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Antimicrobial activity, cytotoxicity, and qualitative phytochemical composition of aqueous and methanolic leaf extracts of *Physalis peruviana* L. (Solanaceae)

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ABSTRACT

Background: The high morbidity and mortality associated with microbial infections and the ineffectiveness of conventional antibiotics due to inappropriate use and resistance warrant alternative stratagems. **Aim**: We investigated the antimicrobial, cytotoxicity, and qualitative phytochemical composition of the aqueous and methanolic leaf extracts of *Physalis peruviana* L. (Solanaceae) based on its ethnomedicinal information. **Methods**: The antimicrobial activity of the extracts was investigated using the disk diffusion technique. The brine shrimp lethality assay method was used to determine the cytotoxicity of the study extracts on brine shrimp nauplii. Qualitative phytochemistry of the study extracts was performed using standard procedures. **Results**: The two extracts did not possess antimicrobial activity against *P. aeruginosa* and were either inactive or slightly inactive against the other microbes at concentrations of ≤ 50 µg/ml. Notably, the aqueous extract exhibited high to very high activities against *E. coli* and *S. aureus* and very high to remarkable activities against *B. cereus.* The methanolic extract showed remarkable activity at concentrations of ≥200 µg/ml against *E. coli*, moderate to very high against *S. aureus*, high to very high against *B. cereus*, and moderate to high against *C. albicans*. The aqueous extract's minimum inhibitory concentrations (MICs) were 50 µg/ml (*E. coli* and *B. cereus*), 100 µg/ml (*S. aureus*), and 200 µg/ml (*C. albicans*). The minimum bactericidal concentration concentrations (MBCs) of the aqueous extract were 100 µg/ml (*E. coli* and *B. cereus*), 200 µg/ml (*S. aureus*), while the minimum fungicidal concentration (MFC) for *C. albicans* was 400 µg/ml. The MICs of the methanolic extract were 25 µg/ml for *E. coli*, *S. aureus*, and *B. cereus* and 100 µg/ml for *C. albicans*, and the MBCs were 100 µg/ml for E. *coli*, *S. aureus*, and *B. cereus*, and the MFC for *C. albicans* was 200 µg/ml. The aqueous was non-toxic, while the methanolic extract was slightly toxic to brine shrimp nauplii, indicating their relative safety. Various phytochemicals were detected in the study extracts, depicting broad pharmacologic activity, including antimicrobial activity. **Conclusion**: The aqueous and methanolic leaf extracts of *P. peruviana* may serve as a source of efficacious and safe novel antimicrobial agents upon further evaluation.

Keywords: Disk diffusion technique; Brine Shrimp Lethality Assay; Minimum Inhibitory Concentration, Minimum Bacterial/Fungicidal Concentration (MBC/MFC), Phytochemicals.

INTRODUCTION

Antibiotic chemotherapy decreases the severity and spread of infectious diseases; however, the wanton usage of antibiotics and the emergence of resistant strains pose a significant health risk [1, 2]. Current epidemiological data shows that low- and medium-income countries, especially those in sub-Saharan Africa, carry over 80 % of global burden diseases, most of which are caused by pathogenic microbes [3, 4]. This is partly attributable to the low hygiene levels, especially in rural and informal settlements, inaccessibility to quality healthcare, and unaffordability to effective therapies, which lead to high morbidity and mortality, especially due to diarrhoea among vulnerable groups [2]. Besides, most conventionally prescribed antibiotics cause severe side effects, limiting their clinical significance [5, 6]. Therefore, considering the drawbacks of conventional chemotherapy, the search for alternative armamentaria, which can effectively treat microbial infections and counter antimicrobial resistance without eliciting adverse effects, is worthwhile.

Various ethnic groups have used medicinal plants to treat microbial infections, among other diseases, for ages [7, 8]. Plant-derived products are a viable source of natural, affordable, safe, and efficacious antimicrobial agents due to the diverse range of bioactive secondary metabolites they produce [9, 10]. Plants primarily synthesise these metabolites as a defence mechanism against biotic and abiotic stress, and their nutritional and, or medicinal benefits are conferred on animals and humans when administered [11, 12]. Research demonstrates that plant-derived amalgams, such as flavonoids, phenols, and tannins, possess antimicrobial activity and other bioactivities [13–15].

However, despite the extensive usage of medicinal plants, and appreciable medicinal value, the most promising plants are yet to be validated empirically.

