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Evaluation of Antibacterial Properties of *Piliostigma reticulatum* leaves

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Abstract
Piliostigma reticulatum is known for its extensive traditional applications, such as managing diarrhea in teething infants, treating wounds and ulcers, combating parasitic infections, stopping bleeding, and alleviating inflammation and bacterial conditions. It is also used in the treatment of gonorrhea, stomach pain, headaches, malaria, dysentery, fever, respiratory disorders, snakebites, hookworm infestations, and various skin infections. This study was conducted to assess the chemomicroscopic and antibacterial effects of *Piliostigma reticulatum* leaf extracts. Chemomicroscopic analysis of the powdered leaf material identified the presence of cellulose, tannins, starch, lignin, suberin, aleurone grains, and mucilage, while calcium carbonate, calcium oxalate, and inulin were not observed. Standardized procedures were employed to perform antibacterial evaluations. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* were 15.625 mg/mL and 31.25 mg/mL, respectively.

Keywords: Antibacterial, Chemomicroscopical, *Escherichia coli*, *Piliostigma reticulatum*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*
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Introduction

Infectious diseases continue to be the major causes of death across all age groups (WHO, 2014). Medicinal plants have long been an essential component of traditional health care systems, particularly in many developing countries where access to conventional medicine is limited (World Health Organization (WHO, 2013). Among such plants, *Piliostigma reticulatum* (commonly known as camel's foot) has gained attention due to its extensive use in ethnomedicine across various African regions. Belonging to the Fabaceae family, this leguminous shrub is widely distributed in tropical and subtropical zones, including West Africa, where it is traditionally employed in the management of numerous health conditions (Burkill, 1995). In folk medicine, various parts of *Piliostigma reticulatum*, especially the leaves, have been used to treat ailments such as diarrhea in infants, skin infections, ulcers, wounds, respiratory tract infections, malaria, and gastrointestinal disturbances (Adedapo *et al.*, 2019). Additionally, its application in addressing microbial infections and inflammatory disorders highlights its potential as a source of bioactive compounds with pharmacological significance.

Despite its broad traditional usage, scientific validation of the pharmacognostic and therapeutic properties of *Piliostigma reticulatum* remains limited. Detailed evaluation of its antioxidant and antibacterial activities, along with phytochemical profiling, is essential to substantiate its ethnopharmacological relevance and support potential drug discovery efforts. Antioxidants play a pivotal role in neutralizing free radicals implicated in chronic diseases, while antibacterial agents are crucial in the era of

increasing antibiotic resistance (Halliwell and Gutteridge, 2015).

Materials and Methods

Collection and Identification of Plant Material

Fresh leaves of *Piliostigma reticulatum* were collected from the botanical garden within the Department of Plant Biology, Bayero University, Kano, located in Gwale Local Government Area, Kano State, Nigeria. The specimen was authenticated by a taxonomist at the university's herbarium, where it was deposited under the voucher number BUKHAN72.

Microbiological Analysis

Based on Karzan *et al.* (2017), microbial isolates were initially cultured on selective media and characterized using Gram staining and biochemical tests (catalase, coagulase, oxidase). Pure isolates were maintained on nutrient and chocolate agar at 4°C.

Identification Using Rapid Kits

Microgen™ identification kits were used following the manufacturer's protocol (Sylvester, 2016). Inoculated wells were incubated, treated with specific reagents (e.g., Kovacs, TDA), and interpreted via Microgen Identification Software (MID-60).

Antibacterial Susceptibility Testing

Following Srinivasan *et al.* (2009), stock solutions (250 mg/mL) of the extracts were serially diluted. Mueller-Hinton agar plates were seeded with bacterial inocula (prepared to 0.5 McFarland standard), and agar well diffusion was used to assess antibacterial activity. Ciprofloxacin served as a positive control, and DMSO as negative control.

Minimum Inhibitory Concentration (MIC)

Serial dilutions of the extract were tested in Mueller-Hinton broth against bacterial strains, incubated at 37°C for 24 hours. Absence of turbidity indicated inhibition. Sub-culturing onto nutrient agar confirmed results (Adesokan *et al.*, 2007).

