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Antimicrobial activity, brine shrimp cytotoxicity and phytochemical screening of *Piliostigma thonningii* (Schumach.) Milne-Redh aqueous and methanol stem bark extracts

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ABSTRACT

Background: The stem bark of *Piliostigma thonningii* is acclaimed for managing various types of diseases by the Kikuyu community of Gaturi, Murang'a County. However, there is limited data on the antimicrobial efficacy and safety of its stem bark extracts. **Aim and objectives:** The study set out to examine the antimicrobial activities, qualitative phytochemical screening, and safety of *Piliostigma thonningii* stem bark extracts in brine shrimp (*Artemia salina*) both in aqueous and methanolic forms. **Materials and Methods:** Standard phytochemical screening techniques were used to study the phytochemicals present in both extracts. Antimicrobial susceptibility of *Bacillus cereus*, *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* to the test extracts was assessed by Agar well diffusion method. Fluconazole and gentamicin were used as standard drugs. The cytotoxic profiles of the extracts were examined using the brine shrimp cytotoxic model, in which vincristine sulphate served as the standard cytotoxic drug. **Results:** According to phytochemical screening, all the extracts were found to possess saponins, glycosides, steroids, alkaloids, terpenoids, phenols, tannins, and anthraquinones. However, tannins were not found in aqueous extract while the methanol extract was devoid of anthraquinones. When evaluated against *Bacillus cereus*, the antibacterial effects of both extracts were notably lower ($p < 0.0001$) than gentamicin across various concentrations. When evaluated against *Staphylococcus aureus*, the aqueous extract exhibited significantly lower antibacterial effects ($p = 0.0100$) compared to gentamicin at 62.5 mg/mL, while the methanol extract exhibited no significant difference ($p = 0.1551$) relative to gentamicin at the same concentration. Neither extract showed antimicrobial effects against *P. aeruginosa*, *E. coli* and *K. pneumoniae*. Fluconazole exhibited superior activity compared to the methanol extract at 0.125 mg per ml ($p = 0.0489$) and 1.0 mg per ml ($p = 0.0207$). In the case of both extracts against test organisms *B. cereus*, *S. aureus* and *C. albicans* there was no difference in MIC and MBC values apart for methanol extract against *B. cereus* whose MIC (15.6mg/ml) and MBC (31.2mg/ml) were markedly lower than that for aqueous extract. It was not possible to identify the crude extracts' minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) against bacteria that were gram negative namely; *K. pneumoniae*, *E. coli*, and *P. aeruginosa* at the tested concentrations, which suggests that the values may be higher than 500 mg/ml or completely ineffective. The standard drugs (gentamycin and fluconazole) were more effective against the tested organisms than both extracts. Gentamicin was more active against gram negative bacteria than gram positive bacteria as was portrayed by the lower MIC and MBC values. The LC_{50} in $\mu\text{g/ml}$ of vincristine sulphate was 15.70, for methanol extract was 7425.46 and for aqueous extract was 1765.20. **Conclusions:** These findings suggest that against these organisms; *Staphylococcus aureus*, *Candida albicans* and *Bacillus cereus*, the antimicrobial effects of *P. thonningii* stem bark extracts are less than those of standard drugs. In addition, the results suggest that extracts of *P. thonningii* stem bark are not cytotoxic to brine shrimp larvae.

Keywords: Agar well diffusion method, Brine shrimp, Minimum inhibitory concentration, Phytochemicals, Minimum bactericidal concentration, Macro broth dilution.

INTRODUCTION

Pathogens of medical significance in sub-Saharan Africa include *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Klebsiella pneumoniae*, *Bacillus cereus*, and *Pseudomonas aeruginosa* [1-6]. But for these pathogens, antimicrobial resistance is becoming an important topic [1, 2, 6] highlighting the necessity of alternatives to the existing antimicrobials. *P. thonningii* belongs to the Fabaceae family of plants. It has a woody, rounded crown, hairy twigs, longitudinally fissured bark, and leathery leaves [7] (Figure 1).

Figure 1 displays the photo of the whole plant of *Piliostigma thonningii* (taken in Murang'a County by PN).



Figure 1: Plant *Piliostigma thonningii*

It is mainly distributed in sub-Saharan Africa and the Middle East especially in Yemen ^[8]. It is used by various communities around the world to manage worm infestation, stomachache, heal wounds, manage snake bites, dysentery, headache, and diarrhea associated with teething in children ^[7-10]. The analgesic, hepatoprotective, antihelmintic, nephroprotective, antiproliferative, cardioprotective, antiprotozoal, and aphrodisiac activities of *P. thonningii* have been well described in the scientific literature ^[11-16].

