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Antibacterial and Antifungal Activities of Saponin Extracts from Three Congolese Medicinal Plants of the Fabaceae Family

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ABSTRACT

Background and Objective: Antimicrobial resistance is a critical global health threat, particularly in Sub-Saharan Africa, where access to effective treatments is very limited. Saponins from Fabaceae plants exhibit broad pharmacological properties, yet several Congolese species remain pharmacologically unexplored. This study evaluated the antibacterial and antifungal activities of saponin-rich extracts from three Congolese Fabaceae: *Millettia laurentii* De Wild., *Millettia dubia* De Wild., and *Pentaclethra eetveldeana* De Wild. & T. Durand. **Materials and Methods:** Saponins were extracted from the trunk and root bark of plant species using 80% methanol maceration and reflux, followed by diethyl ether precipitation. A dialyzed fraction of *M. dubia* was prepared using a 10,000 Da cut-off membrane. Antimicrobial activity was assessed by broth microdilution assay against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442, and *Candida krusei* ATCC 6258. **Results:** Extraction yields ranged from 3.27 to 3.6%. All five extracts selectively inhibited *P. aeruginosa*; the dialyzed *M. dubia* extract showed the greatest potency (MIC = 15.625 µg/mL). No activity was detected against *E. coli* or *S. aureus*. The dialyzed *M. dubia* extract also demonstrated excellent antifungal activity against *C. krusei* (MIC = 15.625 µg/mL), outperforming fluconazole. **Conclusion:** *Millettia dubia* saponins exhibit potent activity against *P. aeruginosa* and *C. krusei* following partial purification, suggesting the antimicrobial potential of this species. Our findings contribute to the valorization of Congolese flora as a potential source of new antimicrobial leads.

KEYWORDS

Saponins, fabaceae, antimicrobial activity, antifungal activity, *Millettia laurentii*, *Millettia dubia*, *Pentaclethra eetveldeana*, Congolese flora

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INTRODUCTION

Antimicrobial resistance (AMR) is one of the most critical public health threats of the 21st century. Driven by the misuse and overuse of antimicrobials in human medicine, veterinary practice, and agriculture, AMR contributes to millions of deaths annually and is projected to become the leading cause of mortality by 2050 if no decisive action is taken, with associated economic losses estimated in the trillions of dollars[1,2]. In Africa, the crisis is compounded by inadequate healthcare infrastructure, limited diagnostics, and restricted access to a new generation of antibiotics[3].

In many rural communities of the Democratic Republic of Congo (DRC) and other African nations, more than 80% of the population relies primarily on traditional herbal remedies for primary healthcare[4,5]. This dependence on traditional plant-based medicines, although born of necessity, has long fueled scientific interest in the pharmacological potential of African medicinal plants. In recent decades, renewed efforts have been directed toward systematically characterizing the phytochemical profiles and biological activities of plant secondary metabolites, including phenolic compounds, alkaloids, terpenes, essential oils, and saponins[6-9]. Among these metabolites, saponins have attracted particular attention because of their structural diversity, amphiphilic nature, and broad range of pharmacological properties, which include anti-inflammatory, antidiabetic, anticancer, antiviral, and antimicrobial activities[10,11]. Saponins are naturally occurring glycosides composed of a lipophilic aglycone linked to one or more hydrophilic sugar chains. Their amphiphilic character confers strong surfactant properties, manifested as stable foam formation in aqueous solutions, a key feature used in preliminary phytochemical screening[12,13]. Their ability to interact with membrane sterols and disrupt lipid bilayer integrity underlies both their hemolytic properties and their antimicrobial potential[14].