The utilisation of medicinal plants has elicited various safety concerns, primarily due to insufficient information about standard preparation procedures, storage, labelling, marketing, and dosage for each disease in various patients [16]. In addition, most countries have insufficient legislative frameworks to guide herbal medicine practice, partly contributing to mushrooming of scoundrel practitioners, thereby jeopardising public health safety. Moreover, the lack of crucial pharmacological information, such as herb-herb and herb-synthetic drug interaction effects and contraindications, among others, may cause deleterious consequences when improperly formulated or combined [17–20]. Therefore, investigating medicinal plant toxicity provides essential information that may help appraise its safety and foster further empirical investigations to validate its medicinal value and drug development.

Therefore, this study was designed to investigate the antimicrobial, cytotoxicity, and qualitative phytochemical composition of the aqueous and methanolic leaf extracts of *Physalis peruviana* L. (Solanaceae) based on its ethnomedicinal background. This herb is known as '*Nathi*' among the Agikuyu community of Kenya, where its leaves are commonly used to treat various microbial infections, including typhoid, pneumonia, inflammation, and spasms, among other diseases [21]. Previous studies indicate fruit and stem bark extracts possess anti-inflammatory and antimicrobial activity [22–24], partly attributable to phytosterols, carotenoids, campesterol, and stigmasterol, among other phytochemicals [25]. However, to the best of our knowledge, the antimicrobial activity and cytotoxicity of *P. peruviana* leaves have not been investigated, hence the present study.

MATERIALS AND METHODS

Plant Collection, Identification, and Processing

The studied plant was identified by its local name ('*Nathi*') with the help of a local herbalist in the natural habitat at Kinangop Sub-County, Njambini Location in Nyandarua County and selected for this study based on its ethnomedicinal usage in treating microbial infections. Voucher specimens were also collected, prepared, and authenticated taxonomically at the Department of Land Resource management and Agricultural Technology (LAMART-UON/HR/250). Then, fresh leaves were sparingly harvested from randomly sampled plants, packaged in well-aerated sisal bags, and transported to the Department of Public Health, Pharmacology, and Toxicology laboratories, at the College of Agriculture and Veterinary Sciences, Kabete Campus, University of Nairobi. The leaves were spread evenly to dry at room temperature for two weeks, after which they were ground into a powder using an electric plant mill. The powdered material was stored in a plastic container on a laboratory shelf awaiting extraction.

Extraction procedure

The extraction procedures described by Harborne [26] and modified by Moriasi *et al.* [27] were followed. For the methanolic extract, 250 g of the ground material was macerated in 1L of methanol and allowed to extract for 48 hours with occasional shaking. The mixture was then decanted carefully and filtered through Whatman filter paper (No. 1) into clean 250 ml conical flasks. The filtrate was concentrated *in vacuo* using a rotary evaporator at 50 ºC and then dried completely in a hot-air oven set at 35 ºC for five days. For the aqueous extract, 50 g of the powdered material was soaked in 500 ml of distilled water and heated at 60 ºC in a water bath for 10 minutes. After that, the mixture was cooled to room temperature, filtered through a Whatman filter paper (No.1), and then lyophilised *in vacuo* using a freeze drier for 48 hours. All the extracts were packaged in well-labelled glass bottles and stored in a refrigerator (4 °C) until use.

Determination of *in vitro* antimicrobial activity of the study extracts

Test Microorganisms

The antimicrobial activity of the aqueous and methanolic leaf extracts of *P. peruviana* was investigated using *Pseudomonas aeruginosa* (ATCC 700603), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25925), *Bacillus cereus* (ATCC 11778), and *Candida albicans* (ATCC 10231). These microbes were provided by the University of Nairobi's Microbiology Laboratory, Department of Public Health, and Faculty of Veterinary Medicine.

Preparation of inoculums

The experimental inoculums were prepared according to the Clinical Laboratory Standards Institute (CLSI) guidelines [28]. Briefly, the bacterial strains were subcultured in Mueller Hinton Agar, while the fungal strain (*C. albicans*) was subcultured in Sabaroud Dextrose Agar for 24 hours at 37 ºC. After that, they were harvested using 5 ml of normal saline, and their absorbances were adjusted to 0.11-0.14 at 530 nm using a spectrophotometer to achieve turbidity equivalent of 0.5 McFarland scale ($1\text{-}5\times10^6$ cfu/ml for the fungal strain and $1\text{-}28\times10^8$ cfu/ml for bacterial strains).