The Kikuyu community of Gaturi, Murang'a County use the stem bark of *P. thonningii* for respiratory, wound, and soft tissue infections caused by microbial organisms. However, data supporting the efficacy and safety of *Piliostigma thonningii* stem bark is lacking. Therefore, the study set out to examine the antimicrobial activities, phytochemical screening, and safety of *Piliostigma thonningii* stem bark extracts in brine shrimp (*Artemia salina*) both in aqueous and methanolic forms.

MATERIALS AND METHODS

Study design

Figure 2 shows the map of Gaturi region of Murang'a County adapted from en.m.wikipedia.org on 3rd June 2024.

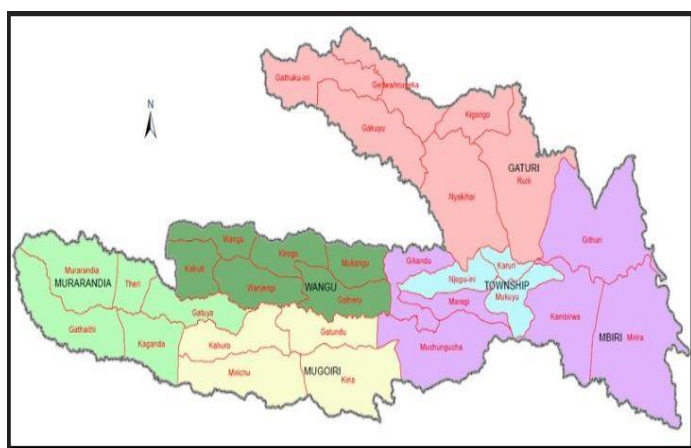


Figure 2: Map of Gaturi region of Murang'a County

The fresh *Piliostigma thonningii* stem barks were obtained from Gaturi region of Murang'a County, Kenya at coordinates -0.686549, 37.161559 (Figure 2). The region is characterized by a tropical dry savanna climate with an altitude of 1,379 meters. All the laboratory work was conducted in the Department of Public Health, Pharmacology and Toxicology-University of Nairobi.

Ethical approval

The Biosafety, Animal Use, and Ethics Committee of University of Nairobi evaluated and approved the current work (FVM BAUEC/2023/532). The National Commission for Science, Technology, and Innovation (NACOSTI), Kenya granted a research permit (NACOSTI/P/24/32994).

Plant collection and identification

On July 5, 2023, *Piliostigma thonningii* was collected from Gaturi region of Murang'a County, and it was recognized at the National Museums of Kenya's East African Herbarium. REF: NMK/BOT/CTX/2/ID/13/2023

Preparation of harvested material

The harvested barks were meticulously washed in flowing tap water to dislodge any adhering soil particles and finally rinsed with distilled water. The clean plant material was then shade-dried. A mechanical mill was used to grind the dry plant material and the powder obtained was stored in air-tight bags awaiting extraction.

Preparation of crude extracts

The method demonstrated by Matará et al. [16] with minimal modifications was used for preparation of the extracts. For aqueous extract, powder weighing five hundred (500) grams was soaked in about 2 ltrs of distilled water in an extraction jar. Maceration was conducted for 72 hours accompanied with vigorous shaking of the extraction jar in the morning and in the evening. The resulting mixture was decanted, and the resulting residue was macerated again in 2 liters of distilled water before being shaken and decanted. One liter of deionized water was further added into the extraction jar, shaken briefly, and decanted. The three filtrates were combined. The combined filtrate then underwent a centrifugation process at 3000 rpm upto a set period of 5 minutes. The supernatant obtained was decanted, then filtered with cotton wool. After being kept in a deep freezer for a whole day, the filtrate was lyophilized at -20° C forming a white powder.

On the other hand, cold maceration technique prepared the methanol extract. A 2400 ml of analytical grade methanol was used to dissolve 500 g of pulverized plant material. For 72 hours, it was incubated at room temperature and occasionally shaken in the morning and in the evening to increase the efficiency of extraction. Whatman paper (No. 1) was used to filter the sample. Concentration of the filtrate was done in a rotary evaporator by evaporating excess methanol at a temperature of 40°C. The concentrate was put in an amber-coloured bottle, enclosed in an aluminum foil with fines holes on the top cover and slowly evaporated in a sand bath which had been attuned to a temperature of 40°C. All the dried extracts were tightly corked and reinforced with a masking tape and stored at -4°C.

Qualitative analysis of phytochemicals.