The Fabaceae family is among the plant families richest in saponins, encompassing numerous genera with demonstrated pharmacological relevance, including *Millettia* and *Pentaclethra*[15,16]. *Millettia laurentii* De Wild., commonly known as Wengé, is a large tropical tree native to the Congo Basin and is listed as an Endangered species by the IUCN due to habitat destruction and overexploitation. Its bark has traditionally been used in the DRC in aqueous extractions for the treatment of convulsive cough and asthma, and has been reported to possess antitumoral, anti-inflammatory, and bactericidal activities[17,18]. *Millettia dubia* De Wild. is a liana or small tree endemic to the DRC, locally known as Bonkelele in Lingala (a national language in DRC), for which no prior pharmacological data have been reported. On the other hand, *Pentaclethra eetveldeana* De Wild. & T. Durand, which is a tree of the African tropical forest distributed from Gabon to Cabinda, is traditionally used in the Republic of Congo and DRC for respiratory diseases, constipation, gastritis, helminthiasis, and hemorrhoids[19-22]. Triterpenoid saponins have been previously isolated and characterized from the trunk bark[20]. Despite the ethnomedical importance of these species, no comparative antimicrobial study has been conducted on all three simultaneously, and *M. dubia* remains entirely unexplored pharmacologically. This study, therefore, aimed to evaluate the saponin content of different plant organs using the foam test; extract and quantify saponin-rich fractions from the most saponin-rich parts; assess the effect of dialysis purification on the antimicrobial activity of *M. dubia* extracts; and evaluate the antibacterial and antifungal activities of all extracts against four clinically relevant microbial strains.

MATERIALS AND METHODS

Study area and duration: Plant material was collected from the campus of the University of Kinshasa (UNIKIN), located in the Commune of Lemba, Kinshasa, Democratic Republic of Congo (4°26 S, 15°19 E). The University of Kinshasa campus is situated in the Kinshasa Plateau Region, characterized by a tropical savanna climate (Köppen Aw) with a mean annual temperature of approximately 25°C and a mean annual rainfall of 1,400 mm, distributed across two rainy seasons (September-December and February-May) and two dry seasons. The campus hosts a diverse collection of indigenous and naturalized woody species,

Fig. 1(a-d): Dried plant materials used for grinding and extraction, (a) *Melia dubia* trunk bark, (b) *Millettia laurentii* trunk bark, (c) *Pseudomonas eetveldeana* trunk bark and (d) *Pseudomonas eetveldeana* root bark

Table 1: Culture conditions used for each microbial strain

Microbial strain	Culture medium	Incubation temperature (°C)
Escherichia coli ATCC 25922	MacConkey agar	35±2
Pseudomonas aeruginosa ATCC 15442	Cetrimide agar	30
Staphylococcus aureus ATCC 25923	Mannitol salt agar	35±2 (aerobic)
Candida krusei ATCC 6258	Sabouraud dextrose agar	35-37

making it a well-established site for the botanical collection of Congolese flora. The study was conducted from January 2023 to March 2024. Bark specimens (*M. laurentii* trunk bark, *M. dubia* root and trunk bark, and *P. eetveldeana* trunk and root bark) were collected in January 2023. Bark collection is feasible year-round and is not constrained by a specific phenological window. Seeds of *M. laurentii* were harvested in May 2023, corresponding to the flowering and fruiting period of the species.

Plant material: *Millettia laurentii* De Wild., *Millettia dubia* De Wild., and *Pentaclethra eetveldeana* De Wild. & T. Durand were collected from the University of Kinshasa campus. Bark specimens (trunk and root) were air-dried at ambient temperature (Fig. 1) and ground into a fine powder. All species were authenticated by Mr. Boniface Lukebakio Nlandu of Institut National d'Etudes et de Recherches Agronomiques (INERA Herbarium, University of Kinshasa). Voucher specimens were deposited under reference numbers GS001 (*M. laurentii* trunk bark), GS002 (*P. eetveldeana* trunk bark), GS003 (*P. eetveldeana* root bark), GS004 (*M. dubia* trunk bark), and GS005 (*M. dubia* root bark).

