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# Antibacterial, Antioxidant activities, and phytochemical content of *Piliostigma thonningii* (Schumach) Milne-Redh and *Piliostigma reticulatum* (DC) Hochst from Lubumbashi, in Democratic Republic of Congo

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## Abstract

**Background**: *Piliostigma thonningii* (Schumach.) Milne-Redh and *Piliostigma reticulatum* (DC.) Hochst. are widely employed in Katangan traditional medicine to treat respiratory and urinary tract disorders. This study evaluated the in vitro antibacterial, antioxidant and safety profiles of crude leaf, stem-bark and root extracts of both species, alongside their phytochemical composition.

**Methods**: Extracts were screened against six bacterial pathogens—*Escherichia coli*, *Salmonella typhi*, *Shigella sonnei*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Streptococcus pneumoniae*—using broth microdilution to determine minimum inhibitory concentrations (MIC) and agar diffusion to measure inhibition zones. Radical-scavenging activity was assessed by the DPPH assay. Total polyphenol and flavonoid contents were quantified spectrophotometrically (expressed as mg gallic acid equivalents [GAE]/g and mg quercetin equivalents [QE]/g, respectively). Acute oral toxicity was conducted according to OECD Guideline 423, with haematological and biochemical parameters monitored post-dosing. Physicochemical quality of the powdered plant drugs was evaluated by total ash and titratable acidity determinations.

**Results**: All extracts exhibited significant antibacterial activity, with MIC values ranging from 15 to 250 µg/mL. The most pronounced activity was observed against *E. coli* and *K. pneumoniae* (MIC = 15.625 µg/mL; Inhibition zone diameter :12–19 mm). Extracts displayed potent antioxidant capacity ($IC_{50}$ = 2.4–5 µg/mL). No acute toxicity or adverse alterations in haematological or biochemical markers were detected at the tested doses. Phytochemical analysis revealed total polyphenol contents of 47–578 mg GAE/g and total flavonoid contents of 30–435 mg QE/g across all organs. The powdered drugs contained 7–10 % total ash and exhibited titratable acidity of 2.3–3.6 mg/g.

**Conclusion**: The demonstrated antibacterial and antioxidant activities, coupled with a favourable safety profile, substantiate the traditional use of *P. thonningii* and *P. reticulatum* in managing bacterial infections and support their further development as phytomedicinal agents.

**Keywords:** Katangan medicinal plant; *E. coli*; *K. pneumoniae*; Gastrointestinal; Urinary and respiratory infections.

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## 1. Introduction

Rising bacterial resistance to conventional antimicrobials constitutes a major global public-health challenge [1,2]. In rural regions of the Democratic Republic of Congo, where access to primary healthcare is limited, traditional medicine remains widespread and, in some areas, represents the sole therapeutic option [3,4]. Among the most frequently used botanicals are *Piliostigma thonningii* (Schumach.) Milne-Redh and *Piliostigma reticulatum* (DC.) Hochst., traditionally employed against malaria, respiratory infections, Ulcers, boils, toothache, wounds, syphilitic cancer, gingivitis, Hemorrhoids, diarrhea gastrointestinal and inflammatory disorders [3,5,6]. However, preparation methods and dosage regimens vary between ethnic groups and lack standardization both across Africa [7,8] and within the DRC [3,9,10].

Phytochemical investigations of *P. thonningii* have documented alkaloids, flavonoids, tannins, coumarins, steroids and terpenoids [8,11–13], whereas *P. reticulatum* appears to share the same metabolite classes [14,15] but remains poorly characterized quantitatively and without a comprehensive thin-layer chromatography (TLC) fingerprint.

*In vitro* studies report promising antibacterial and antioxidant activities for crude extracts of both species [7,14,16,17], yet critical pathogens such as *Klebsiella pneumoniae* and *Shigella sonnei* are often omitted and activity is not consistently linked to specific phytochemical groups. Regarding safety, an ethanolic leaf extract of *P. thonningii* exhibited oral and intraperitoneal $LD_{50}$ values > 5000 mg/kg in rats[18], a finding confirmed by an aqueous leaf extract with no acute toxicity in mice [19]. Similarly, a methanolic extract of *P. reticulatum* was found to have an oral $LD_{50}$ > 5000 mg/kg in Swiss mice [20]. However, these safety data do not extend across all three principal organs of either species.

The present study aims to characterize the antibacterial and antioxidant activities, to profile phytochemical content and to evaluate acute oral toxicity of methanolic extracts from leaves, stem bark and root bark of *P. thonningii* and *P. reticulatum* collected in Lubumbashi, DRC, by determining minimum inhibitory and bactericidal concentrations against *Escherichia coli*, *Salmonella typhi*, *Shigella sonnei*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Streptococcus pneumoniae*, quantifying total phenolics, flavonoids and tannins, qualitatively identifying alkaloids, anthraquinones, coumarins, flavonoids, polyphenols, saponins, steroids, tannins and terpenoids and establishing a TLC fingerprint, measuring DPPH radical-scavenging activity to calculate $IC_{50}$ values, and estimating oral $LD_{50}$ in Swiss mice according to OECD guideline 423.

## 2. Material and Methods

### 2.1. Plant material

*Piliostigma thonningii* (PITO; 11°34′12″ S, 27°22′44″ E; altitude 1,250 m) and *Piliostigma reticulatum* (PIRE; 11°30′26″ S, 27°27′55″ E; altitude 1,260 m) were collected in January 2021 from the INERA Kipopo research station, Haut-Katanga Province, Democratic Republic of the Congo. Botanical identification was confirmed at the Kipopo Herbarium, where voucher specimens were deposited under accession numbers PITO: KIP193744229 and PIRE: KIP193744229.

Following air-drying at ambient temperature (25 °C), the samples were ground using an electric stainless-steel mill (Plymix PX-MFC 90 D, Belgium). A 350 g portion of the resulting powder was macerated in 1 L of methanol (Sigma-Aldrich, CAS 67-56-1) at 25 °C for 72 hours with continuous agitation. The extract was then filtered through Whatman No. 1 filter paper (Whatman, USA) and concentrated under reduced pressure (180 mbar) at 50 °C using a rotary evaporator (Büchi R-210, Switzerland).

### 2.2. Experimental Animals

Eighteenth male Swiss albino mice weighing between 25 to 35 g were used in this study. The animals were kept at an ambient temperature of 25 ◦ C and a light/dark cycle of 12 h in cages at the Faculty of Pharmacy in Lubumbashi. They had free access to water and normal food throughout the experiment. The experiment was carried out according to the principles described in the "Guide to the care and use of laboratory animals," 8th edition, prepared by the National Academy of Sciences (National Research Council of the National Academies). Every effort was made to minimize animal suffering and the number of animals used for the study. Ethics approval was obtained from University in Lubumbashi.

## 2.3. Determination of physicochemical parameters

### *2.3.1. Loss on drying (LOD)*

Loss on drying was determined by accurately weighing 0.500 ± 0.001 g of finely pulverized, air-dried plant material into a pre-weighed flat-bottomed capsule (50 mm diameter × 30 mm height). The capsule was dried in a convection oven at 105 °C for 3 h, cooled in a desiccator to room temperature for 30 min, and re-weighed to constant mass. Loss on drying was expressed as the percentage weight loss relative to the initial sample mass:

$$\textbf{LOD (\%)} = \frac{\textbf{Wi} - \textbf{Wf}}{\textbf{Wi}} \textbf{ x 100 \% (1)}$$

where *W*i and *W*f are the weights of the capsule plus sample before and after drying, respectively.