The methods described by Sofowora, ^[17] and Trease and Evans, ^[18] were utilized to determine the qualitative presence of glycosides, tannins, terpenoids, flavonoids, saponins, alkaloids, anthraquinones and phenols. Colour changes were used for examination and qualitatively noted as (+) when the phytochemical of interest was present and (–) when the secondary metabolite was absent in the extracts.

Antimicrobial activity assay

Bacillus cereus of ATCC No. 11778, *Candida albicans* of ATCC No. 10231, *Escherichia coli* of ATCC No. 25922, *Klebsiella pneumoniae* of ATCC No. 700603, *Pseudomonas aeruginosa* of ATCC No. 27853, and *Staphylococcus aureus* of ATCC No. 25923 were obtained from

the Bacteriology Unit of the Department of Public Health, Pharmacology and Toxicology, University of Nairobi.

Inoculum preparation and standardization

The Central Laboratory Standard institute (CLSI) guidelines for cultivating microorganisms were followed, albeit with minor modifications made in accordance with the Balouri et al technique [19]. 10 mls of sterile saline was used to suspend a loopful of each pure culture of microorganisms which was vortexed and adjusted to a turbidity of roughly 1×10^6 cfu/ml using MacFarland standard.

Agar well diffusion method

The width of the zones of inhibition was assessed in accordance with CLSI guidelines with minor modifications as per Balouri et al. [19] protocol. A standardized amount of sterile Mueller Hinton agar (MHA) was placed on the plate and allowed to spread evenly. Four wells that were evenly distributed on the plates were bored using an aseptic cork borer. The inoculum of each test microorganism was streaked employing a sterile wire loop under a standardized procedure as described by CLSI. The plugs were then removed from the wells. Each well on a specific plate was filled with the test extracts. The test doses ranged from 500 to 62.5 mg/ml under 2-fold serial dilutions. The test substance was allowed to disperse into MHA for about 1 hour before incubation for 24 hours at 37°C but *Candida albicans* were incubated at room temperature

Macrobroth dilution method

The MICs and the MBCs of the test microbial organisms was assessed using macrobroth dilution method according to CLSI guidelines modified by the protocols of Matara et al. [16]. Two-fold serial dilutions of the two extracts which ranged from 500mg/ml to 1.95mg/ml were evaluated against the test microbes. 0.1mls of each inoculum (test microorganism under test) was inoculated using a micropipette into the tubes containing the plant extracts. The tubes containing bacteria organisms were incubated for 24 hours at 37°C whereas *Candida albicans* was incubated at room temperature. Petri dishes containing a standardized amount of MHA were swabbed with a standardized inoculum of each test microorganism from both extracts. The Petri dishes swabbed with bacterial organisms were incubated for 24 hours at 37°C while that of *Candida albicans* was incubated at room temperature. The concentration that enabled the least growth of microorganisms was taken as the MIC while the least concentration that did not enable growth of the microorganisms was taken as the MBC.

Brine shrimp lethality bioassay (BSLA)

The protocol demonstrated by Meyer et al. [21] and Clarkson et al. [22] was used to examine the cytotoxicity levels of the two extracts in brine shrimp; minor adjustments were permitted in accordance with Nguta et al. [23] technique. *A. salina* larvae were hatched in a trough that contained about 38.5% w/v of marine solution. Ten *A. salina* larvae were selected from the trough and placed into each of the twenty-five plastic tubes that were set on a test tube rack (n=250). The larvae were exposed in 10 µg/ml (n=50), 100 µg/ml (n=50), and 1000 µg/ml (n=50) of the extracts, vincristine sulphate (positive control, n=50) and deionized water (negative control, n=50). The concentration of the extracts and vincristine sulphate which was responsible for 50% death of *A. salina* larvae was determined by probit regression analysis. Toxicity was summarized using Meyer et al. and Clarkson et al. toxicity criterion.

Data analysis

The findings of the composition of the phytochemicals present in the extracts were summarized in a tabular format. The determination of the lethality (median lethal concentration: LC₅₀) exhibited by the studied extracts and vincristine sulphate was achieved by Probit

regression analysis (SPSS version 25). Comparison of the antimicrobial profiles of the two extracts and the two conventional antimicrobial drugs was done by the two-way ANOVA and Tukey's test as post hoc analysis.

RESULTS

Table 1 shows the phytochemical screening of the methanol and aqueous extracts of *P. thonningii* stem bark of Gaturi region. The aqueous and methanol extracts contained alkaloids, terpenoids, steroids, phenols, saponins, and glycosides but the aqueous extract was devoid of tannins while the methanol extract was devoid of anthraquinones (Table 1).

