 23.0 ± 4.79 mm, 14.5 ± 3.80 mm, and 22.0 ± 4.69 mm against E. coli, E. cloacae, and K. pneumoniae, respectively. Ciprofloxacin (10 µg), used as a positive control, exhibited higher or comparable activity across all isolates, with inhibition zones of 22.1 ± 0.3 mm for *E. coli*, 19.9 ± 0.7 mm for *E. cloacae*, and 21.3 ± 0.5 mm for *K. pneumoniae*. Although the extract was less potent than ciprofloxacin, especially against *E. cloacae*, it demonstrated significant antibacterial effects at higher concentrations, notably against *E. coli* and *K. pneumoniae*.

Table 3. Mean Zones of Inhibition (mm) of *Piliostigma thonningii* Extract at Varying Concentrations against Gram-Negative Uropathogens

Agent	**Concentration (mg/mL)**	***E. coli* (mm)**	***E. cloacae* (mm)**	***K. pneumoniae* (mm)**
P. thonningii Extract	100	17.5 ± 4.18	3.0 ± 1.73	9.5 ± 3.08
	200	18.5 ± 4.30	7.0 ± 2.64	13.5 ± 3.67
	300	22.0 ± 4.69	13.0 ± 3.60	18.0 ± 4.24

	400	23.0 ± 4.79	14.5 ± 3.80	22.0 ± 4.69
Ciprofloxacin (10 µg/disc)	–	22.1 ± 0.3	19.9 ± 0.7	21.3 ± 0.5

The extract exhibited a concentration-dependent inhibitory effect against all tested Gram-negative isolates, with the highest activity recorded at 400 mg/mL. The greatest inhibition was observed against *E. coli* (23.0 ± 4.79 mm), followed by *K. pneumoniae* (22.0 ± 4.69 mm), and the lowest was against *E. cloacae* (14.5 ± 3.80 mm). These findings are in agreement with Igwe et al. (2025) and Ipav et al. (2014), who reported significant activity of *P. thonningii* extracts against various Gram-negative bacteria, including uropathogens. Although ciprofloxacin exhibited stronger and more consistent activity, especially against *E. cloacae*, the extract demonstrated promising inhibition zones that are considered biologically relevant at higher concentrations. The observed antimicrobial activity may be attributed to the synergistic effects of the phytochemicals identified, particularly flavonoids, terpenoids, and alkaloids, which are known to possess antimicrobial, anti-inflammatory, and antioxidant properties.[37]. However, the reduced efficacy compared to ciprofloxacin suggests that while *P. Thonningii* holds therapeutic promise; further purification and characterization of its bioactive compounds are necessary to enhance its clinical potential.

3.4. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the antimicrobial agent against the tested Gram-negative bacteria

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the antimicrobial agent against the tested Gram-negative bacteria are summarized in Table 4. The MIC and MBC values for *Escherichia coli* were 18.5 ± 4.30 mg/ml and 17.6 ± 3.31 mg/ml, respectively, indicating that the agent effectively inhibits and kills the bacterium at similar concentrations. *Enterobacter cloacae* exhibited slightly lower MIC (14.5 ± 3.80 mg/ml) and MBC (15.21 ± 2.33 mg/ml) values, suggesting a higher susceptibility to the antimicrobial compound. For *Klebsiella pneumoniae*, the MIC (16.21 ± 11 mg/ml) and MBC (18.0 ± 4.24 mg/ml) values were comparable, although the larger standard deviation in the MIC reflects some variability in bacterial response. Overall, the proximity of MIC and MBC values across all tested organisms indicates that the antimicrobial agent possesses a bactericidal effect rather than merely bacteriostatic. These findings demonstrate the potential of the tested compound as an effective antibacterial agent against these clinically important Gram-negative pathogens.