### *2.3.2. Total ash (TA)*

One gram of the pulverized drug was placed in a porcelain crucible. The test sample was distributed evenly within the crucible. The sample was subjected to an oven drying process for one hour at a temperature range of 100-105°C. After that, it was incinerated in a muffle furnace at 600 ± 25°C. It is important that the sample does not ignite at any point during this process. Once the sample had been incinerated, it was allowed to cool in a desiccator before being weighed using an analytical balance. Subsequently, the crucible was permitted to cool in a desiccator before being weighed on an analytical balance. The total ash content was calculated as follows [21]:

$$\textbf{TA } (\%) = \frac{\textbf{m}}{\textbf{n}} \textbf{ x 100 \% (2)}$$

**m** is the weight after incineration, **n** is the test sample.

### *2.3.3. Hydrochloric acid soluble ash (HAA)*

To ascertain the ash content, 15 mL of distilled water and 10 mL of hydrochloric acid were added to the residue obtained during the total ash determination. Subsequently, the crucible was covered with a watch glass and boiled gently for ten minutes. Following this, the crucible was allowed to cool. Subsequently, the residue was filtered through an ash-free filter paper, with the filtrate subsequently washed with hot water until it reached a neutral pH. Subsequently, the filtrate was transferred to a dry, tared crucible. Subsequently, the crucible containing the filter paper was subjected to an oven firing for 24 hours. Subsequently, the crucible was subjected to a further calcination process for six hours at a temperature of 600°C. Following cooling in a desiccator, the crucible containing the ash was reweighed, and the hydrochloric ash was calculated as follows [21,22]:

$$\textbf{AA } (\%) = \frac{\textbf{P1}}{\textbf{P2}} \textbf{ x 100 \% (3)}$$

P1 is the weight after calcination and P2 the weight before calcination.

### *2.3.4. Sulfuric acid soluble ash (SAA)*

In order to determine the sulfuric-acid-soluble ash, 2.000 ± 0.005 g of air-dried, finely powdered plant material was accurately weighed into a pre-heated, pre-weighed silica crucible (550 °C, 30 min), cooled in a desiccator and ashed in a muffle furnace at 550 ± 25 °C until constant mass (mass change < 0.5 mg) was achieved. After cooling in a desiccator, the total ash weight ($W_1$) was recorded. The ash was then moistened with 1 mL of 2 M $H_2SO_4$ and gently boiled under reflux with 25 mL of the same acid for 5 min. The hot suspension was filtered through ashless filter paper and the insoluble residue washed repeatedly with hot distilled water until the filtrate tested negative for sulfate (0.1 M $BaCl_2$). The filter paper plus residue were dried at 105 °C for 1 h, returned to the same crucible, ignited at 550 °C for 15 min, cooled in a desiccator and weighed to obtain the acid-insoluble ash weight ($W_2$) [23]. The sulfuric-acid-soluble ash content was calculated as:

$$\textbf{SAA } (\%) = \frac{\textbf{W1} - \textbf{W2}}{\textbf{2.000}} \textbf{ x 100 \% (4)}$$

All determinations were performed in triplicate and results expressed as mean ± SD.

*2.3.5. Determination of Titratable Acidity and Alkalinity*

The titratable acidity and alkalinity indices, expressed as milligrams of titrant per gram of sample, correspond to the amount of 0.1 M NaOH or HCl required to neutralize the free acid or base in 1 g of dried plant material. For determination, 10.0 g of finely powdered drug was dissolved in 50 mL of a 1:1 (v/v) mixture of 96 % ethanol and light petroleum ether previously neutralized with 0.1 M NaOH (for acidity) or 0.1 M HCl (for alkalinity); after addition of 0.5 mL of phenolphthalein, titration was performed with 0.1 M NaOH until a faint pink endpoint persisted for 30 s (acid index) or with 0.1 M HCl until the pink color just vanished (alkalinity index), and the volume of titrant consumed was used to calculate the index.

$$\mathbf{A}_{\mathbf{Index}}\ (\mathbf{mg/g}) = \frac{\mathbf{VxMxEM\ x1000}}{\mathbf{10.0}} \quad \mathbf{(5)}$$

Where *V*= volume of titrant (mL); *M* = molarity of titrant (mol/L); EW = equivalent weight of NaOH (40 mg/mmol) or HCl (36.5 mg/mmol); 10.0 = mass of sample (g) used in the assay.

*2.3.6. Preliminary phytochemical screening*

The whole plant powders were analyzed for the presence of alkaloids, coumarins, flavonoids, saponins, steroids, tannins, terpenoids, and phenols. This was conducted using standard tube reactions, as previously reported [24–26].

*2.3.7. Determination of total phenol, flavonoid and tannin content*

The total phenolic content of each sample was quantified using a Folin-Ciocalteu method [27]. The procedure involved the preparation of a solution of extract (100 µg . $mL^{-1}$) or gallic acid (100-600 µg . $mL^{-1}$) in 1 mL of 10% (w/v) Folin-Ciocalteu reagent, which was then mixed with 2.5 mL of the solution. Subsequently, 2.0 mL of $Na_2CO_3$ (75%) was added to the mixture, which was then incubated at 50 ºC for 10 minutes with intermittent stirring. Subsequently, the sample was cooled, and the absorbance was measured using a UV spectrophotometer (Shimazu, UV-1800) at 765 nm against a blank. The results were expressed in milligrams of gallic acid equivalents per gram of dry extract (mg GAE.$g^{-1}$) using a calibration curve (y = 0.0696x + 0.0113, $R^2$ = 0.998; linearity range, 1–1200 mg. $mL^{-1}$).

As previously described, the total flavonoid content was quantified using an aluminum trichloride assay [28]. Briefly, An aliquot of 1 mL extract solution (100 µg . $mL^{-1}$) or quercetin (25-250 µg. $mL^{-1}$) was combined with 0.2 mL of a 10% (w/v) $AlCl_3$ solution in methanol, 0.2 mL of a 1 M potassium acetate solution, and 5.6 mL of distilled water. The mixture was incubated for 30 minutes at room temperature, after which the absorbance was measured at 415 nm against the blank. The results were expressed in milligrams of quercetin equivalents per gram of dry extract (mg QE.$g^{-1}$) using a quercetin calibration curve (y = 0.006 x + 0.004, $R^2$ = 0.996; linearity range, 0.1 to 150 mg. $mL^{-1}$).

The total condensed tannin content was determined using a vanillin method, as previously described [29]. Briefly, 1 mL of a solution of the extract (100 µg/mL) or gallic acid (100-600 µg/mL) was mixed with 5 mL of a vanillin solution (0.5 g reagent and 200 mL 4% HCl in methanol). The absorbance at 500 nm, following a 20-minute reaction time, was quantified using a spectrophotometer (SP 2000 UV, Bel Photonics®, Piracicaba, SP, Brazil) in comparison to a blank. The results were expressed in milligrams of gallic acid equivalents per gram of plant dry extract (mg GAE.g-1ED) using a calibration curve based on gallic acid (y = 0.005 x + 0.0012, $R^2$ = 0.997; linearity range, 1 - 100 mg. $L^{-1}$).