Table 2 shows the effect of methanol and aqueous extracts of *P. thonningii* stem bark in brine shrimp larvae over 24 hours. The LC₅₀ in µg/ml of aqueous extract was 1765.20, for methanol extract was 7425.46 and for vincristine sulphate was 15.70 (Table 2).

Table 3 shows the results of Agar well diffusion method as mean zones of inhibition of test extracts and standards when tested against the microbial pathogens. Both the extracts were bioactive against *Bacillus cereus*, *Staphylococcus aureus* and *Candida albicans* but not against *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The reference drugs gentamicin and fluconazole produced markedly larger inhibition zones than the extracts under test implying that the microbial pathogens were more susceptible to them than the extracts (Table 3).

Table 4 shows the results for macrobroth dilution method of the test extracts and standards when tested against microbial pathogens. Positive control of bacterial organisms was gentamycin 40mg/ml while that for *Candida albicans* was fluconazole 2mg/ml. The MICs and MBCs of both extracts against *C. albicans*, *S. aureus*, and *B. cereus* were 250mg/ml and 500mg/ml respectively. However, the MIC (15.6mg/ml) and MBC (31.2mg/ml) for methanol extract against *B. cereus* was markedly lower than for aqueous extract. The MICs and MBCs for *K. pneumoniae*, *E. coli*, and *P. aeruginosa* could not be determined at the tested concentrations which suggests that the values may be higher than 500mg/ml or completely ineffective. The standards drugs were more effective than both the extracts. Gentamycin was more active against gram negative bacteria than gram positive bacteria as shown by lower MIC and MBC values (Table 4).

Figure 3 shows the effect of different doses of vincristine, methanol and aqueous extracts of *P. thonningii* stem bark. The mean quantity of brine shrimp that died after being exposed to 10 µg/ml of vincristine sulphate was considerably more (p=0.0252) than the mean quantity of brine shrimp that died after being exposed to an equivalent dosage of the other extracts. When brine shrimp were exposed to 100 and 1000 µg/ml of vincristine sulphate, the mean deaths occurred significantly (p<0.0001) more than when brine shrimp were exposed to an equal amount of each extract. Nevertheless, no statistically significant difference in the mean number of brine shrimp that died after being exposed to the investigated extracts at 10 µg/ml (p>0.9999), 100µg/ml (p=0.9595) and 1000 µg/ml (p=0.1485) was observed (Figure 3).

Figure 4 shows the antibacterial effect of gentamicin, methanol and aqueous extracts of *P. thonningii* stem bark against *Bacillus cereus*. The stem bark extracts from this plant species demonstrated a substantially weaker (p=0.0001) antibacterial activity against *B. cereus* than gentamicin at all tested concentrations, which ranged from 62.5 mg/ml to 500mg/ml under two (2) fold serial dilutions (Figure 4).

Figure 5 shows the antibacterial effect of gentamicin, methanol and aqueous extracts of *P. thonningii* stem bark against *Staphylococcus aureus*. The observed antibacterial effect of the two studied extracts against *S. aureus* was significantly lower (p=0.0100) than the antibacterial effect of gentamicin at 62.5 mg/ml. The mean difference in antibacterial effect between gentamicin and methanol extract at

62.5mg/ml ($p=0.1551$) was nonsignificant. Furthermore, at 62.5mg/ml, no discernible difference ($p=0.4113$) in the antibacterial activity of the *P. thonningii* methanol and aqueous extracts was observed. When compared to the antibacterial action of gentamicin at dosages ranging from 125 to 500 mg/ml under two (2) fold serial dilutions, the observed antibacterial activities of the methanol and aqueous extracts against *S. aureus* were significantly lower ($p=0.0001$). Nevertheless, at 500mg/ml no discernible difference ($p=0.4590$) between the antibacterial action of methanol and the aqueous extract was noted (Figure 5).

Figure 6 shows the antifungal effect of fluconazole, methanol and aqueous extracts of *P. thonningii* stem bark against *Candida albicans*. The antifungal activity of fluconazole at 1.0 mg/ml ($p=0.2113$) and 0.125 mg/ml ($p=0.0564$) did not significantly differ from that of the aqueous extract. The antifungal effect exhibited by fluconazole was significantly greater than that exhibited by methanol extract at 0.125 mg/ml ($p=0.0489$) and 1.0 mg/ml ($p=0.0207$). The antifungal activity of *P. thonningii* stem bark extracts evaluated at 0.125 mg/ml ($p=0.9525$), and 1.0 mg/ml ($p=0.4876$) did not differ significantly from one another (Figure 6).