The disk diffusion assay procedure

The disk diffusion technique [28–30] was used to determine the antimicrobial susceptibility of the microbial strains to the test extracts. In brief, each extract was weighed (0.8 g), separately dissolved in 1.4 % of dimethylsulphoxide (DMSO) (10 ml), and vortexed vigorously to yield 800 µg/ml concentrations. After that, the stock concentrations of each extract were serially diluted two-fold to yield concentrations of 800 µg/ml, 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, and 6.25 µg/ml, respectively. Then, 20 µl of each extract concentration were aspirated and carefully dispensed on sterile 6 mm diameter disks, prepared from Whatman papers, and placed equidistantly on petri dish plates inoculated with 1 ml of respective microbial inoculum. The experiments were performed in triplicate with DMSO as a negative control and Ciprofloxacin (10 µg) of Fluconazole (10 µg) as a positive control. The plates were incubated at 37 ºC for 24 hours in an incubator, after which respective growth inhibition zone diameters were measured and recorded.

Furthermore, the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were determined according to a previously described with slight modifications. The MIC was defined as the lowest extract concentration that could inhibit microbial growth, while the MBC/MFC was defined as the lowest extract concentration that could completely suppress microbial growth in freshly inoculated agar plates after 24 hours of incubation [28].

Examination of *in vitro* cytotoxicity of the study extracts

The brine shrimp lethality assay method, described by Meyer *et al.* [31] was used to examine the cytotoxicity of the study extracts. Briefly, 1 g of *Artemia salina* nauplii cysts (Sanders Great SaltLake, Brine Shrimp Company LC, USA.) were dispensed in an artificially prepared sea containing 3 % of marine salt into the dark compartment of the holding plastic container, which had two compartments (the light and dark compartments) separated by a plastic sheet on which 2 mm wide holes were poked. The light compartment was illuminated by a bulb, and the setup was allowed to settle for 48 hours at 25-29 ºC. After that, 10 nauplii were carefully using a Pasteur pipette and transferred into test tubes containing 5 ml of serial concentrations (0.0 µg/ml, 0.01 µg/ml, 0.1 µg/ml, 1 µg/ml, 10 µg/ml, 100 µg/ml, and 1000 µg/ml in 1% DMSO) of each extract or vincristine sulphate (positive control) in five replicates. The test tubes were incubated at room temperature for 24 hours, after which the number of survivors was recorded and used to compute the percentage mortality in each tube. Moreover, the median lethal concentration (LC_{50}) was defined as the concentration of the test extract, which could cause a 50 % mortality of the nauplii, and was derived from a line of best-fit plots (simple linear regression) of percentage mortality against the concentration.

Qualitative phytochemical screening of the study extracts

The presence or absence of alkaloids, quinones, anthraquinones, cyanogenic glycosides, cardiac glycosides, flavonoids, reducing sugars, tannins, saponins, terpenoids, phenolics, and phytosterols was determined according to the procedures described by Harborne [26] and Kathare *et al.* [32]

Data management and statistical analysis

Quantitative data from the antimicrobial activity and cytotoxicity assays were tabulated on a spreadsheet (Microsoft 365), arranged, and then exported to GraphPad Prism version 9.5.1 for analysis. The data were descriptively analysed and expressed as mean± standard error of the mean (SEM) ($\bar{x} \pm SEM$). Afterward, inferential statistics using One-Way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* were performed to determine the significance of differences among means and for pairwise comparisons and separation of means. Besides, unpaired student t-test statistic was used to determine the significance of differences between the antimicrobial and cytotoxicity of the two studied plant extracts at each concentration. The LC_{50} values of the study extracts were determined using a simple linear regression analysis of percentage mortality against concentration. During inferential statistical analyses, means with P<0.05 were considered significant. Qualitative data from qualitative phytochemical screening was only tabulated.

Ethical approval

This study was ethically approved by the Faculty of Veterinary Medicine's Biosafety, Animal Care and Use Committee (FVM-BAUEC) of the University of Nairobi (REF: FVM BAUEC/2023/423).

RESULTS

Antimicrobial activity of the aqueous and methanolic leaf extracts of *P. peruviana*

Growth inhibition zones

Our findings showed no significant differences between and among growth inhibition zones produced by the aqueous and methanolic leaf extracts of *P. peruviana* at all concentrations (P>0.05); however, the positive control (ciprofloxacin) produced a significantly larger growth inhibition zone on *P. aeruginosa* than the two extracts (P<0.05; Table 1).

The growth inhibition zones produced by the aqueous leaf extract of *P. peruviana* on *E. coli* at concentrations of ≤50 µg/ml were not significantly different (P>0.05) but were significantly smaller than those recorded at higher concentrations (P<0.05; Table 1). Similarly, differences in growth inhibition zones produced by the aqueous extract on *E. coli* at concentrations of 100 µg/ml and 200 µg/ml were insignificant (P>0.05; Table 1). Besides, the growth inhibition zones produced by the methanolic extract at concentrations of 200-800 µg/ml were not significantly different (P>0.0.5); however, these zones were significantly larger than those produced at lower concentrations (P<0.05). At 6.25-25 µg/ml concentrations, the methanolic extract produced significantly smaller growth inhibition zones on *E. coli* than at higher concentrations (P<0.05), as shown in Table 1. Moreover, the methanolic extract produced significantly larger growth inhibition zones than the aqueous extract at all concentrations on plates inoculated with *E. coli* (P<0.05; Table 1). The positive control antibiotic (Ciprofloxacin) produced significantly larger growth inhibition zones on *E. coli* than those of the two extracts at all concentrations (P<0.05; Table 1).