## 2.4. DPPH assay

The antioxidant activity of the samples was evaluated using the DPPH assay, as previously described [30]. Briefly, 50 µL of extracts (or positive control) were prepared at different dilutions of order 2 in methanol from 100 g. $mL^{-1}$ solution, was interacted with 1950 µL of 0.002% DPPH in a 96-well plate (Nunc WVR, Germany). Following a 30-minute incubation period in the dark, the solution was read at 492 nm (Thermo Fisher Scientific Inc., Waltham, USA). The assays were conducted in triplicate, with the 0.002% DPPH solution as the negative control. The percentage of antioxidant activity was calculated using the following formula :

$$\text{AOA (\%)} = \frac{\mathbf{(Ab-Ae)}}{\boldsymbol{Ab}}\boldsymbol{x}\ \mathbf{100}\ \text{\% (6)}$$

Where, (Ab) is the absorbance measured in the presence of the negative control, (Ae) is the absorbance measured in the presence of the extract, and (AOA) is the calculated percentage of inhibition. The experiment was performed in triplicate.

In this study, the antioxidant activity of the extracts was categorized as follows: very strong when $IC_{50}$ < 50 µg/mL, strong when 50 µg/mL ≤ $IC_{50}$ ≤ 100 µg/mL, moderate when 100 µg/mL < $IC_{50}$ < 250 µg/mL, weak: 250 µg/mL < $IC_{50}$ < 500 µg/mL, and absent when $IC_{50}$ ≥ 500 µg/mL [21,30].

### 2.5. Anti-bacterial activity test

Antibacterial activity was assessed by the macrodilution test in a tube, by determining MIC, and by macrodiffusion on a disk, by determining DZI [21,31–33].

The bacterial strains included in this study were *Escherichia coli (E coli)* ATCC 25922*, Salmonella typhi (S typhi)* ATCC 14028*, Shigella sonnei (S sonnei)* ATCC 25931*, Staphylococcus aureus (S aureus)* ATCC 6538*, Klebsiella pneumoniae (K pneumoniae)* ATCC 13883 et *Streptococcus pneumoniae (S pneumoniae)* ATCC 49619. The bacterial strains were obtained from the Lubumbashi Provincial Laboratory, where tests were conducted using an agar diffusion method to ascertain the diameter of the zone of inhibition (DZI). Additionally, the broth dilution method was employed in triplicate to ascertain the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC) of the extract [21].

These distinct parameters (DZI, MIC, and MBC) were utilized to delineate the antimicrobial activity of the extracts. The microorganisms selected for this study were based on their prevalence in locally observed instances of bacterial gastroenteritis.

For susceptibility testing, 20 mL of Muller-Hinton agar was poured into sterile Petri dishes, and allowed to stand for 20 minutes. Subsequently, 1 mL of a bacterial suspension containing $10^8$ CFU (colony-forming units) per mL was evenly distributed over the entire surface of each culture medium. Blotting paper discs (with a diameter of 6 mm) were impregnated with 5 µL of extracts (with a concentration of 50 µg. $mL^{-1}$), and placed on the surface of the solidified, infected medium. Subsequently, the Petri dishes were incubated at 37°C for 48 hours in an oven. The susceptibility of the germs to the extracts was estimated by measuring the diameter (in millimeters) of the zone of inhibition induced by different concentrations of plant extracts around the discs. Each experiment was repeated three times. The Extracts were categorized as follows according to their MIC values: very active (MIC < 100 µg/mL), moderately active (100 ≤ MIC < 500 µg/mL), weakly active (500 ≤ MIC ≤ 1000 µg/mL), and inactive (MIC > 1000 µg/mL) [21,31–33].

To determine MIC, 100 µL of each extract at a concentration of 1 mg/mL, dissolved in methanol (petroleum ether), was combined with 1900 µL of culture medium. Subsequently, eight successive dilutions of order 2 (ranging from 500 µg. $mL^{-1}$ to 1.9 µg. $mL^{-1}$) were prepared for each extract and placed in different 5 mL aseptic tubes. After that, 1000 µL of standard inoculum was added to each tube, and the mixture was incubated for 24 h at 37°C. The growth of microorganisms was observed visually. The minimum inhibitory concentration (MIC) was determined as the lowest concentration at which the extract prevented visible bacterial growth [34]. Extracts were categorized as follows according to their MIC values: very active (MIC < 100 µg. $mL^{-1}$), moderately active (100 ≤ MIC < 500 µg. $mL^{-1}$), weakly active (500 ≤ MIC ≤ 1000 µg . $mL^{-1}$), and inactive (MIC > 1000 µg.$mL^{-1}$).

To determine the minimum bactericidal concentration (MBC), samples were taken from tubes that exhibited no visible growth, as had been used to determine the minimum inhibitory concentration (MIC). Inoculation was conducted in Petri dishes on Mueller Hinton agar medium, and incubation was performed at 37°C for 24 hours. The growth of microorganisms was monitored visually, and the minimum bactericidal concentration (MBC), defined as the lowest concentration at which the extract prevented visible bacterial growth after transplantation, was determined [35]. The effect of the extract was determined according to the ratio between the minimum bactericidal concentration (MBC), and the minimum inhibitory concentration (MIC). A bactericidal effect was considered present when the ratio was ≤ 2, while a bacteriostatic effect was observed when the ratio was >2 [32]. The experiment was performed in triplicate.

### 2.6. Acute oral toxicity

Acute oral toxicity of methanolic extracts from the leaves, stem bark and root bark of *Piliostigma thonningii* (PITO) and *Piliostigma reticulatum* (PIRE) was evaluated in male Swiss albino mice (25 ± 2 g, n = 6 per group) according to OECD Guideline 423. After a 3-h fast, each group received a single oral gavage dose of 5 000 $mg{\cdot}kg^{-1}$ of the respective extract dissolved in 0.9 % NaCl (control group: vehicle alone) and was monitored individually for 14 days for mortality, clinical signs of toxicity and changes in body weight. On day 15, following overnight fasting, mice were anesthetized and blood was collected by cardiac puncture into EDTA and serum tubes; animals were then euthanized, and the heart, liver, kidneys and spleen were excised, blotted dry and weighed to determine relative organ weights (organ weight/body weight × 100). Serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and γ-glutamyltransferase (γ-GT) were quantified spectrophotometrically using commercial

reagent kits (Elabscience®), and hematological parameters (white blood cell count, red blood cell count, hemoglobin concentration, hematocrit and platelet count) were measured with an automated hematology analyzer (AR-9000, ARI group, China). Data are presented as mean ± SEM.

### 2.7. Statistical analysis

All the tests were carried out in triplicate and the results are expressed as mean ± Standard deviation (SD). In bivariate analysis, the T Student Test was used and in multivariate analysis, the one-way ANOVA test. The significance level of the test was set at 95%.

## 3. Results

### 3.1. Sensitivity of bacteria to different extracts

In agar-well diffusion assays, methanolic extracts of *Piliostigma thonningii* (PITO) and *Piliostigma reticulatum* (PIRE) inhibited the growth of all tested pathogens, yielding inhibition-zone diameters (IZDs) of 10.1 ± 0.4 mm (PITO vs. *Shigella sonnei*) to 19.7 ± 0.1 mm (PIRE vs. *Salmonella typhi*). By the criteria of > 10 mm IZD for antibacterial activity [21,36], both extracts qualify as active. *Escherichia coli* and *Klebsiella pneumoniae* were particularly susceptible, exhibiting IZDs > 12 mm across all treatments. Notably, PIRE displayed broad-spectrum efficacy, with IZDs exceeding 19 mm against every strain tested. Overall, PIRE demonstrated superior antibacterial potency compared to PITO (Table 1).