Table 4: Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Organism	**Minimum Inhibitory Concentration (MIC) (mg/ml)**	**Minimum Bactericidal Concentration (MBC) (mg/ml)**
Escherichia coli	18.5 ± 4.30	17.6 ± 3.31
Enterobacter cloacae	14.5 ± 3.80	15.21 ± 2.33
Klebsiella pneumonia	16.21 ± 11.00	18.0 ± 4.24

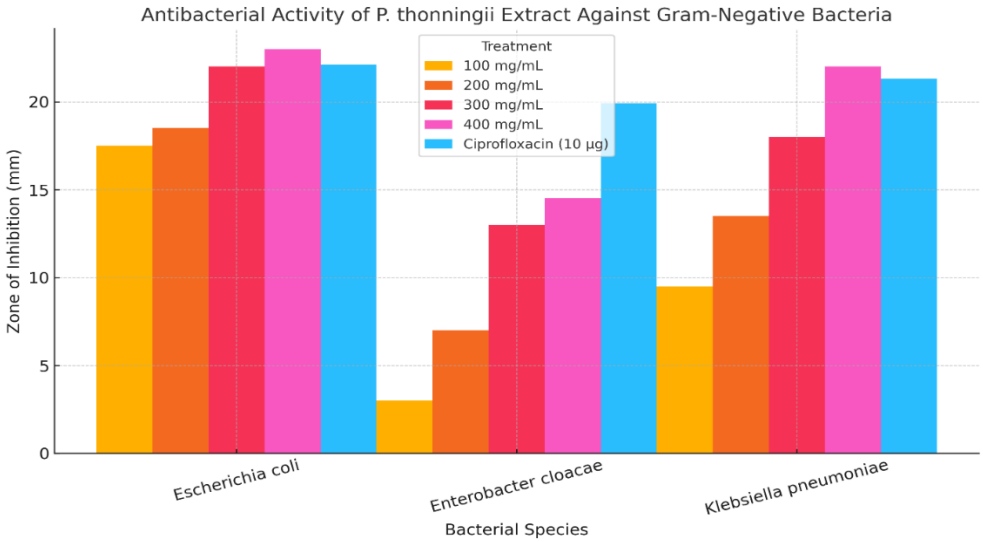

Figure 1: Antibacterial Activity of *Piliostigma thonningii* Extract gram Gram-negative bacteria

While this study has provided meaningful insights into the antibacterial potential of *Piliostigma thonningii* leaf extract, several some must be acknowledged. First, the use of crude extracts limits the precision of attributing antimicrobial activity to specific phytochemical constituents, as the extract contains a complex mixture of compounds with varying bioactivities. Secondly, the study exclusively focused on Gram-negative bacterial isolates from urine samples, thereby excluding other clinically relevant pathogens such as Gram-positive bacteria and fungi. Furthermore, the results are restricted to in vitro conditions and may not directly translate to therapeutic efficacy in living organisms. Lastly, the study did not assess the cytotoxic or systemic safety profile of the extract, which is a critical prerequisite for its development as a pharmacological agent.

CONCLUSION

The findings of this study demonstrate that *Piliostigma thonningii* leaf extract exhibits a concentration-dependent inhibitory effect against clinically significant Gram-negative uropathogens. The most susceptible organism was *Escherichia coli*, followed by *Klebsiella pneumoniae* and *Enterobacter cloacae*. Phytochemical screening confirmed the presence of several secondary metabolites, notably saponins and terpenoids, which are known to possess antimicrobial properties. Although ciprofloxacin exhibited superior antimicrobial activity, the extract showed promising zones of inhibition, especially at higher concentrations, thereby supporting its traditional use in the treatment of infections. These results not only validate the ethnomedicinal applications of *P. thonningii* but also highlight its potential as a source of natural antimicrobial compounds. However, further research is required to isolate, characterize, and optimize its bioactive components for therapeutic use. It is essential to conduct toxicity assessments (both in vitro and in vivo) to determine the safety profile of the extract.