We observed no significant differences between and among the growth inhibition zones produced by the aqueous and methanolic leaf extracts of *P. peruviana* on *S. aureus* at concentrations of between 6.25-100 µg/ml (P>0.05; Table 1). The two extracts produced significantly larger growth inhibition zones on *S. aureus* at higher concentrations than at lower concentrations (P<0.05; Table 1). Nevertheless, a comparison between the growth inhibition zones produced by the study plant's aqueous and methanolic leaf extracts revealed no significant difference (P>0.05; Table 1). As shown in Table 1, the positive control antibiotic formed a significantly larger growth inhibition zone on *S. aureus* than the study extracts at all concentrations (P<0.05; Table 1).

The aqueous leaf extract of *P. peruviana*, at concentrations of <50 µg/ml and between 200 µg/ml and 400 µg/ml, and the methanolic extract at concentrations of <25 µg/ml showed significantly smaller growth inhibition zones than those recorded at higher concentrations on *B. cereus* (P<0.05; Table 1). Further, no significant differences between the growth inhibition zones produced by the aqueous and those produced by the methanolic leaf extracts of *P. peruviana* were observed at all concentrations except at 400 µg/ml were observed (P>0.05; Table 1). However, the methanolic leaf extract of *P. peruviana* produced a significantly larger growth inhibition on *B. cereus* than that produced by the aqueous extract (P<0.05; Table 1). The reference antibiotic (ciprofloxacin) produced a significantly larger growth inhibition zone than the plant extracts at all concentrations in this study (P<0.05; Table 1).

The antifungal effects of the studied plant extracts on *C. albicans* were also investigated in this study. The results showed no significant differences among the growth inhibition zones produced by the aqueous and methanolic leaf extracts of *P. peruviana* at concentrations <200 µg/ml on *C. albicans* (P>0.05; Table 1). Likewise, the growth inhibition zones produced by the two extracts at concentrations of 400 µg/ml and 800 µg/ml, and those recorded between the aqueous and methanolic extracts at all concentrations, were not significantly different (P>0.05; Table 1). However, the positive control antifungal

drug (Fluconazole) produced a significantly larger growth inhibition than those produced by the two extracts (P<0.05; Table 1).

Table 1: Growth inhibition zones produced by the aqueous and methanolic leaf extracts of *P. Peruviana* on various microbes