Chemicals, reagents, and microbial strains: Distilled water, 80% methanol, and diethyl ether were used for all extractions. Dimethyl sulfoxide (DMSO, 5%), tryptic soy broth (TSB), Mueller-Hinton broth (MHB), resazurin sodium salt, phosphate-buffered saline (PBS), and standard microbiological media were of analytical grade. Ciprofloxacin and fluconazole (Sigma-Aldrich) served as positive controls. Reference microbial strains, including *Staphylococcus aureus* ATCC® 25923™, *Escherichia coli* ATCC® 25922™,

Pseudomonas aeruginosa ATCC® 15442™, and *Candida krusei* ATCC® 6258™, were supplied by the Microbiology Laboratory, Faculty of Pharmaceutical Sciences, University of Kinshasa. Culture conditions are described in Table 1.

Foam test: Two grams of dried powder were macerated in 30 mL of distilled water at ambient temperature for 24 hrs, heated at 70°C for 90 min, and filtered. The filtrate was vigorously shaken for 5 min in a 15-cm test tube, and the foam column height was measured immediately and at 15 and 30 min. Results were scored as: (++) strongly positive (column >10 cm, stable after 15 min); (+) positive (column >10 cm, gradual degradation); (0) weakly positive (<10 cm); and (–) negative.[23]

Saponin extraction: Dried plant powder was macerated in 500 mL of 80% methanol for 6 days, then subjected to reflux extraction at 80°C for 3 hrs[24]. After filtration, the extract was concentrated by evaporation and further reduced under vacuum to approximately 120 mL. Saponins were precipitated by the addition of three volumes of diethyl ether, collected by Büchner filtration, and dried under vacuum.

$$\text{Extraction yeild (\%)} = \frac{\text{Dry extract weight (g)}}{\text{Intitial dry plant material weight (g)}} \times 100$$

Dialysis purification of M. dubia extract: One gram of crude *M. dubia* saponin extract was dissolved in 100 mL of distilled water and transferred to a pre-soaked cellophane membrane (10,000 Da cut-off, Amicon). Dialysis was performed against 500 mL of distilled water with gentle agitation for 24 hrs, with water replaced every 4 hrs[25]. The retentate was recovered and freeze-dried. A loss of 320 mg (32%) was recorded, yielding 680 mg of dialyzed extract.

Antimicrobial activity (microdilution assay): Antimicrobial activity was evaluated by broth microdilution in 96-well polystyrene microplates according to CLSI guidelines[26]. Each extract was dissolved in 5% DMSO and diluted to 4000 µg/mL in TSB. Two-fold serial dilutions yielded final concentrations of 2000-15.625 µg/mL per well (200 µL total volume per well). Growth and sterility controls were included on each plate. After incubation at appropriate temperatures (35±2°C for *S. aureus* and *E. coli*; 35±2°C for *P. aeruginosa*; 35°C for *C. krusei*), 10 µL of resazurin (0.01%, w/v) was added, and plates were re-incubated for 4 hrs. The MIC was the lowest concentration, showing no color change from blue to pink[27]. Experiments were performed in triplicate across three independent sessions.

RESULTS AND DISCUSSION

Foam test and saponin distribution: The foam test results are presented in Table 2. A strongly positive result (++) was obtained from the trunk bark of *M. laurentii* (column height 11 cm, stable after 30 min) and root bark of *M. dubia* (11 cm). In contrast, seeds of *M. laurentii* and trunk bark of *M. dubia* showed negative or weakly positive results, respectively. Both trunk and root bark of *P. eetveldeana* showed positive responses (+), with column heights of 5 and 4 cm, respectively. These results indicate preferential accumulation of saponins in bark organs, consistent with reports from other Fabaceae[14]. The findings guided the selection of plant material: Trunk bark for *M. laurentii*, root bark for *M. dubia*, and both bark types for *P. eetveldeana*, in agreement with prior phytochemical characterizations[20].