**Table 1** Inhibition zone diameter of methanolic extracts of leaves, stem bark, root of *Piliostigma thonningii* and *Piliostigma reticulatum* at 500 µg. $mL^{-1}$.

| Simple | *E. coli* | *S. typhi* | *S. sonnei* | *K. pneumoniae* | *S. aureus* | *S. pneumoniae* |
|---|---|---|---|---|---|---|
| CIP | 26.0 ± 1.2 | 24.1 ± 0.4 | 18.1 ± 0.2 | 23.0 ± 0.1 | 23.1 ± 0.4 | 23.1 ± 0.4 |
| PITOF | 12.1 ± 0.2 | 12.7 ± 0.1 | 12.1 ± 0.2 | 12.2 ± 0.2 | 12.2 ± 0.6 | 12.2 ± 0.8 |
| PITOT | 12.4 ± 0.1 | 10.3 ± 0.2 | 10.1 ± 0.4 | 12.4 ± 0.2 | 10.1 ± 0.2 | 10.1 ± 0.2 |
| PITOR | 16.1 ± 0.2 | 15.7 ± 0.1 | 15.1 ± 0.2 | 16.2 ± 0.2 | 15.2 ± 0.6 | 15.2 ± 0.8 |
| PIREF | 18.1 ± 0.2 | 18.7 ± 0.1 | 18.1 ± 0.2 | 18.2 ± 0.2 | 18.2 ± 0.6 | 18.2 ± 0.8 |
| PIRET | 18.4 ± 0.1 | 18.3 ± 0.2 | 18.1 ± 0.4 | 18.1 ± 0.2 | 18.1 ± 0.2 | 18.1 ± 0.2 |
| PIRER | 19.1 ± 0.2 | 19.7 ± 0.1 | 19.1 ± 0.2 | 19.2 ± 0.2 | 19.2 ± 0.6 | 19.2 ± 0.8 |

***Legend*** - PITOF: *Piliostigma thonningii* leaves; PITOT : *Piliostigma thonningii* stem bark; PITOR: *Piliostigma thonningii* root; PIREF: *Piliostigma reticulatum* leaves; PIRET : *Piliostigma reticulatum* stem bark; PIRER: *Piliostigma reticulatum* root; Results expressed as mean ± standard deviation with n= 3; Ciprofloxacin (CIP) was used as a positive control (DZI: 18-28 mm) and the dilution solvent (5% ) from each extract was used as negative control. No zone of inhibition was observed in the negative control.

### 3.2. Minimum inhibitory concentration, minimum bactericidal concentration and effect of extracts

Methanolic extracts prepared from the leaves, stem bark and root bark of *Piliostigma thonningii* (PITO) and *Piliostigma reticulatum* (PIRE) revealing minimum inhibitory concentrations (MICs) of 15.625–250 µg/mL. Both PITO and PIRE completely inhibited the growth of *Escherichia coli* and *Klebsiella pneumoniae* at 15.625 µg/mL, indicative of strong efficacy against uropathies commonly implicated in cystitis as well as pathogens responsible for lower respiratory tract infections. Notably, the PIRE extract exhibited superior potency against *K. pneumoniae*, *Staphylococcus aureus* and *Streptococcus pneumoniae* compared with its PITO counterpart, positioning it as a promising lead for the development of novel anti-pneumonic therapeutics (Table 2).

**Table 2** Minimum inhibitory concentration (MIC in µg. $mL^{-1}$) and minimum bactericidal concentration (MBC in µg/mL) of methanolic extracts of *Piliostigma thonningii and Piliostigma reticulatum*.

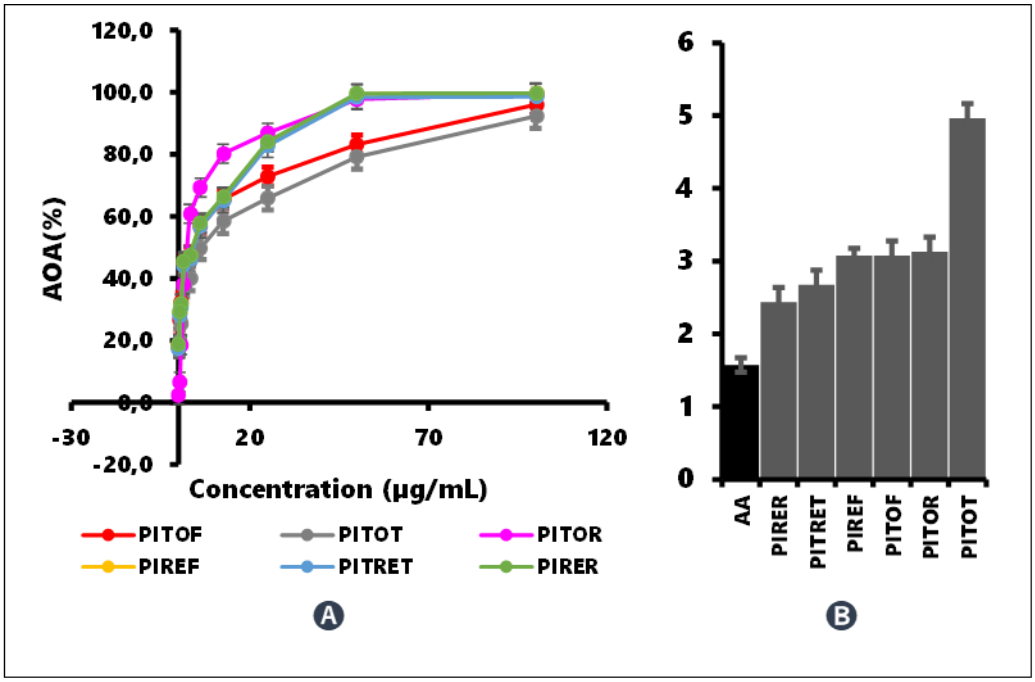
| Simple | Bacterial Strain | CMI (µg/mL) | CMB (µg/mL) | CMB/CMI | Effects |
|---|---|---|---|---|---|
| PITOF | *E. coli* | 250 | 250 | 1 | BC |
| | *S. typhi* | 125 | 250 | 2 | BC |
| | *S. sonnei* | 250 | 250 | 1 | BC |
| | *K. pneumoniae* | 62.5 | 125 | 2 | BC |
| | *S. aureus* | 125 | 125 | 1 | BC |
| | *S. pneumoniae* | 125 | 125 | 1 | BC |
| PITOT | *E. coli* | 15.625 | 15.625 | 1 | BC |
| | *S. typhi* | 31.25 | 62.50 | 2 | BC |
| | *S. sonnei* | 250 | 250 | 1 | BC |
| | *K. pneumoniae* | 15.625 | 15.625 | 1 | BC |
| | *S. aureus* | 250 | 250 | 1 | BC |
| | *S. pneumoniae* | 15.625 | 15.625 | 1 | BC |
| PITOR | *E. coli* | 15.625 | 15.625 | 1 | BC |
| | *S. typhi* | 125 | 125 | 1 | BC |
| | *S. sonnei* | 250 | 250 | 1 | BC |
| | *K. pneumoniae* | 15.625 | 15.625 | 1 | BC |
| | *S. aureus* | 125 | 125 | 1 | BC |
| | *S. pneumoniae* | 125 | 125 | 1 | BC |
| PIREF | *E. coli* | 15.625 | 15.625 | 1 | BC |
| | *S. typhi* | 31.25 | 62.50 | 2 | BC |
| | *S. sonnei* | 250 | 125 | 1 | BC |
| | *K. pneumoniae* | 15.625 | 15.625 | 1 | BC |
| | *S. aureus* | 15.625 | 15.625 | 1 | BC |
| | *S. pneumoniae* | 15.625 | 12.5 | 1 | BC |
| PIRET | *E. coli* | 15.625 | 15.625 | 1 | BC |
| | *S. typhi* | 15.625 | 31.25 | 2 | BC |
| | *S. sonnei* | 6.3 | 6.3 | 1 | BC |
| | *K. pneumoniae* | 15.625 | 15.625 | 1 | BC |
| | *S. aureus* | 15.625 | 15.625 | 1 | BC |
| | *S. pneumoniae* | 15.625 | 15.625 | 1 | BC |
| PIRER | *E. coli* | 31.25 | 31.25 | 1 | BC |
| | *S. typhi* | 15.625 | 15.625 | 1 | BC |
| | *S. sonnei* | 250 | 250 | 1 | BC |
| | *K. pneumoniae* | 15.625 | 15.625 | 1 | BC |
| | *S. aureus* | 15.625 | 15.625 | 1 | BC |