Concentration (µg/ml)	Growth inhibition zone diameter (mm)									
	Pseudomonas aeruginosa		*Escherichia coli*		*Staphylococcus aureus*		*Bacillus cereus*		*Candida albicans*	
	Aq. PP	**MeOH.PP**	**Aq. PP**	**MeOH.PP**	**Aq. PP**	**MeOH.PP**	**Aq. PP**	**MeOH.PP**	**Aq. PP**	**MeOH.PP**
800	$6.00\pm0.00^{b}_{A}$	$6.00\pm0.00^{b}_{A}$	$17.67\pm0.33^{b}_{B}$	$22.67\pm0.88^{b}_{A}$	$19.00\pm0.58^{b}_{A}$	$18.33\pm0.33^{b}_{A}$	$21.00\pm0.58^{b}_{A}$	$23.00\pm0.58^{b}_{A}$	$9.67\pm0.33^{b}_{B}$	$13.67\pm0.33^{b}_{A}$
400	$6.00\pm0.00^{b}_{A}$	$6.00\pm0.00^{b}_{A}$	$15.33\pm0.33^{c}_{B}$	$21.00\pm0.58^{b}_{A}$	$15.00\pm0.58^{c}_{A}$	$15.00\pm0.58^{c}_{A}$	$17.33\pm0.67^{c}_{B}$	$20.33\pm0.33^{c}_{A}$	$9.00\pm0.00^{b}_{B}$	$11.67\pm0.33^{bc}_{A}$
200	$6.00\pm0.00^{b}_{A}$	$6.00\pm0.00^{b}_{A}$	$13.00\pm0.58^{d}_{B}$	$20.00\pm0.58^{b}_{A}$	$12.00\pm0.58^{d}_{A}$	$12.33\pm0.33^{d}_{A}$	$15.33\pm0.33^{c}_{A}$	$16.67\pm0.33^{d}_{A}$	$8.33\pm0.33^{bc}_{A}$	$9.67\pm0.33^{cd}_{A}$
100	$6.00\pm0.00^{b}_{A}$	$6.00\pm0.00^{b}_{A}$	$11.00\pm0.58^{d}_{B}$	$15.00\pm0.58^{c}_{A}$	$8.00\pm0.58^{e}_{A}$	$9.50\pm0.29^{f}_{A}$	$11.00\pm0.58^{d}_{A}$	$13.00\pm0.58^{e}_{A}$	$6.00\pm0.00^{c}_{A}$	$7.67\pm0.33^{de}_{A}$
50	$6.00\pm0.00^{b}_{A}$	$6.00\pm0.00^{b}_{A}$	$7.00\pm0.00^{e}_{B}$	$12.00\pm0.58^{d}_{A}$	$6.00\pm0.00^{e}_{A}$	$7.33\pm0.33^{f}_{A}$	$7.00\pm0.58^{e}_{A}$	$9.67\pm0.33^{f}_{A}$	$6.00\pm0.00^{c}_{A}$	$6.50\pm0.50^{e}_{A}$
25	$6.00\pm0.00^{b}_{A}$	$6.00\pm0.00^{b}_{A}$	$6.00\pm0.00^{e}_{B}$	$8.67\pm0.33^{e}_{A}$	$6.00\pm0.00^{e}_{A}$	$6.00\pm0.00^{f}_{A}$	$6.00\pm0.00^{e}_{A}$	$6.67\pm0.33^{g}_{A}$	$6.00\pm0.00^{c}_{A}$	$6.00\pm0.00^{e}_{A}$
12.5	$6.00\pm0.00^{b}_{A}$	$6.00\pm0.00^{b}_{A}$	$6.00\pm0.00^{e}_{A}$	$6.67\pm0.33^{e}_{A}$	$6.00\pm0.00^{e}_{A}$	$6.00\pm0.00^{f}_{A}$	$6.00\pm0.00^{e}_{A}$	$6.00\pm0.00^{g}_{A}$	$6.00\pm0.00^{c}_{A}$	$6.00\pm0.00^{e}_{A}$
6.25	$6.00\pm0.00^{b}_{A}$	$6.00\pm0.00^{b}_{A}$	$6.00\pm0.00^{e}_{A}$	$6.00\pm0.00^{e}_{A}$	$6.00\pm0.00^{e}_{A}$	$6.00\pm0.00^{f}_{A}$	$6.00\pm0.00^{e}_{A}$	$6.00\pm0.00^{g}_{A}$	$6.00\pm0.00^{c}_{A}$	$6.00\pm0.00^{e}_{A}$
Positive control	33.67 ± 0.88^{a}		35.67 ± 0.88^{a}		26.67 ± 0.67^{a}		25.33 ± 0.67^{a}		21.67 ± 1.45^{a}	

Values are presented as $\bar{x}\pm$SEM; Means with the same lowercase alphabet within the same column are not significantly different (P>0.05; One-Way ANOVA with Tukey's *post hoc*) and means with the same uppercase alphabet between Aq.PP and MeOH.PP within the same concentration and microbe are not significantly different (P>0.05; Un-paired t-test); Aq.PP: Aqueous leaf extract of *Physalis peruviana*; MeOH.PP: Methanolic leaf extract of *Physalis peruviana*; Positive control: Ciprofloxacin (10 µg) for *P. aeruginosa, E. coli, S. aureus,* and *B. cereus*, and Fluconazole (10µg) for *C. albicans.*

Minimum inhibitory concentrations (MICs) and Minimum bactericidal concentrations (MBCs)

Further, the minimum inhibitory concentrations (MIC) and minimum bactericidal/fungicidal (MBC/MFC) were determined in this study. The aqueous leaf extract of *P. peruviana* had MICs of 50 µg/ml for *E. coli* and *B. cereus,* 100 µg/ml for *S. aureus,* and 200 µg/ml for *C. albicans* (Table 2). The MBC of the aqueous extract was 100 µg/ml for *E. coli* and *B. cereus,* 200 µg/ml for *S. aureus*, while the MFC for *C. albicans* was 400 µg/ml (Table 2). The MICs of the methanolic extract were 25 µg/ml for *E. coli*, *S. aureus,* and *B. cereus* and 100 µg/ml for *E. C. albicans* (Table 2). The MBCs of the methanolic extract were 100 µg/ml for E. *coli, S. aureus,* and *B. cereus*, and the MFC for *C. albicans* was 200 µg/ml (Table 2).