REFERENCE

[1] A. Sofowora, E. Ogunbodede, and A. Onayade, “The role and place of medicinal plants in the strategies for disease prevention.” *African J. Tradit. Complement. Altern. Med. AJTCAM*, vol. 10, no. 5, pp. 210–229, Aug. 2013, doi: 10.4314/ajtcam.v10i5.2.

[2] N. Chaachouay and L. Zidane, “Plant-Derived Natural Products: A Source for Drug

Discovery and Development," *Drugs Drug Candidates*, vol. 3, no. 1, pp. 184–207, 2024, doi: 10.3390/ddc3010011.

[3] C. C. Davis and P. Choisy, "Medicinal plants meet modern biodiversity science," *Curr. Biol.*, vol. 34, no. 4, pp. R158–R173, 2024, doi: 10.1016/j.cub.2023.12.038.

[4] S. Parham *et al.*, "Antioxidant, antimicrobial and antiviral properties of herbal materials," *Antioxidants*, vol. 9, no. 12, pp. 1–36, 2020, doi: 10.3390/antiox9121309.

[5] A. Najmi, S. A. Javed, M. Al Bratty, and H. A. Alhazmi, "Modern Approaches in the Discovery and Development of Plant-Based Natural Products and Their Analogues as Potential Therapeutic Agents," *Molecules*, vol. 27, no. 2, 2022, doi: 10.3390/molecules27020349.

[6] M. B. Hailemariam, Z. Woldu, Z. Asfaw, and E. Lulekal, "Ethnobotany of an indigenous tree Piliostigma thonningii (Schumach.) Milne-Redh. (Fabaceae) In the arid and semi-arid areas of South Omo Zone, southern Ethiopia," *J. Ethnobiol. Ethnomed.*, vol. 17, no. 1, p. 44, Jul. 2021, doi: 10.1186/s13002-021-00469-6.

[7] I B. Abubakar, I. Malami, A. Muhammad, T. Salihu Shinkafi, D. Shehu, and P. Maduabuchi Aja, " A review of the medicinal uses and biological activity of Piliostigma thonningii (Schum). Milne-Redh. " *RPS Pharm. Pharmacol. Reports*, vol. 3, no. 1, pp. 1–15, 2024, doi: 10.1093/rpsppr/rqae004.

[8] K. Boualam, B. Ndiaye, H. Harhar, M. Tabyaoui, N. Ayessou, and K. Taghzouti, "Study of the Phytochemical Composition, the Antioxidant and the Anti-Inflammatory Effects of Two Sub-Saharan Plants: Piliostigma reticulatum and Piliostigma thonningii." *Adv. Pharmacol. Pharm. Sci.*, vol. 23, no. 5, p. 5549478, 2021, doi: 10.1155/2021/5549478.

[9] M. Afolayan *et al.*, "Phytochemical study of Piliostigma thonningii, a medicinal plant grown in Nigeria," *Med. Chem. Res.*, vol. 27, no. 10, pp. 2325–2330, 2018, doi: 10.1007/s00044-018-2238-1.

[10] B. Nethathe, L. M. Ramphinwa, A. Selekane Motadi, and F. K. Matlakala, "Scoping review of ethnobotanical studies on Piliostigma thonningii (Schumach.) Milne-Redh. in Sub-Saharan Africa," *Front. Pharmacol.*, vol. 16, no. 2, pp. 1–12, 2025, doi: 10.3389/fphar.2025.1575548.

[11] M. Afolayan *et al.*, "Phytochemical study of Piliostigma thonningii, a medicinal plant grown in Nigeria." *Med. Chem. Res.*, vol. 27, no. 10, pp. 2325–2330, Oct. 2018, doi: 10.1007/s00044-018-2238-1.

[12] P. N. Ngari, J. M. Nguta, and I. O. Mapenay, "Antimicrobial activity, brine shrimp cytotoxicity and phytochemical screening of Piliostigma thonningii (Schumach .) Milne-Redh aqueous and methanol stem bark extracts," *J. Phytopharm.*, vol. 13, no. 5, pp. 366–373, 2024, doi: 10.31254/phyto.2024.13505.