| Simple | Bacterial Strain | CMI (µg/mL) | CMB (µg/mL) | CMB/CMI | Effects |
|---|---|---|---|---|---|
| | *S. pneumoniae* | 15.625 | 15.625 | 1 | BC |

***Legend*** - CIP : Ciprofloxacin; PITOF: *Piliostigma thonningii* leaves; PITOT : *Piliostigma thonningii* stem bark; PITOR: *Piliostigma thonningii* root; PIREF: *Piliostigma reticulatum* leaves; PIRET : *Piliostigma reticulatum* stem bark; PIRER: *Piliostigma reticulatum* root; BC: Bactericide; Ciprofloxacin was employed as a positive control, demonstrating MIC values below 5 µg/mL, whereas the uninoculated culture medium and the 5% methanol vehicle served as negative controls and showed no detectable antibacterial activity.

### 3.3. Antioxidant activity

Methanolic extracts of the leaves, stem bark and root bark of Piliostigma thonningii (PITO) and P. reticulatum (PIRER) were assessed for DPPH radical-scavenging activity. Both extracts exhibited a clear dose–response relationship over 5–100 µg/mL, achieving 2.4–99.7 % inhibition and fitting an exponential decay model ($R^2 > 0.99$). $IC_{50}$ values of 2.4 µg/mL (PIRER) and 5.0 µg/mL (PITO) classify them as highly active antioxidants (threshold < 50 µg/mL), although they were less potent than the ascorbic acid reference ($IC_{50}$ = 1.6 µg/mL). Notably, the root bark extract of P. reticulatum (PIRER) displayed the strongest radical-scavenging efficacy, highlighting its promise as a source of natural antioxidant compounds (Figure 1).




***Legend*** – PITO: *Piliostigma thonningii*; PIRE : *Piliostigma reticulatum*; PITOF: *Piliostigma thonningii* leaves; PITOT : *Piliostigma thonningii* stem bark; PITOR: *Piliostigma thonningii* root; PIREF: *Piliostigma reticulatum* leaves; PIRET : *Piliostigma reticulatum* stem bark; PIRER: *Piliostigma reticulatum* root; AA: Ascorbic acid used as positive control; AOA: Antioxidant activity.

**Figure 1** Antioxidant activity of methanolic extracts of *Piliostigma thonningii* and *Piliostigma reticulatum* expressed as percentage DPPH inhibition ⓐ and as $IC_{50}$ ⓑ; ((X )¯± S, n=3).

### 3.4. Phytochemical groups identified

Phytochemical screening of secondary metabolites in the leaves, stem bark and root bark of *Piliostigma thonningii* and *Piliostigma reticulatum* revealed the widespread presence of coumarins, flavonoids, polyphenols, tannins and terpenoids, whereas anthraquinones and saponins were uniformly absent. Notably, alkaloids were detected solely in the leaves and root bark of *P. reticulatum* (Table 3).

**Table 3** Phytochemical groups of antibacterial interest identified in leaves, stem bark, and root of *Piliostigma thonningii* and *Piliostigma reticulatum*.

| Group | Tube Solution Test | PITOF | PITOT | PITOR | PIREF | PIRET | PIRER |
|---|---|---|---|---|---|---|---|
| Alkaloids | Dragendorff | - | - | - | + | + | + |
| | Mayer | - | - | - | + | - | + |

| Group | Tube Solution Test | PITOF | PITOT | PITOR | PIREF | PIRET | PIRER |
|---|---|---|---|---|---|---|---|
| | Hager | - | - | - | + | - | + |
| | Bertrand | - | - | - | + | - | + |
| | Sonnenschein | - | - | - | + | - | + |
| Anthraquinones | Bornträger | - | - | - | - | - | - |
| Coumarins | Sodium hydroxide solution test | + | + | + | + | + | + |
| Flavonoids | Shinoda | + | + | + | + | + | + |
| Polyphenols | Ferric Chloride Test | + | + | + | + | + | + |
| Saponins | Foam test | - | - | - | - | - | - |
| Steroids | Liebermann-Burchard | - | + | + | + | + | + |
| Tannins | Braemer | + | + | + | + | + | + |
| Terpenoids | Liebermann-Burchard | + | + | + | + | + | + |
| | Hirschson | + | + | + | + | + | + |

***Legend*** - Present: + ; Absent: - ; *Dragendorff's reagent*: $Bi(NO_3)_3$ + KI + tartaric acid ($C_4H_6O_6$) ; *Mayer's reagent*: $HgCl_2$ + KI ; *Bertrand's reagent*: potassium sodium tartrate ($KNaC_4H_4O_6$) + $CuSO_4{\cdot}5H_2O$ + $Fe_2(SO_4)_3/H_2SO_4$ + $KMnO_4$ ; *Sonnenschein's reagent*: phosphomolybdic acid ($H_3PMo_{12}O_{40}$) ; *Shinoda reagent*: Mg + HCl ; *Bornträger's reagent*: $FeCl_3$ + HCl ; *Liebermann–Burchard reagent*: acetic anhydride ($C_4H_6O_3$) + $H_2SO_4$ ; *Braemer's reagent*: $FeCl_3$ ; *Hirschhorn's reagent*: trichloroacetic acid ($CCl_3CO_2H$) ; Bate–Smith test: conc. HCl. PITOF: *Piliostigma thonningii* leaves; PITOT : *Piliostigma thonningii* stem bark; PITOR: *Piliostigma thonningii* root; PIREF: *Piliostigma reticulatum* leaves; PIRET : *Piliostigma reticulatum* stem bark; PIRER: *Piliostigma reticulatum* root.

### 3.5. Total phenolic, flavonoids and total tannins content

Total phenolic and tannin contents were quantified against gallic acid standards and expressed as mg GAE·$g^{-1}$ of extract. Phenolic concentrations ranged from 47.21 ± 0.12 to 577.24 ± 1.89 mg GAE·$g^{-1}$, while tannin levels varied between 21.30 ± 0.21 and 169.30 ± 0.17 mg GAE·$g^{-1}$. Total flavonoids, measured as quercetin equivalents (mg QE·$g^{-1}$), spanned 30.01 ± 0.31 to 447.12 ± 0.41 mg QE·$g^{-1}$. In both *Piliostigma thonningii* and *P. reticulatum*, leaf and root-bark extracts consistently exhibited higher polyphenol contents than stem-bark extracts. Notably, *P. thonningii* leaves contained the highest levels of flavonoids and total phenolics, whereas tannins were most abundant in the stem bark of *P. reticulatum* (Table 4).