Table 2: Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFC) of the aqueous and methanolic leaf extracts of *P. peruviana*

Microorganism	Plant extract	MIC (µg/ml)	MBC/MFC (µg/ml)
P. aeruginosa	Aq.PP	-	-
	MeOH.PP	-	-
E. coli	Aq.PP	50	100
	MeOH.PP	25	100
S. aureus	Aq.PP	100	200
	MeOH.PP	50	100
B. cereus	Aq.PP	50	100
	MeOH.PP	50	100
C. albicans	Aq.PP	200	400
	MeOH.PP	100	200

MIC: Minimum Inhibitory Concentration; MBC/MFC: Minimum Bactericidal Concentration/Minimum Fungicidal Concentration; Aq.PP: Aqueous leaf extract of *Physalis peruviana*; MeOH.PP: Methanolic leaf extract of *Physalis peruviana*

Cytotoxicity of the aqueous and methanolic leaf extracts of *P. peruviana*

Percentage mortality of brine shrimp nauplii

The results revealed no significant differences between the percentages of mortalities of brine shrimp nauplii exposed to vincristine sulphate (1 µg/ml) and 1000 µg/ml of the aqueous leaf extract of *P. peruviana* (P>0.05; Table 3). Likewise, the difference between the percentage mortality of the brine shrimp nauplii exposed to 10 µg/ml and 100 µg/ml of the aqueous or methanolic leaf extract of *P. peruviana* was statistically insignificant *(*P>0.05; Table 3). No mortality was recorded in brine shrimp nauplii exposed to 0.01 µg/ml of the two extracts and 0.1 µg/ml of the methanolic leaf extract of *P. peruviana* (Table 3). Further, the percentage of mortality of brine shrimp nauplii exposed to 0.1 µg/ml, 100 µg/ml, and 1000 µg/ml of the aqueous leaf extract of *P. peruviana* were significantly higher than those recorded in brine shrimp nauplii exposed to similar concentrations of the methanolic leaf extract of *p. peruviana* (P<0.05; Table 3).

Table 3: Percentage of mortality in brine shrimp nauplii exposed to the aqueous and methanolic leaf extracts of *P. peruviana*

Concentration (µg/ml)	% Mortality	
	MeOH.PP	Aq. PP
0.01	$0.00\pm0.00^{E}{}_{a}$	$0.00\pm0.00^{E}{}_{a}$
0.1	$2.00\pm1.00^{D}{}_{a}$	$0.00\pm0.00^{E}{}_{b}$
1	$10.00\pm3.16^{C}{}_{a}$	$4.00\pm2.45^{D}{}_{a}$
10	$18.00\pm3.74^{BC}{}_{a}$	$10.00\pm0.00^{CD}{}_{a}$
100	$34.00\pm4.00^{B}{}_{a}$	$16.00\pm2.45^{C}{}_{b}$
1000	$74.00\pm4.00^{A}{}_{a}$	$34.00\pm4.00^{B}{}_{b}$
Vincristine sulphate (1 µg)	88.00 ± 5.83^{A}	88.00 ± 5.83^{A}

Values are presented as $\bar{x} \pm SEM$ of five replicates (n=5); Means with different Uppercase superscript letters within the same column are significantly different (P<0.05; One-Way ANOVA with Tukey's *post hoc*) and means with different lowercase subscript letters within the same row are significantly different (P<0.05; Unpaired student t-test). Aq.PP: Aqueous leaf extract of *Physalis peruviana*; MeOH.PP: Methanolic leaf extract of *Physalis peruviana*.

Median lethal concentration (LC$_{50}$) Values

Furthermore, the concentration of the study extract or reference drug, which could cause a 50 % mortality of the brine shrimp nauplii (LC_{50}), was determined from a simple linear regression of percentage mortality against concentration ($Y = 0.03064X + 4.279$ for the aqueous extract; $Y = 0.06679X + 9.113$ for the methanolic extract; $Y = 90.56X - 3.130$ for vincristine). As the results in Table 4 show, the LC_{50} of the aqueous leaf extract of *P. peruviana* was higher (1492.20 µg/ml) than that of the methanolic extract of the same plant, which was 612.17. The reference drug (Vincristine sulphate) had a very low LC_{50} value of 0.59 µg/ml (Table 4)

Table 4: LC_{50} values of the aqueous and methanolic leaf extracts of *P. peruviana* on brine shrimp nauplii

Drug	LC_{50} Values (µg/ml)
MeOH.PP	612.17
Aq.PP	1492.20
Vincristine (1 µg/ml)	0.59

Aq.PP: Aqueous leaf extract of *P. peruviana*; MeOH.PP: Methanolic leaf extract of *P. peruviana*

Qualitative phytochemical composition of the aqueous and methanolic extracts of *P. peruviana*

Table 5 shows the results of qualitative phytochemical analysis of the aqueous and methanolic leaf extracts of *P. peruviana.* The results showed that out of the tested phytochemicals, only the quinolones and phytosterols were absent (Table 5).