Saponin extraction yields: Extraction yields ranged from 3.27 to 3.6%, corresponding to dry extract masses of 5.8-11.1 g per batch (Fig. 2a-b). The highest absolute yield was obtained from *P. eetveldeana* trunk bark (11.1 g; 3.4%), while *P. eetveldeana* root bark gave the highest percentage yield (3.6%). *Millettia. laurentii* trunk bark yielded 8.96 g (3.5%), and *M. dubia* root bark yielded 5.8 g (3.27%). All extracts appeared as amorphous brownish or golden residues, consistent with saponin-enriched fractions[24]. These yields fall within the range commonly reported for crude saponin fractions obtained by methanol

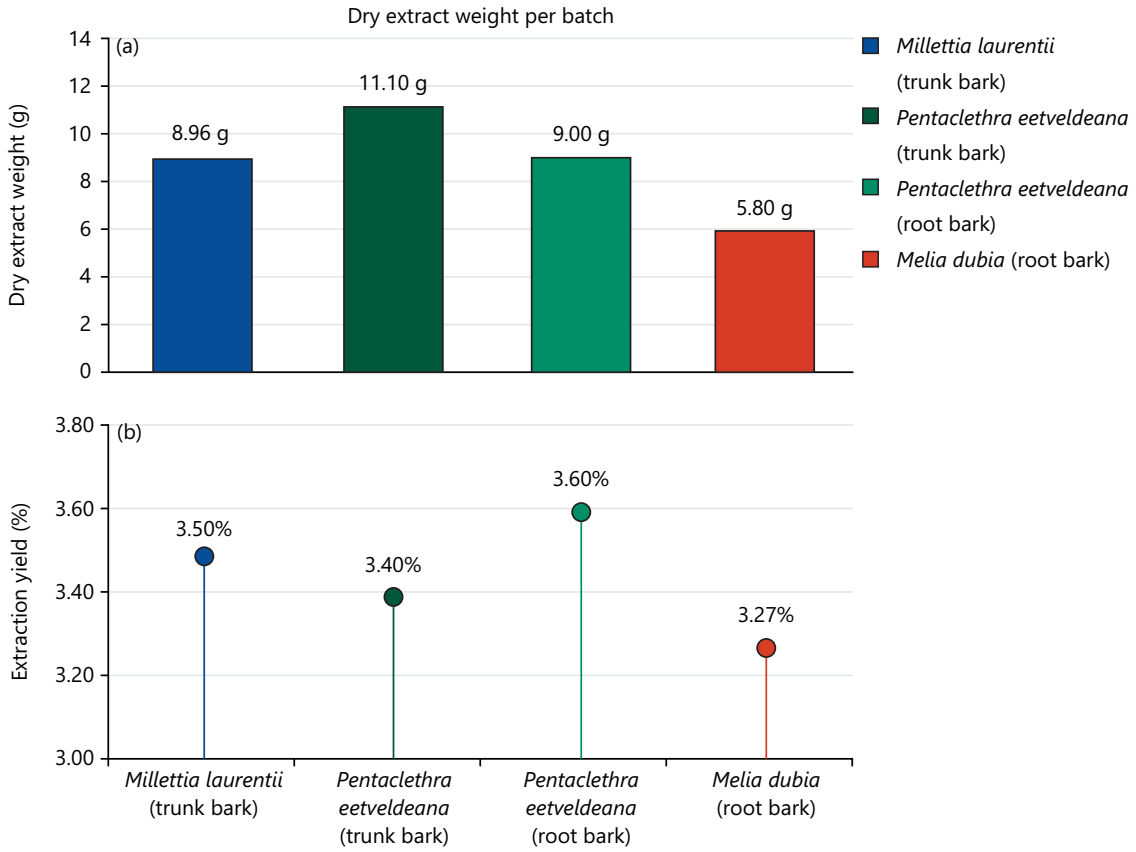

Fig. 2(a-b): Dry extract weight (g) and extraction yield (%) of saponin-rich fractions obtained from the bark of three Congolese Fabaceae species, (a) Absolute dry extract weight per batch and (b) Extraction yield (%) expressed as a percentage of the initial dry starting material