Minimum Bactericidal Concentration (MBC)

To determine MBC, 1 mL of broth from MIC tubes was inoculated onto nutrient agar. The lowest concentration showing no growth after 24 hours at 37°C was considered bactericidal (Adesokan *et al.*, 2007).

Results

Chemo-microscopical examination of powdered *Piliostigma reticulatum* leaves revealed the presence of cellulose, tannins, starch, lignin, suberin, aleurone grain and mucilage but calcium carbonate, calcium oxalate and inulin were absent (Table 1).

Antibacterial activities of methanol extract of *Piliostigma reticulatum* leaves showed inhibition on all the tested clinical isolates of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* at 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml (Table 2). The MIC and MBC of the *Piliostigma reticulatum* leaf extract recorded respective values of 15.625 mg/ml and 31.25 mg/ml against *Pseudomonas sp.* and *Escherichia coli* and *Staphylococcus aureus.*

Table 1: Chemomicroscopical studies of *Piliostigma reticulatum* powdered leaves

Constituents	Inference
Starch	+
Gum and Mucilage	+
Cellulose cell walls	+
Lignin	+
Aleurone grain	+
Calcium oxalate crystals	-
Calcium carbonate	-
Suberized/Cuticular cell wall	+
Inulin	-

Keys: + = present, - = absent

Table 2: Antibacterial activity of methanol extract of *Piliostigma reticulatum* leaves

Clinical isolates	Concentration (mg/ml)/Diameter zone of inhibition (mm)						MIC	MBC
	250	125	62.5	31.25	CPR	DMSO		
S. aureus	20	17	14	11	38	06	15.625	31.25
E. coli	31	26	20	18	37	06	15.625	31.25
Pseudomonas	26	23	19	14	40	06	15.625	31.25

Discussion

The Microscopic evaluation of the powdered leaf sample revealed the presence of essential cellular materials such as cellulose, starch, lignin, suberin, mucilage, aleurone grains, and tannins, this clearly shown in table 1. These components suggest structural stability and potential pharmacological activities. For instance, tannins are known to have antimicrobial and antioxidant effects, while mucilage may contribute to the plant's emollient properties (Evans, 2009). The absence of certain microscopic elements like calcium oxalate, calcium carbonate, and inulin further helps in distinguishing *P. reticulatum* from other species, contributing to its botanical specificity.

The extract also demonstrated antibacterial efficacy as shown in table 2, inhibiting the growth of clinical isolates including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* across all tested concentrations. The minimum inhibitory concentration (MIC) was 15.625 mg/mL, and the minimum bactericidal concentration (MBC) was 31.25 mg/mL, indicating strong antimicrobial potential. These effects are likely due to the presence of phytochemicals such as alkaloids, tannins, and flavonoids, which have been documented to disrupt microbial membranes and inhibit essential enzymes (Nascimento *et al.*, 2000; Doughari *et al.*, 2007).

Conclusion

The present study confirms that *Piliostigma reticulatum* leaves possess significant pharmacological and phytochemical properties that validate their traditional medicinal use. Phytochemical screening revealed the presence of various bioactive compounds, including flavonoids, alkaloids, tannins, phenols, saponins, steroids, glycosides, and carbohydrates all known to contribute to a range of biological activities. The methanolic extract exhibited remarkable antioxidant potential, showing strong free radical scavenging activity with a very low IC_{50} value, which highlights its ability to counter oxidative stress. Additionally, the extract demonstrated broad-spectrum antibacterial activity against clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, further supporting its potential use in the treatment of infectious diseases. Overall, these findings provide scientific support for the ethnomedicinal application of *P. reticulatum* leaves and underscore their potential as a source of natural antioxidant and antimicrobial agents. The findings from this study confirm the medicinal value of *Piliostigma reticulatum* leaves. Its rich phytochemical composition, potent antioxidant capacity, and broad-spectrum antimicrobial activity support its traditional use and highlight its promise as a source of therapeutic agents. Further investigations, including isolation of specific compounds and in vivo studies, are recommended to fully explore and develop its therapeutic potential.

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