Table 5: Qualitative phytochemical composition of the aqueous and methanolic leaf extracts of *P. peruviana*

Phytochemical group	Aq. PP	MeOH. PP
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Glycosides	+	+
Flavonoids	+	+
Phenols	+	+
Anthraquinones	+	+
Cardiac glycosides	+	+
Coumarins	+	+
Quinones	-	-
Phytosterol	-	-

Aq.PP: Aqueous leaf extract of *P. peruviana*; MeOH.PP: Methanolic leaf extract of *P. peruviana*; +: Present; -: Absent

DISCUSSION

Considering the high morbidity and mortality rates caused by microbial infections, and the ineffectiveness of currently used antibiotics in eradicating the infections, compounded by their adverse effects [33], alternative strategies are required [34–36]. This study investigated the antimicrobial, cytotoxicity, and qualitative phytochemistry of the aqueous and methanolic extracts of *P. peruviana* as potential sources of alternative antimicrobials.

We appraised the antimicrobial activity of the study extracts based on the growth inhibition zones produced on each tested microbial strain according to a previously described criterion [36, 37]. Growth inhibition zone diameters measuring 6-8 mm denote slight antimicrobial activity, while those measuring 9-12 mm, 13- 15 mm, 16-19 mm, and ≥20 mm indicate moderate, high, very high, and remarkable antimicrobial activity, respectively [36, 37]. Based on this criterion, the aqueous and methanolic leaf extracts of *P. peruviana* were inactive against *P. aeruginosa* at all the tested concentrations. Generally, the study plant's aqueous and methanolic leaf extracts at concentrations of ≤ 50 µg/ml were either inactive or slightly inactive against *E. coli, S. aureus, B. cereus,* and *C. albicans.* However, aqueous extract demonstrated high to very high activity against *E. coli* and *S. aureus* and very high to remarkable activity against *B. cereus.* Besides, the methanolic extract showed remarkable antimicrobial activity at ≥200 µg/ml concentrations against *E. coli,* moderate to very high against *S. aureus,* high to very high against *B. cereus,* and moderate to high against *C. albicans*. These findings are comparable to those of the aqueous and methanolic stem bark extracts of the same plant collected from Murang'a County reported by Kathare *et al.* [38]. and further corroborate those of Higaki *et al.* [39] and Gostok and Zengin [22].

Previous studies indicate that plant extracts with MIC and MBC/MFC values below 1000 µg/ml may be potential sources of antimicrobial agents, and values ≤100µg/ml depict significant antimicrobial efficacy [40–42]. Accordingly, we examined the efficacy of the study extracts based on their MIC and MBC/MFC values to appraise their antimicrobial potential. Our findings showed that the methanolic extract had significant antimicrobial efficacy (MIC= 25-100 µg/ml; MBC= 50-200 µg/ml) against the experimental strains. Similarly, the aqueous extract demonstrated significant antimicrobial efficacy against all the tested strains (MIC= 50-100 µg/ml; MBC=100-200 µg/ml)

except *C. albicans* (MIC=200 µg/ml; MBC 400 µg/ml). Therefore, these results suggest significant antimicrobial efficacy of the studied extracts against various microorganisms and are consistent with previous reports [7, 43, 44].

Ample research has demonstrated that various phytochemicals exert the antimicrobial efficacy of plant extracts, which may act singly or in association with others to avert microbial growth or their existence [45–47]. For instance, tannins, flavonoids, and phenols, among other phytochemicals, are responsible for the antimicrobial activity of various plants [14, 36, 45, 48]. Therefore, the observed antimicrobial effects, especially at higher concentrations, were attributed to various antimicrobial-associated phytochemicals in the extracts. The low efficacy or inefficacy of the extracts against *P. aeruginosa* and other microbes at lower concentrations may be due to the absence or low concentrations of responsible amalgams. Moreover, the varied antimicrobial activities may be attributable to the differences in the composition of phytochemical types, and microbial dynamics cumulatively influence drug agent potency [42, 49–51]. Nonetheless, further studies aimed at demystifying the mechanisms through which the studied plant extracts exert their antimicrobial efficacy may be insightful in discovering alternative antimicrobials to curb antimicrobial resistance.