Table 1: The phytochemical screening of *P. thonningii* stem bark extracts in methanol and aqueous form

Plant Metabolite	Name of the test	Aqueous Extract	Methanol Extract
Alkaloids	Dragendorff's	+	+
Antraquinones	Borntranger's	+	-
Flavonoids	Alkaline reagent	+	+
Glycosides	Keller-killiani	+	+
Phenols	Ferric chloride	+	+
Saponins	Froth	+	+
Steroids	Salkowski	+	+
Tannins	Ferric chloride	-	+
Terpenoids	Salkowski	+	+

(+) the sign was an indicator of the presence of secondary metabolite while (-) was an indicator of the absence of secondary metabolite.

Table 2: The 24-hour effects of *P. thonningii* stem bark extracts of aqueous and methanolic forms on brine shrimp larvae

Test substance	Mortality observed per test dose (doses in µg/ml)			LC ₅₀ in µg/mL with 95% Confidence interval (CI)	Cytotoxicity criteria	
	10	100	1000		Meyer's toxicity index	Clarkson's toxicity index
Aqueous stem bark extract of <i>P. thonningii</i>	0	9	19	1765.20 (799.38-9049.98)	Non-cytotoxic	Non-cytotoxic
Methanol stem bark extract of <i>P. thonningii</i>	0	8	12	7425.46 (1768.35-881798.23)	Non-cytotoxic	Non-cytotoxic
Vincristine sulphate	10	50	50	15.70 (No CI)	Cytotoxic	Highly cytotoxic

Table 3: Results for Agar well diffusion method as mean zones of inhibition of test extracts and standards against the microbial pathogens

Mean zones of inhibition					
Test extracts				Reference standards	
Microorganism	Conc mg/ml	Methanol extract	Aqueous extract		
<i>Bacillus cereus</i>	500	21.07±0.20	18.62±0.22	Gentamycin 40mg/ml	
	250	21.10±0.35	18.44±0.02	29.23±0.02	
	125	20.08±0.13	17.52±0.03	28.47±0.07	
	62.5	18.38±0.08	17.91±0.05	27.77±0.07	
<i>Staphylococcus aureus</i>	500	20.61±0.16	20.28±0.15	26.23±0.00	
	250	20.61±0.05	19.17±0.01	25.23±0.02	
	125	19.17±0.17	17.60±0.04	23.12±0.02	
	62.5	18.91±0.18	18.55±0.11	21.15±0.00	
<i>Escherichia coli</i>	500	NI	NI	19.43±0.04	
	250	NI	NI	28.97±0.5	
	125	NI	NI	28.28±0.05	
	62.5	NI	NI	25.99±0.19	

<i>Klebsiella pneumoniae</i>	500	NI	NI	27.26±0.33
	250	NI	NI	26.26±0.15
	125	NI	NI	25.83±0.3
	62.5	NI	NI	24.23±0.2
<i>Pseudomonas aeruginosa</i>	500	NI	NI	31.05±0.33
	250	NI	NI	30.36±0.47
	125	NI	NI	28.57±0.11
	62.5	NI	NI	27.35±0.59
				Fluconazole 2mg/ml
<i>Candida albicans</i>	500	13.69±0.11	20.01±0.12	42.82±0.00
	250	16.19±0.04	17.81±0.00	39.06±0.00
	125	15.06±0.17	15.11±0.13	36.22±0.02
	62.5	14.58±0.01	14.95±0.02	36.23±0.00

NI: No inhibition (at tested concentrations)

Table 4: Results for macrobroth dilution method showing the minimum inhibitory and minimum bactericidal concentrations of the test extracts and standards

Microbial strain	Aqueous extract		Methanol extract		Positive control	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>B. cereus</i>	250	500	15.6	31.2	0.075	0.15
<i>S. aureus</i>	250	500	250	500	1.2	2.5
<i>C. albicans</i>	250	500	250	500	0.25	0.5
<i>E. coli</i>	NE	NE	NE	NE	<0.075	<0.075
<i>K. pneumoniae</i>	NE	NE	NE	NE	<0.075	<0.075
<i>P. aeruginosa</i>	NE	NE	NE	NE	<0.075	<0.075

NE: Not Effective (at test ed concentrations)