[13] A. Michael and A. Sarah, "Assessment of Antitrypanosomal Activities of Crude Extracts and Fractions from Piliostigma thonningii Schum. Leaves in Vitro," *J. Biochem. Phytomedicine*, vol. 3, no. 1, pp. 46–52, 2024, doi: 10.34172/jbp.2024.10.46.

[14] M. Makosa, S. Sithole, and S. Mukanganyama, "Evaluation of the Antimicrobial Effect of the Extracts of the Pods of Piliostigma thonningii (Schumach .) Milne-Redh. (Fabaceae)," *Adv. Pharmacol. Pharm. Sci.*, vol. 5, no. 27, 2021, doi: https://doi.org/10.1155/2021/6616133.

[15] D. W. Malgwi, M. H. Shagal, R. D. Adamu, B. F. Dowell, and A. State, "Phytochemical analysis, isolation and biological studies of piliostigma thonnigii stem bark extract," *Niger. Res. J. Chem. Sci.*, vol. 12, no. 1, pp. 93–106, 2024.

[16] S. S. Ipav, K. Dasofunjo, T. T. Sar, and C. E. Hilary, "Antimicrobial and Bioactive Potentials of Extracts of Piliostigma thonningii Leaves," *Niger. J. Pure Appl. Sci.*, vol. 5, pp. 22–27, 2014.

[17] S. N. Mathias, S. C. Ofoegbu, M. Lawal, A. Hamidu, and A. S. Ajayi, "Wound Healing Potentials of Methanolic Leaf Extract of Piliostigma thonningii Schum," *Niger. J. Basic Appl. Sci. (December*, vol. 30, no. 2, pp. 147–153, 2022, doi: DOI: http://dx.doi.org/10.4314/njbas.v30i2.20.

[18] O. U. Igwe, M. U. Ejezie, and I. E. Otuokere, "Chemical composition, antimicrobial and docking studies of chloroform extracts of piliostigma thonningii leaves and seeds," *J. Chem. Soc. Nigeria*, vol. 50, no. 1, pp. 178–193, 2025.

[19] M. S. Bader, M. Loeb, D. Leto, and A. A. Brooks, "Treatment of urinary tract infections in the era of antimicrobial resistance and new antimicrobial agents," *Postgrad. Med..*, vol. 132, no. 3, pp. 234–250, Apr. 2020, doi: 10.1080/00325481.2019.1680052.

[20] G. Mancuso, A. Midiri, E. Gerace, M. Marra, S. Zummo, and C. Biondo, "Urinary Tract Infections: The Current Scenario and Future Prospects," *Pathogens*, vol. 12, no. 4, 2023, doi: 10.3390/pathogens12040623.

[21] S. Whelan, B. Lucey, and K. Finn, "Uropathogenic Escherichia coli (UPEC)-Associated Urinary Tract Infections: The Molecular Basis for Challenges to Effective Treatment," *Microorganisms*, vol. 11, no. 9, 2023, doi: 10.3390/microorganisms11092169.

[22] S. Teferi *et al.*, "Uropathogenic bacterial profile and antibiotic susceptibility pattern of isolates among gynecological cases admitted to Jimma Medical Center, South West Ethiopia," *Sci. Rep..*, vol. 13, no. 1, pp. 1–11, 2023, doi: 10.1038/s41598-023-34048-4.

[23] M. C. Cheesbrough, *District laboratory practice in Tropical Countries, Cambridge University Press, Part 2*, 2006.

[24] J. G. Cappuccino and N. Sherman, *Microbiology: A Laboratory Manual*. Pearson, 2014. [Online]. Available: https://books.google.com.ng/books?id=6W2JMQEACAAJ

[25] C. and L. S. I. CLIS, *CLSI M100-ED29: 2021 Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition*, 30th ed., vol. 40, no. 1. 2020.