Although herbal preparations have been utilised to treat microbial infections since antiquity with appreciable success, their safety profiles remain elusive [52, 53]. The insufficiency of crucial data regarding preparation procedures, storage, labelling, marketing, dosage regimens for various diseases, contraindications, herbal preparation-synthetic drug, and herb-herb interaction effects, and the lack of proper herbal medicine practice legislation in many Countries raise serious public safety concerns, as inappropriate use may cause life-threatening sequelae [16, 53–55]. Thus, determining the toxicity and safety of herbal preparations is worthwhile and offers crucial data that aids the empirical validation of the reported efficacy and prevent potential toxicity when these preparations are consumed, and directs the course of further research [17, 19, 56]. Hence, this study investigated the cytotoxicity of the aqueous and methanolic leaf extracts of *P. peruviana*, which the Agikuyu people of Nyandarua County prominently use to manage microbial infections in humans and animals [57].

We used the standard brine shrimp lethality assay technique to determine the cytotoxicity of the study extracts and appraise their safety [31]. The results showed a concentration-dependent increase in the percentage mortality of the brine shrimp *nauplii* exposed to the studied plant extracts. Besides, the differences between the percentage mortalities of the two extracts at lower concentrations (10 µg/ml) were insignificant; however, the methanolic extracts were significantly more cytotoxic than the aqueous extract at higher concentrations. The results further showed that the aqueous leaf extract of the study plant had an LC_{50} value of 1492.20 µg/ml, which was higher than 1000 µg/ml, denoting its safety, while that of the methanolic extract was < 1000 µg/ml (612.17 µg/ml) indicating its weak cytotoxicity. Generally, plant extracts having LC_{50} values of >100 µg/ml are of low cytotoxicity; thus, the two extracts were deemed safe [32]. Our findings corroborate those reported earlier on the same plant's stem bark [21] and fruit extracts [58], further fostering its safety, considering the longstanding usage in traditional medicine. The safety of these extracts is attributable to the absence or low concentrations of toxic phytochemicals [59, 60]. Upon further evaluation, it is anticipated that these extracts may be safe in higher animals and humans and that this information may be instrumental in validating their bio-efficacy in managing various diseases.

Research demonstrates that phytochemicals drive the bioactivity of medicinal plants in treating various diseases [61, 62]. The synthesis of these phytocompounds, commonly known as secondary metabolites, is evoked by biotic and abiotic stress, which faces wild plants and serves a protective role [13]. Mainly, antioxidant phytocompounds, especially flavonoids, phenols, tannins, and coumarins, exhibit a broad spectrum of pharmacologic activity, including antimicrobial efficacy [62]. In this study, qualitative phytochemical analysis of the aqueous and methanolic leaf extracts of *P. peruviana* revealed the presence of various phytochemicals associated with antimicrobial activity. Thus, the antimicrobial effects of the aqueous and methanolic leaf extracts of *P. peruviana* reported herein are attributable to the presence of tannins, phenols, flavonoids, and coumarins, whose bioactivity has been demonstrated previously [25]. Besides, the toxicity of medicinal plants is usually attributable to the presence of alkaloids, anthraquinones, and some glycosides [63]. This suggests that the concentration-dependent increase in percentage mortality of brine shrimp *nauplii* exposed to the studied plant extracts is due to the toxic amalgams, whose increase in concentration increases toxicity. The generally low cytotoxic efficacy of the two extracts implies that the detected toxicity-associated phytochemicals were of low concentrations. Nevertheless, further toxicological investigations should be conducted using other model organisms to fully establish the studied plant extracts' safety levels and toxicity profiles.

CONCLUSIONS AND RECOMMENDATIONS

Based on the findings, this study concluded that the aqueous and methanolic leaf extracts of *P. peruviana* possess antimicrobial activity against *S. aureus*, *E. coli*, *B. cereus*, and *C. albicans* and do not have antimicrobial activity against *P. aeruginosa*. Besides, the aqueous leaf extract of *P. peruviana* is non-toxic to brine shrimp nauplii, while the methanolic extract has weak toxicity. Considering the findings reported herein, further antimicrobial investigations using other microbial strains and determination of the specific mechanisms through which the studied plant extracts exert their activity are recommended. Moreover, extensive toxicological investigations should be conducted using other advanced model organisms to fully establish the safety of the studied extracts, individually and when combined. Furthermore, the specific antimicrobial phytochemicals should be isolated and characterised as potential lead compounds for drug development.

Availability of data and materials

All data is presented within the manuscript; however, any additional information may be provided by the authors upon reasonable request.

Competing interests

The authors declare that there are no competing interests/conflicts of interest on this publication.

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Authors' contributions

Samuel M Njoroge, James Mbaria, and Gabriel Aboge conceived the research idea and designed the experiments. Samwel Njoroge

performed the experiments, analysed the data, and wrote the manuscript with the guidance of Gervason Moriasi. All authors reviewed and approved the final draft for submission and publication.

Conflict of interest

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