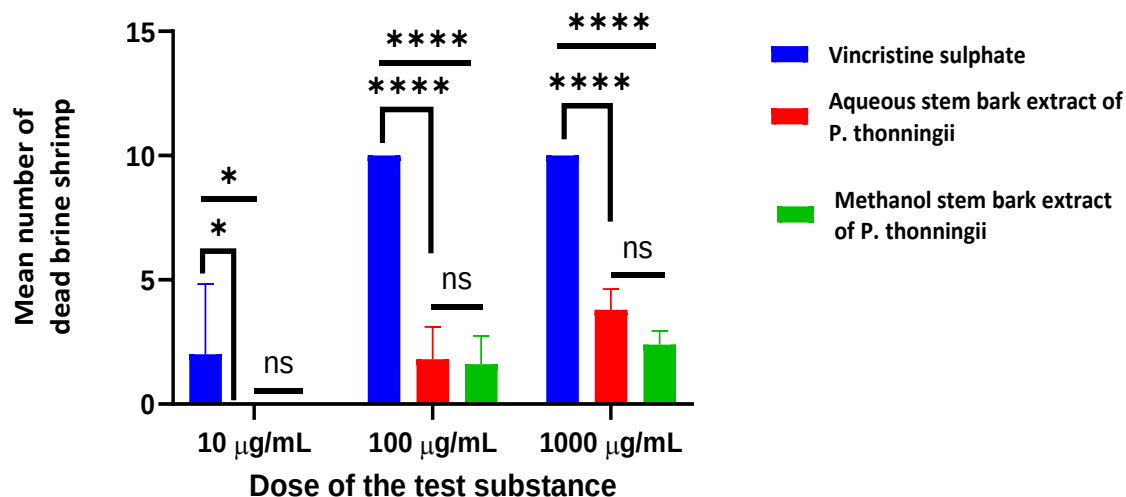


Figure 3: Comparison of the effects of different dosages of *P. thonningii* stem bark extracts in aqueous, methanolic and that of vincristine sulphate on brine shrimp larvae

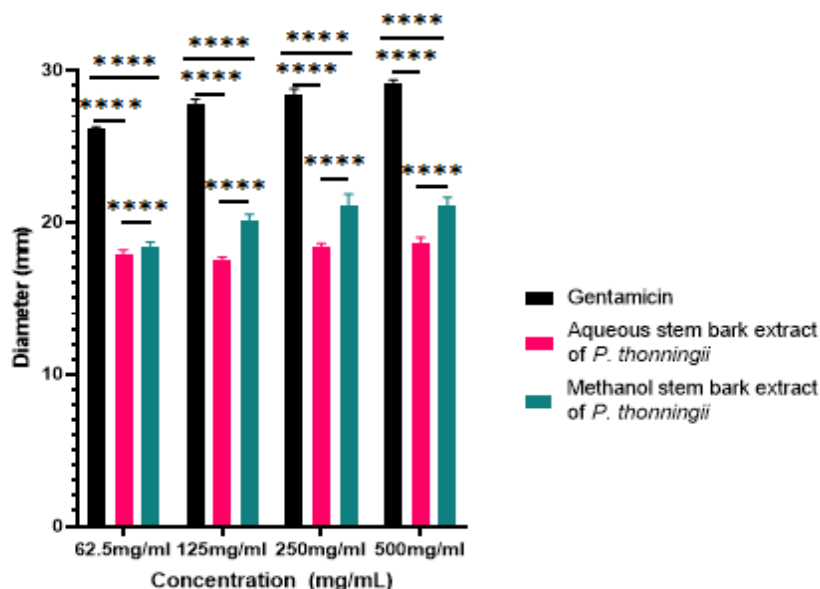


Figure 4: Comparison of antibacterial effects of *P. thonningii* stem bark extracts in aqueous, methanolic and that of gentamicin against *Bacillus cereus*