Table 2: Foam column height and stability of plant organs from three Congolese Fabaceae species as indicators of saponin content

Plant species	Organ	Column height (cm)	Structure after 15 min	Structure after 30 min	Score
Millettia laurentii	Trunk bark	11	Intact	Very slightly modified	++
Millettia laurentii	Seeds	1.5	Intact	Intact	–
Melia dubia	Trunk bark	5	1.0 cm	Gradually collapses	0
Melia dubia	Root bark	11	1.9 cm	Tends to remain stable	++
Pentaclethra eetveldeana	Trunk bark	5	1.0 cm	Tends to remain stable	+
Pentaclethra eetveldeana	Root bark	4	1.9 cm	Tends to remain stable	+

(++): Strongly positive, (+): Positive, (0): Weakly positive and (–): Negative

maceration and precipitation[10]. It is important to note that saponin content was not quantified spectrophotometrically in the crude extracts; yields are reported as gravimetric extraction yields (%), which reflect total precipitable material rather than pure saponin mass. Additionally, the hemolytic activity was not assessed for any extract, including the dialyzed *M. dubia* fraction. Given that saponins are classically hemolytic due to their interaction with membrane cholesterol, the absence of hemolysis data may limit the interpretation of the selectivity and safety of the active fractions. Future work should incorporate quantitative saponin determination, for example, using a vanillin-sulfuric acid or β-sitosterol calibration method, to allow accurate inter-species and inter-organ comparisons of saponin loading.

Antibacterial activity: The MIC values for all extracts are presented in Table 3. All five extracts demonstrated selective inhibition of *P. aeruginosa*, while no activity was detected against *E. coli* or *S. aureus* at concentrations up to 2000 µg/mL. Against *P. aeruginosa*, the dialyzed *M. dubia* extract was by far the most potent (MIC = 15.625 µg/mL), eight-fold lower than its crude counterpart (MIC = 125 µg/mL). *Pentaclethra eetveldeana* root bark showed the next best activity (MIC = 31.250 µg/mL), followed by *M. laurentii* trunk bark (MIC = 62.5 µg/mL). Although these

Table 3: Minimum inhibitory concentrations (MICs, µg/mL) of saponin-rich extracts and controls against tested microbial strains

Pathogen	*Melia dubia* (EHAER)	*Melia dubia* Dialyzed	*Millettia laurentii* (EHAET)	*Pentaclethra eetveldeana* (EHAER)	*Pentaclethra eetveldeana* (EHAET)	Control
Pentaclethra aeruginosa	125	15.625	62.5	31.25	125	2.5 (CIP)
Escherichia coli	>2000	>2000	>2000	>2000	>2000	2.0 (CIP)
Staphylococcus aureus	>2000	>2000	>2000	>2000	>2000	5.0 (CIP)
Candida krusei	2000	15.625	1000	250	1000	2000 (FLU)

EHAER: Hydroalcoholic extract of root bark, EHAET: Hydroalcoholic extract of trunk bark, CIP: Ciprofloxacin, FLU: Fluconazole. Values >2000 indicate no inhibitory activity at the highest tested concentration. All experiments were performed in triplicate across three independent sessions

values remain higher than those of ciprofloxacin (MIC = 2.5 µg/mL), they are consistent with values reported for crude saponin fractions from other species[28]. The resistance of *E. coli* and *S. aureus* is consistent with observations that the antibacterial activity of bidesmosidic saponins becomes apparent only after hydrolysis to more active monodesmosidic forms[29]. Both the aglycone nature and sugar chain composition critically influence membrane disruption capacity[12,30,31]. The selective activity against *P. aeruginosa* may relate to specific membrane composition differences, which make this bacterium more vulnerable to saponin-mediated disruption[14].