**Table 4** Total phenols, flavonoids and tannins content in leaves, of *Piliostigma thonningii* and *Piliostigma reticulatum*

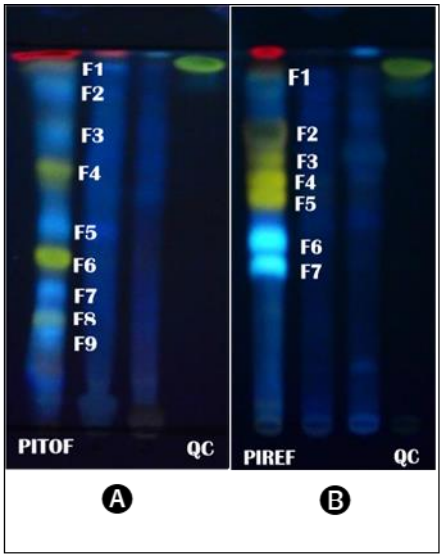
| Simple | Total Flavonoids (mg EQC/100g) | Total polyphenols (mg EAG/100g) | Total tannins (mg EAG/100g) | Ratio TPC/TTC |
|---|---|---|---|---|
| PITOF | 435.90 ± 1.59 | 577.24 ± 1.89 | 113.12 ± 0.12 | 5.1 |
| PITOT | 030.01 ± 0.31 | 047.21 ± 0.12 | 021.30 ± 0.21 | 2.2 |
| PITOR | 030.03 ± 0.28 | 078.18 ± 0.11 | 034.36 ± 0.20 | 2.3 |
| PIREF | 447.12 ± 0.41 | 471.13 ± 0.13 | 140.30 ± 0.24 | 3.4 |
| PIRET | 038.51 ± 0.27 | 078.15 ± 0.11 | 031.23 ± 0.12 | 2.5 |
| PIRER | 296.12 ± 0.70 | 471.21 ± 0.12 | 169.30 ± 0.17 | 2.8 |

*Legend* - PITOF: *Piliostigma thonningii* leaves; PITOT : *Piliostigma thonningii* stem bark; PITOR: *Piliostigma thonningii* root; PIREF: *Piliostigma reticulatum* leaves; PIRET : *Piliostigma reticulatum* stem bark; PIRER: *Piliostigma reticulatum* root

### 3.6. TLC Profile

Thin-layer chromatographic fractionation of the methanolic leaf extracts yielded nine fractions from *Piliostigma thonningii*, four of which (F1, F4 and F6) were identified as quercetin-type flavonoids, and seven fractions from *P. reticulatum*, five of which (F1–F5) exhibited flavonoid profiles. Fractions fluorescing sky-blue under UV illumination were tentatively assigned to phenolic acids. These data reveal that, despite their similar morphology, the two species'

leaves are phytochemically distinct, a divergence that may underlie their differing applications in traditional medicine (Figure 2).




***Legend*** **-** Mobile phase: ethyl acetate–formic acid–glacial acetic acid–water (10.0:1.1:1.1:2.6, v/v/v/v). Stationary phase: silica gel 60 F254 TLC plates (Merck; 5 × 10 cm, glass-backed). Detection: UV illumination at 360 nm following spray-derivatization with Neu's reagent. Quercetin (10 µg) was used as a positive control.

**Figure 2** Thin-Layer Chromatography Profile of the Leaves of *Piliostigma thonningii* (PITOF) and *Piliostigma reticulatum* (PIREF)

### 3.7. Dry matter, Ash, total acidity, pH

Titratable acidity/alkalinity of the samples ranged from 2.3 to 3.6 mg $g^{-1}$. Total ash content varied between 6.1 and 9.7 %, whereas hydrochloric acid–soluble ash was 0.6 % and sulfuric acid–soluble ash ranged from 2.1 to 2.4 %. Loss on drying, determined at 105 °C, was 95–98 %, and the pH of the extracts spanned 3.8 to 7.8 (Table 5).

**Table 5** Moisture, ash and acidity in *Piliostigma thonningii and Piliostigma reticulatum*

| Parameters | Titratable acidity/Alkalinity | Ash HCl | Ash $H_2SO_4$ | Total Ash | LOD | pH |
|---|---|---|---|---|---|---|
| | $mg.g^{-1}$ | % | % | % | % | $mol.L^{-1}$ |
| PITOF | 2.3 ± 0.1 | 0.6 ± 0.2 | 2.1 ± 0.4 | 7.1 ± 0.1 | 95.9 ± 0.4 | 3.8 ± 0.4 |
| PITOT | 2.6 ± 0.1 | 0.6 ± 0.1 | 2.2 ± 0.1 | 9.7 ± 0.4 | 98.2 ± 0.1 | 3.9 ± 0.1 |
| PITOR | 2.3 ± 0.1 | 0.6 ± 0.3 | 2.1 ± 0.4 | 7.1 ± 0.1 | 97.9 ± 0.4 | 3.8 ± 0.4 |
| PIREF | 3.6 ± 0.2 | 0.6 ± 0.1 | 2.3 ± 0.1 | 8.7 ± 0.4 | 97.2 ± 0.1 | 7.8 ± 0.1 |
| PIRET | 3.3 ± 0.1 | 0.6 ± 0.2 | 2.3 ± 0.4 | 6.1 ± 0.1 | 98.9 ± 0.4 | 7.7 ± 0.4 |
| PIRER | 3.6 ± 0.1 | 0.6 ± 0.1 | 2.4 ± 0.1 | 8.7 ± 0.4 | 97.3 ± 0.1 | 7.8 ± 0.1 |

***Legend*** **-** Results expressed as mean ± standard deviation; n = 3; 95% confidence interval; analysis of variances: a if p <0.01; b if p <0.001; c if p <0.0001; Loss on drying (LOD). PITOF: *Piliostigma thonningii* leaves; PITOT : *Piliostigma thonningii* stem bark; PITOR: *Piliostigma thonningii* root; PIREF: *Piliostigma reticulatum* leaves; PIRET : *Piliostigma reticulatum* stem bark; PIRER: *Piliostigma reticulatum* root.

### 3.8. Acute toxicity

The PIRE and PITO orally administered at a dose of 5000 mg/kg in male mice did not induce any death or toxic symptoms in treated mice. All animals displayed normal behavior throughout the study and survived until the end of the 14-day experiment period. During the entire observation period, they did not present any significant clinical alteration. Furthermore, no significant difference was observed between the weight gain in these groups in relation to the controls (Table 6). Hematological analyzes showed that PITO and PIRE did not cause significant changes of any of the parameters (Figure 3 and 4) and did not alter renal or hepatic function, remaining within the reference range. As a result, the $LD_{50}$ of PIRE and PITO was greater than 5000 mg/kg of body weight.