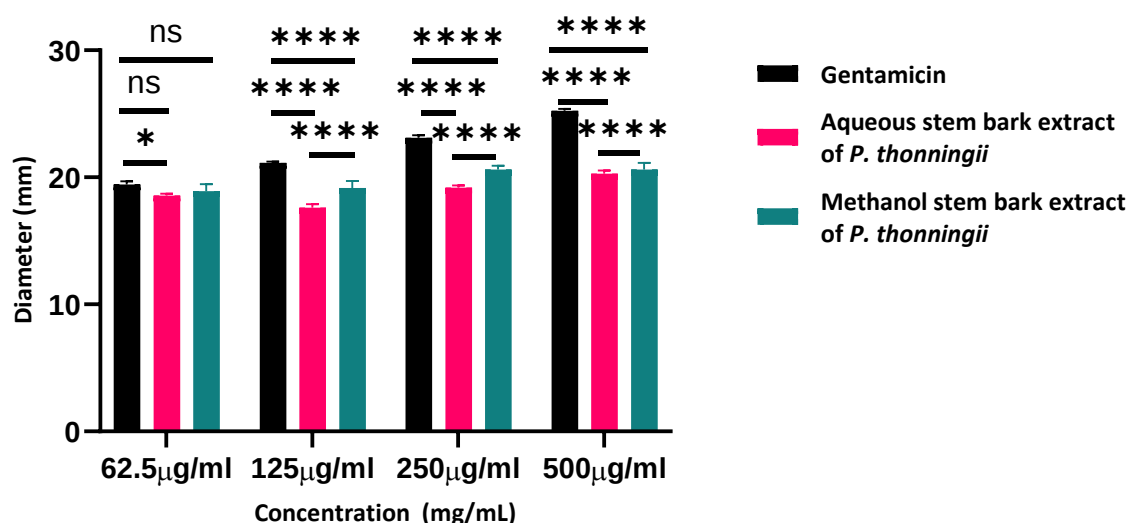


Figure 5: Comparison of antibacterial effects of *P. thonningii* stem bark extracts in aqueous, methanolic and that of gentamicin against *Staphylococcus aureus*

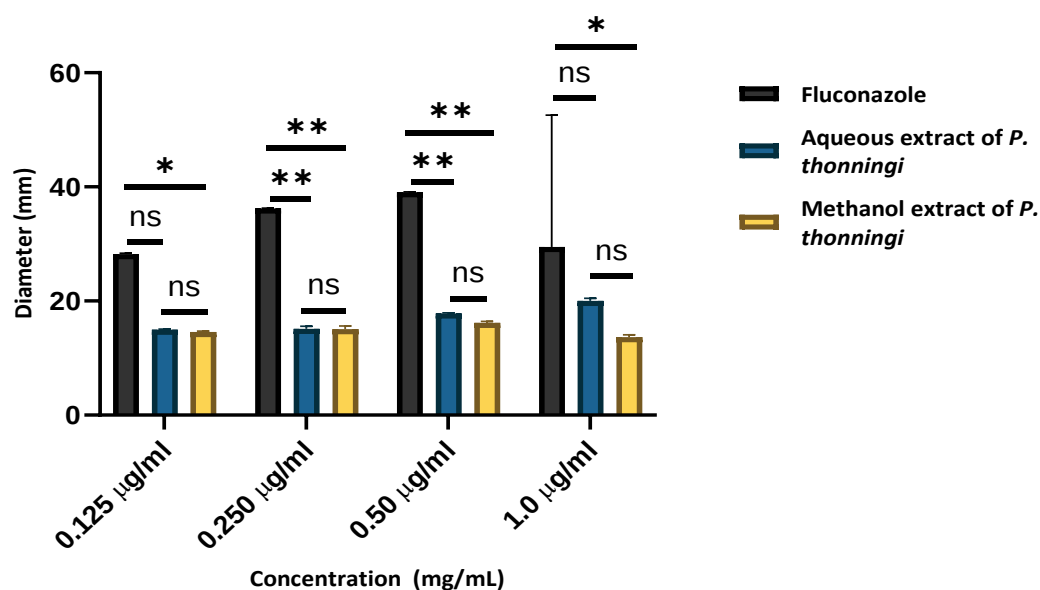


Figure 6: Comparison of antifungal effects of *P. thonningii* stem bark extracts in aqueous, methanolic and that of fluconazole against *Candida albicans*