Antifungal activity: The dialyzed *M. dubia* extract demonstrated exceptional antifungal potency (MIC = 15.625 µg/mL), lower than the fluconazole control (MIC = 2000 µg/mL). *Pentaclethra eetveldeana* root bark also showed appreciable antifungal activity (MIC = 250 µg/mL), while *M. laurentii* and crude *M. dubia* showed weaker effects (MIC = 1000 and 2000 µg/mL, respectively). The superior antifungal activity reflects saponin enrichment and removal of interfering low-molecular-weight compounds during dialysis. Saponins are known to disrupt fungal membranes by interacting with ergosterol, forming pores that compromise membrane integrity[14,32]. This mechanism explains the stronger antifungal relative to antibacterial activity, as fungal membranes are sterol-rich while bacterial (particularly Gram-negative) membranes typically lack or contain different sterols. These findings are consistent with potent antifungal activities reported for triterpenoid saponins from other organisms[33-35].

Effect of dialysis purification on antimicrobial activity: Dialysis purification dramatically enhanced *M. dubia* extract activity: MIC against *P. aeruginosa* dropped from 125 to 15.625 µg/mL (8-fold); MIC against *C. krusei* dropped from 2000 to 15.625 µg/mL (128-fold). This improvement reflects two complementary effects: Removal of low-molecular-weight impurities (sugars, salts, organic acids) that compete with saponins in membrane interactions, and enrichment in saponin concentration per unit mass. Comparable purification-dependent improvements have been reported by Hassan *et al.*[30] for saponin fractions from guar, quillaja, yucca, and soybean. Stuardo and Martín[25] similarly showed that partial alkaline hydrolysis of quinoa saponins dramatically improved antifungal activity, mechanistically analogous to the enrichment achieved here. Our results indicate that the antimicrobial potential of *M. dubia* saponins may be substantially underestimated when crude extracts alone are tested, and that individual saponin compounds are likely to have even lower MIC values than 15.625 µg/mL. Further work, including isolation of individual saponin compounds by chromatographic fractionation, is necessary to confirm the observed activity and better determine structure-activity relationships.

CONCLUSION

This study constitutes a comparative evaluation of the antimicrobial activities of saponin-rich extracts from *Millettia laurentii*, *Millettia dubia*, and *Pentaclethra eetveldeana* from the Democratic Republic of Congo. All five extracts selectively inhibited *Pseudomonas aeruginosa*, while no activity was detected against *E. coli* or *S. aureus*. Most importantly, dialyzed *M. dubia* saponins demonstrated outstanding activity against both *P. aeruginosa* and *C. krusei*, outperforming fluconazole, consistent with the antimicrobial potential of individual saponin constituents recently isolated from this species by preparative HPLC, NMR spectroscopy, and mass spectrometry. These findings identify *M. dubia* as a priority candidate for

phytochemical drug discovery targeting resistant *P. aeruginosa* and *C. krusei* infections and support the valorization of Congolese Fabaceae as a source of novel antimicrobial leads. Future work should focus on the evaluation of dialyzed fractions from *M. laurentii* and *P. eetveldeana* to determine whether purification equally enhances their activities; mechanistic studies to elucidate the mode of action of the active saponins against *P. aeruginosa* and *C. krusei*; and cytotoxicity and *in vivo* pharmacological evaluation to establish the safety profiles required for preclinical development.

SIGNIFICANCE STATEMENT

This study highlights the potential of saponin-rich bark extracts from selected Congolese Fabaceae species as promising sources of antimicrobial agents. The observed selective activity against *Pseudomonas aeruginosa*, together with the enhanced potency following dialysis, particularly in *Millettia dubia*, demonstrates the importance of extract purification in revealing strong bioactive compounds. The superior antifungal performance of dialyzed *M. dubia* compared to fluconazole further supports its therapeutic relevance. These findings identify *M. dubia* as a high-priority candidate for future phytochemical isolation and development of novel agents targeting resistant bacterial and fungal pathogens.

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