[26] F. F. Yannick Stéphane, B. K. Jean Jules, L. N. Bruno, G. El-Saber Batiha, and I. Ali, "Extraction of Bioactive Compounds from Medicinal Plants and Herbs," in *Natural Medicinal Plants*, H. El-Shemy, Ed., Rijeka: IntechOpen, 2021. doi: 10.5772/intechopen.98602.

[27] T. Sharma, B. Pandey, B. K. Shrestha, G. M. Koju, R. Thusa, and N. Karki, "Phytochemical Screening of Medicinal Plants and Study of the Effect of Phytoconstituents in Seed Germination," *Tribhuvan Univ. J.*, vol. 35, no. 2, pp. 1–11, 2020, doi: 10.3126/tuj.v35i2.36183.

[28] S. Dubale, D. Kebebe, A. Zeynudin, N. Abdissa, and S. Suleman, "Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia.," *J. Exp. Pharmacol.*, vol. 15, pp. 51–62, 2023, doi: 10.2147/JEP.S379805.

[29] B. Kowalska-Krochmal and R. Dudek-Wicher, "The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance.," *Pathog. (Basel, Switzerland)*, vol. 10, no. 2, Feb. 2021, doi: 10.3390/pathogens10020165.

[30] C. I. Owuama, "Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method," *African J. Microbiol. Res.*, vol. 11, no. 23, pp. 977–980, 2017.

[31] V. N. Kumar and C. Saikumar, "Isolation and Identification of Uropathogenic E. coli and

its Antimicrobial Susceptibility Pattern with Special Reference to Extended Spectrum Beta-Lactamases (Esbl)," *J. Pharm. Res. Int.*, vol. 33, no. 24A, pp. 56–67, 2021, doi: 10.9734/jpri/2021/v33i24a31431.

[32] T. A. Ajani *et al.*, "Urinary tract infections and antimicrobial susceptibility pattern among female students in a tertiary institution in southwest Nigeria - A cross sectional study," *J. Clin. Sci.*, vol. 17, no. 4, pp. 131–135, 2020, [Online]. Available: https://journals.lww.com/jocs/fulltext/2020/17040/urinary_tract_infections_and_antimicrobial.9.aspx

[33] S. Usin and O. Daramola, "Phytochemical Analysis and Evaluation of Ethanol and Aqueous Extracts of Piliostigma thonningii Leaf for in vitro Antioxidant," *Niger. J. Biochem. Mol. Biol.*, vol. 37, no. 1, pp. 72–81, 2022.

[34] J. S. Câmara, R. Perestrelo, R. Ferreira, C. V Berenguer, J. A. M. Pereira, and P. C. Castilho, "Plant-Derived Terpenoids: A Plethora of Bioactive Compounds with Several Health Functions and Industrial Applications—A Comprehensive Overview," *Molecules*, vol. 29, no. 16. pp. 38–61, 2024. doi: 10.3390/molecules29163861.

[35] O. M. Seyi, "Phytochemicals , Toxicity , Antioxidant and Antimicrobial Activities of Active Secondary Metabolites of Stem Bark of Piliostigma Thonningii (Schum) Milne-Redh," *Int. J. Adv. Acad. Res.*, vol. 8, no. 5, pp. 15–27, 2022.

[36] Q. O. Nurudeen *et al.*, "Hydroethanolic extract of Piliostigma thonningii leaves extenuates the severity of diarrhoea in female Wistar rats.," *J. Complement. Integr. Med.*, vol. 21, no. 1, pp. 26–37, Mar. 2024, doi: 10.1515/jcim-2023-0205.

[37] W. Huang *et al.*, "Biosynthesis Investigations of Terpenoid, Alkaloid, and Flavonoid Antimicrobial Agents Derived from Medicinal Plants.," *Antibiot. (Basel, Switzerland)*, vol. 11, no. 10, Oct. 2022, doi: 10.3390/antibiotics11101380