DISCUSSION

The present findings corroborate previous reports indicating the rich phytochemical diversity of *P. thonningii* stem bark extracts [23-28]. The aqueous and methanol extracts contained alkaloids, terpenoids, steroids, phenols, saponins, and glycosides but the aqueous extract was devoid of tannins while the methanol extract was devoid of anthraquinones. The secondary metabolites identified are responsible for the observed antimicrobial activities [29-31]. Moreover, this study established that the aqueous and methanol stem bark extracts of *P. thonningii* were effective against *Bacillus cereus*, *Staphylococcus aureus*, and *Candida albicans* but not *Escherichia coli* and *Klebsiella pneumoniae*. The findings of this study contradict the research findings by Seyi et al. [24] who researched on the antimicrobial properties of *Piliostigma thonningii* stem bark collected in Nigeria and reported that the hexane and methanol extracts were effective against *Pseudomonas spp*, *Salmonella spp*, *Staphylococcus aureus*, *Klebsiella spp*, and *Escherichia coli*. It is not clear why there was such a huge discrepancy between the two studies especially considering that the phytochemical composition of the extracts in our study and those in Seyi et al. study was relatively similar. It was also observed that although there were notable antimicrobial properties against the studied pathogens, the activity was much less pronounced in comparison to the standard drugs used. Okwute et al. [25] in his study of methanol crude extract of Nigerian species, found that the phytochemicals present were almost similar to those of the present study. However, in his study, the crude extract was active against two organisms among those that were under test namely; *E. coli* and *S. aureus* and but not against *K. pneumoniae* and *C. albicans*. These findings also contradict the present study where the methanol extracts were not active against *E. coli* and but active against *C. albicans*. Therefore, it can be suggested that differences in phytogeographical region plays a major role in the pharmacological properties of plant species as asserted by Nirala et al. [32]. Few studies have reported the MICs and MBCs of *P. thonningii* stem bark. Akinpelu and Obuotor, [26] evaluated the MICs of methanol extract of *P. thonningii* stem bark against microbial pathogens. The MIC of *Staphylococcus aureus* was 0.635mg/ml while that for *Escherichia coli* was 10.0mg/ml which were significantly lower than the MICs determined from the current study. Comparative analysis with standard antibiotics and antifungals provides invaluable insights into the efficacy of *P. thonningii* extracts relative to conventional therapeutic agents. Additionally, the comparable antifungal activity of fluconazole relative to the aqueous and methanol extracts *P. thonningii* stem bark suggests potential synergistic effects or alternative mechanisms of action warranting further exploration. The fact that aqueous and methanol extracts displayed antimicrobial activity gives support to the traditional usage of this plant against microbial infections. However, invitro tests are not enough necessitating the need for in vivo tests to support this. This plant may be used as an antimicrobial agent following extensive toxicological research and in-depth in vivo antimicrobial evaluation, particularly in remote areas where access to health facilities is limited and standard medications are expensive or unavailable.

The cytotoxicity of aqueous and methanol extracts of *Piliostigma thonningii* stem bark were much lower than what was reported for the hexane and methanol extracts of the same anatomical part of the same plant by Seyi et al. [24]. This may have something to do with the phytochemical composition of the extracts which is a consequence of the type of solvent used and differences in geographical regions of plant species [31-32]. It may be important to perform a quantitative phytochemical analysis of the studied extracts. While several studies have documented the cytotoxic potential of different plant parts of *Piliostigma thonningii* in *Artemia salina* [23, 33-34], this is the first study that provides quantitative data elucidating the differential cytotoxicity between vincristine, the aqueous and methanol extracts of *Piliostigma thonningii* stem bark. Moreover, this study extends this knowledge by demonstrating a significantly higher cytotoxicity of the aqueous extract compared to the methanol extract underscoring the importance of solvent choice in extraction of bioactive compounds and warrants further investigation into the specific cytotoxic constituents of *P.*

thonningii. Comparative analysis with standard antibiotics and antifungals provides valuable insights into the efficacy of *P. thonningii* extracts relative to conventional therapeutic agents. Additionally, the comparable antifungal activity of fluconazole relative to the aqueous and methanol extracts *P. thonningii* stem bark suggests potential synergistic effects or alternative mechanisms of action warranting further exploration. The observed differences in phytochemicals present, antimicrobial activity, and cytotoxicity underscore the complexity of *P. thonningii* extracts and highlight the importance of standardized extraction methods and comprehensive phytochemical profiling. The lack of activity against specific pathogens and the differential cytotoxicity between extracts necessitate further investigation into the underlying mechanisms and therapeutic potential of the phytochemicals in *P. thonningii*. When taken collectively, these findings contribute to the growing repertoire of scientific evidence backing the medicinal value of *P. thonningii* and provide a foundation for future research aimed at exploring its therapeutic potential for combating microbial infections.

CONCLUSION

The aqueous and methanol extracts of *Piliostigma thonningii* stem bark have a rich diversity of phytochemical compounds that conferred antimicrobial action against the namely organisms; *Bacillus cereus*, *Candida albicans* and *Staphylococcus aureus* but not *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*. Moreover, the extracts are non-cytotoxic to *Artemia salina* at the doses tested. Future studies should investigate the mechanism of antimicrobial action of the extracts with a focus on the isolation of key phytochemicals.

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Authors' contributions

PN: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, visualization, writing original draft, and writing review and editing. PN, JN, and IM: Conceptualization, investigation, methodology, supervision, validation, writing review, and editing. PN: Formal analysis, validation, visualization, writing review, and editing. All authors read and approved the final manuscript.

Conflict of interest

The authors declared no conflict of interest.

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None declared.

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