**Table 6** Effects of acute oral administration of extracts of PIRE and PITO on the body weights of Swiss mice with a dose of 5000 mg/kg.

| Group | Extract Dose mg/kg | Initial Weight (1st Day) | Final Weight (14th Day) | Δ | Ratio |
|---|---|---|---|---|---|
| PIRER | 5000 | 31.68 ± 3.22 | 32.43 ± 0.49 | +0.75 | 1.02 |
| PITOF | 5000 | 23.42 ± 0.57 | 25.11 ± 1.30 | +1.69 | 1.07 |
| PIREF | 5000 | 28.11 ± 1.56 | 29.64 ± 2.71 | +1.53 | 1.05 |
| PITRET | 5000 | 31.68 ± 3.23 | 32.43 ± 0.50 | +0.75 | 1.02 |
| PITOR | 5000 | 23.42 ± 0.58 | 25.11 ± 0.30 | +1.69 | 1.07 |
| PITOT | 5000 | 28.12 ± 0.56 | 28.84 ± 1.73 | +0.72 | 1.02 |
| Control | NaCl 0.9% | 27.83 ± 1.78 | 29.43 ± 1.21 | +1.60 | 1.06 |

Legend - PITOF: *Piliostigma thonningii* leaves; PITOT : *Piliostigma thonningii* stem bark; PITOR: *Piliostigma thonningii* root; PIREF: *Piliostigma reticulatum* leaves; PIRET : *Piliostigma reticulatum* stem bark; PIRER: *Piliostigma reticulatum* root.

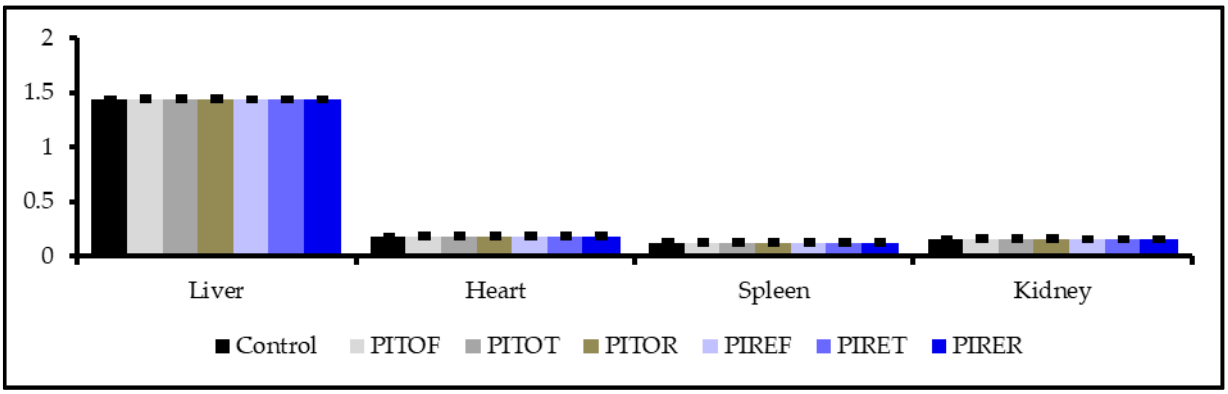



**Figure 3** Organ Weights (Liver, Heart, Kidney and Spleen) Following Exposure to Various Extracts in Acute Toxicity Assessment; PITOF: *Piliostigma thonningii* leaves; PITOT : *Piliostigma thonningii* stem bark; PITOR: *Piliostigma thonningii* root; PIREF: *Piliostigma reticulatum* leaves; PIRET : *Piliostigma reticulatum* stem bark; PIRER: *Piliostigma reticulatum* root

### 3.9. Biochemical profile of Piliostigma thonningii and Piliostigma reticulatum

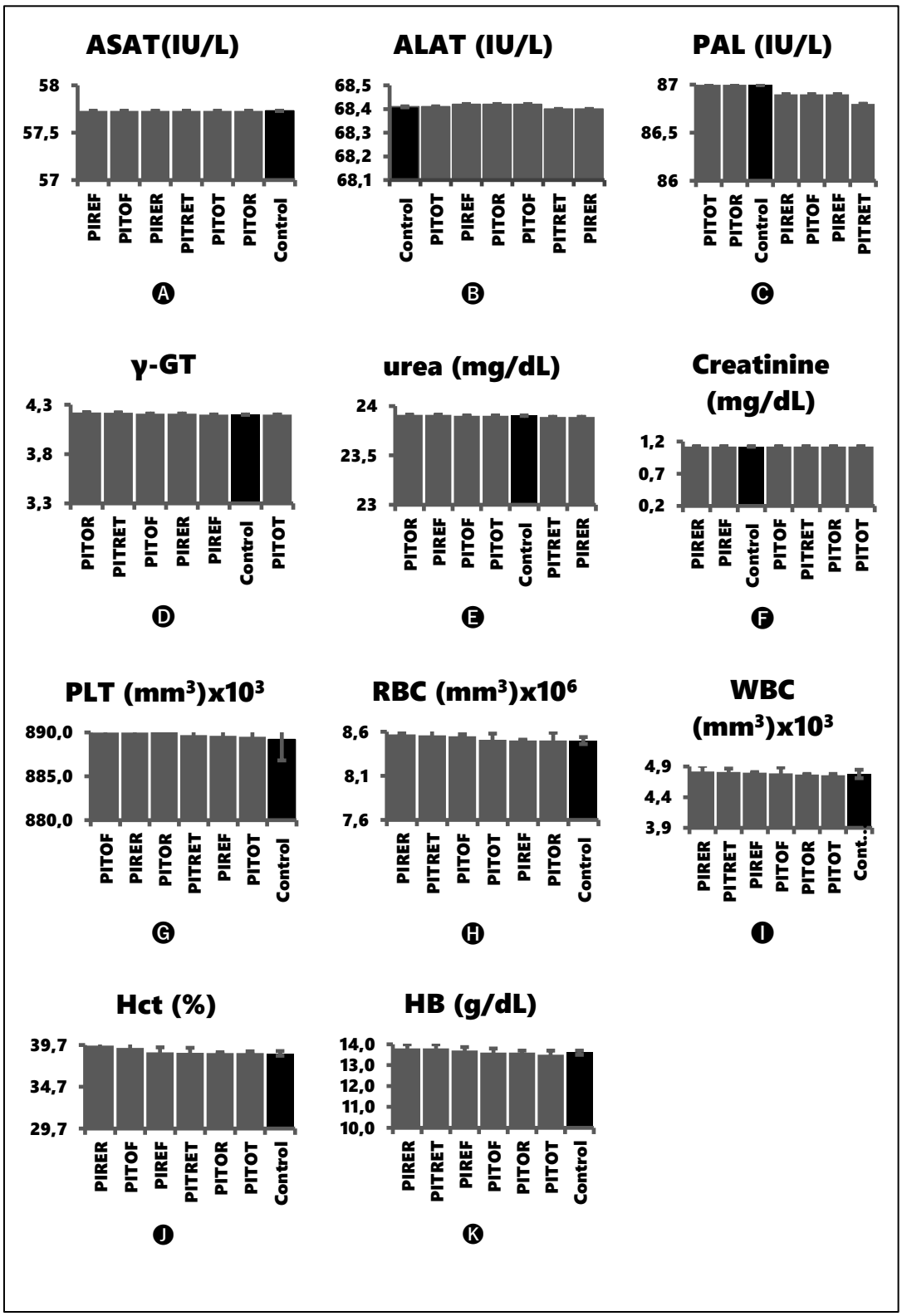



**Figure 4** Biochemical and hematological parameters in mice exposed to a 5000 mg/kg dose of methanolic extracts of *Piliostigma thonningii* and *Piliostigma reticulatum. PITOF: Piliostigma thonningii leaves; PITOT : Piliostigma thonningii stem bark; PITOR: Piliostigma thonningii root; PIREF: Piliostigma reticulatum leaves; PIRET : Piliostigma reticulatum stem bark; PIRER: Piliostigma reticulatum root*

## 4. Discussion

This investigation provides the first evidence of *in vitro* antibacterial activity of leaf, stem-bark and root-bark extracts of *Piliostigma thonningii* against *Klebsiella pneumoniae*, together with its phytochemical characterization (ash content and TLC fingerprint) and an acute oral toxicity assessment in *Mus musculus* at 5 000 mg/kg. It further establishes, for the first time, the antibacterial efficacy of *Piliostigma reticulatum* against *Salmonella typhi*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Shigella sonnei*, and confirms that oral administration of its stem- and root-bark extracts at 5 000 mg/kg in Swiss mice elicits no acute toxic effects.

### 4.1. Antibacterial and antioxidant potential of *Piliostigma thonningii* and *Piliostigma reticulatum*.

Our methanolic extracts of *Piliostigma thonningii* (PITO) and *P. reticulatum* (PIRE) exhibited MICs of 15.625–250 µg/mL, with complete inhibition of *Escherichia coli* and *Klebsiella pneumoniae* at 15.625 µg/mL. These values are substantially lower than the 16– 18.5 mg/mL MICs reported for *P. thonningii* leaf extracts [37], highlighting the improved efficacy achieved through our optimized microdilution protocol.

Notably, the *P. reticulatum* extract (PIRE) demonstrated superior efficacy against *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Streptococcus pneumoniae* (MIC = 15.625 µg/mL for all), markedly outperforming the 10–15 mg/mL range previously reported [38,39]. Phytochemical screening in the present study further revealed an elevated content of flavonols, methylated quercetins and condensed tannins—polyphenols well-documented for their ability to disrupt bacterial membranes and inhibit essential enzymatic processes [7].

Taken together, these findings position *P. reticulatum* as a prime candidate for bioassay-guided fractionation, isolation of active constituents and synergy studies with conventional antibiotics—particularly against multidrug-resistant pneumococcal and staphylococcal strains. Meanwhile, *P. thonningii* remains a valuable source of anti-uropathogenic agents given its potent activity against *E. coli* and *K. pneumoniae*.

### 4.2. Phytochemical content of Piliostigma thonningii and Piliostigma reticulatum

Building on foundational work [40,41], and recent analyses of *P. thonningii,* and *P. reticulatum* [7,14,15], comprehensive screening of leaves, stem- and root-bark revealed ubiquitous coumarins, flavonoids, polyphenols, tannins and terpenoids, with anthraquinones and saponins uniformly absent. This phenolic-rich profile typifies Fabaceae chemistry and underpins traditional antioxidant and antimicrobial uses, whereas the absence of anthraquinones and saponins rules out laxative or surfactant activities.

Quantitative analysis showed that total phenolics ranged from 47.2 to 577.2 mg GAE/g extract, tannins from 21.3 to 169.3 mg GAE/g, and flavonoids from 30.0 to 447.1 mg QE/g (Table 4). These levels surpass those reported by Boualam et al. [14], who found 56.5–74.7 µg GAE/mL in similar methanolic extracts, and correlate with the potent antioxidant (DPPH $IC_{50}$ = 2.5–5 µg/mL) and antibacterial (MIC = 15–250 µg/mL) activities observed (Table 2, Figure 1). Such high phenolic and condensed tannin contents are known to disrupt microbial membranes and chelate essential metal ions, underpinning both free radical scavenging and broad-spectrum antimicrobial effects [14].

Marked organ-specific variation in polyphenolic distribution was observed, with leaves and root bark consistently containing higher levels of phenolic compounds than stem bark. In particular, the leaves of *Piliostigma thonningii* showed the greatest accumulation of total phenols and flavonoids, whereas the stem bark of *Piliostigma reticulatum* was distinguished by its elevated tannin content. Such spatial heterogeneity in secondary metabolite profiles may account for the divergent ethnopharmacological uses recorded in traditional medicine: leaf decoctions are predominantly employed for their anti-inflammatory and antimicrobial efficacy, while stem bark preparations are traditionally applied as astringents and antidiarrheal remedies [7,14,42].

Thin-layer chromatographic fractionation of methanolic leaf extracts further delineated bioactive constituents. In *P. thonningii*, nine fractions were obtained, of which F1, F4 and F6 were identified as quercetin-type flavonoids. In *P. reticulatum,* five of seven fractions (F1–F5) exhibited flavonoid signatures. Sky-blue fluorescence under UV illumination tentatively assigned to phenolic acids suggests the presence of hydroxycinnamic and hydroxybenzoic acid derivatives. These compounds have been implicated in synergistic antimicrobial interactions and may contribute to the lower MIC values observed for *P. reticulatum* in parallel studies [43].

Despite morphological similarities, the two species display distinct phytochemical fingerprints. This divergence likely drives their unique ethnopharmacological applications—from wound-healing poultices of *P. thonningii* leaves to gastrointestinal tonics derived from *P. reticulatum* bark. Future work should focus on isolating these flavonoid and

phenolic fractions to evaluate their individual pharmacodynamics, as well as exploring potential synergisms that may inform standardized herbal formulations.

### 4.3. Oral acute toxicity of *Piliostigma thonningii* and *Piliostigma reticulatum*

In acute oral toxicity tests conducted in male Swiss mice according to OECD Guideline 423, single-dose administration of 5000 mg/kg PIRE and PITO provoked neither mortality nor behavioral, hematological or biochemical disturbances, establishing an $LD_{50}$ > 5000 mg/kg (Globally Harmonized System of Classification and Labelling of Chemical : GHS Category 5). These findings corroborate the work of Doh *et al.* [19], who similarly observed no adverse effects after gavaging mice with an aqueous leaf extract of *Piliostigma thonningii* at 6000 mg/kg, whereas no acute oral toxicity data for *Piliostigma reticulatum* have been reported to date. When compared with other African antibacterial phytotherapies, the safety profile of PIRE/PITO parallels ($LD_{50}$ > 5000 mg/kg; no toxic signs) that the leaves of *Moringa oleifera* Lam [44] and *Ceiba pentandra* (L.) Gaertn [45], exceeds the toxicity threshold of the leaves of *Garcinia huillensis* Baker ($LD_{50}$ = 2625.00 and 2717.39 mg/kg; GHS Category 5) [46].

## 5. Conclusion

Our findings demonstrate that both *Piliostigma thonningii* and *P. reticulatum* extracts exhibit significant antibacterial and antioxidant activities without acute toxicity at 5 000 mg/kg in murine models; given that multiple antibacterial and antioxidant compounds have already been isolated from *P. thonningii*, future efforts should prioritize bioassay-guided fractionation of *P. reticulatum* to identify its active constituents, and for both species, comprehensive standardization studies—including validation of extraction methods, quantification of phytochemical markers, and establishment of reproducible dosage parameters—are essential to advance their development as safe, effective phytotherapeutics.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

The authors declare that they have not known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### *Author contributions*

Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software and Writing - original draft: Bashige Chiribagula valentin, Ngoy Kalonda Nathan, Muhona Melman, Biayi Benaja Supervision: Okusa Ndjolo Philippe, Writing - review & editing: Bakari Amuri Salvius, Okusa Ndjolo Philippe.

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