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Research Article

Antimicrobial, Cytotoxicity, Acute Oral Toxicity and Qualitative Phytochemical Screening of the Aqueous and Methanolic Extracts of *Physalis peruviana* L (*Solanaceae*)

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ABSTRACT

Pathogenic microbes are the major causes of morbidity and mortality globally, especially in children and in immunocompromised individuals. Despite the successes of antimicrobial therapy, various challenges, including antimicrobial resistance, therapeutic failure, deleterious side effects, high costs, and inaccessibility, hinder health and wellbeing, necessitating the need for alternative and complementary approaches. Medicinal plants have, for a long time, played an integral role in meeting the primary healthcare needs of over 80% of the global population, especially in low- and middle-income countries. However, despite the rich ethnomedical evidence of utilization there are insufficient empirical scientific data to validate and authenticate the therapeutic potential of medicinal plants. *Physalis peruviana* (*Solanaceae*) is used by the Agikuyu community of Kenya to treat malaria, pneumonia, typhoid, among other health conditions. Even though this plant has been used since antiquity to treat microbial-associated infections, there is no enough scientific proof of its pharmacologic efficacy against microbial infections. Moreover, the safety levels and toxicity profiles of herbal preparations of *P. Peruviana* are not adequately demystified scientifically. As a result, the current study investigated the antimicrobial, cytotoxicity, acute oral toxicity, and qualitative phytochemical composition of the aqueous and methanolic bark extracts of *P. Peruviana* and potential sources of alternative, efficacious, safe, and affordable antimicrobials. The disc diffusion and the Broth microdilution techniques were used to evaluate the antimicrobial activity of the studied plant extracts on selected microbial strains (*Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, and Candida albicans*). The brine shrimp lethality test was used to determine the cytotoxicity of the studied plant extracts. At the same time, the acute oral toxicity effects were investigated as per the guidelines of the Organization for Economic Co-operation and Development (OECD) outlined in document number 425. Qualitative phytochemical screening was performed using standard procedures. The aqueous bark extract of *P. Peruviana* exhibited slight antimicrobial activity against *S. typhimurium* and *E. coli*, slight to moderate activity against *S. aureus*, and moderate to high activity against *C. albicans*, in a concentration-dependent manner. Besides, the methanolic bark extract of *P. Peruviana* showed slight antimicrobial activity against *S. typhimurium* and slight to moderate activities against *E.coli*, *S. aureus*, and *C. albicans* microbial strains. Moreover, both of the studied plant extracts did not show any observable signs of acute oral toxicity effects in Winstar rats, and cytotoxicity in brine shrimp *Nauplii*. The studied plant extracts showed the presence of antimicrobial- associated phytochemicals. Further studies to establish specific mode(s) through which the studied plant extracts exert their antimicrobial activity should be done. Moreover, the antimicrobial effects of the studied plant extracts on other microbial strains of clinical significance should be evaluated. Additionally, extensive safety and toxicity evaluation of the studied plant extracts should be undertaken. Quantitative phytochemical evaluation, isolation, characterization and development of antimicrobial compounds from the studied plant extracts should also be done in future studies.

Keywords: Antimicrobial activity; *Physalis peruviana*; Disk diffusion; Broth Microdilution; Brine shrimp lethality assay; Acute oral toxicity

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INTRODUCTION

Microbial infections are among the major causes of morbidity and mortality in humans globally, especially in vulnerable groups like young children, pregnant women, the elderly, and the immunocompromised [1-3]. Furthermore, the increased rate of antimicrobial resistance to the available medications has caused significant healthcare challenges [4,5]. The persistence of microbial infections during and after the treatment cycles has precipitated an overuse of antibiotics leading to other unprecedented outcomes, including resistance, adverse effects, and therapeutic failure [6].

Various synthetic antibiotic drugs currently prescribed in conventional medicine for the treatment of microbial infections cause detrimental effects, thereby hindering their therapeutic significance [7-9]. Additionally, the presumably potent antimicrobial agents are arguably expensive and inaccessible, especially to low-income earners [10]. Therefore, due to the inexorable challenges posed by microbial infections, and the failures of conventional antimicrobial therapies, the need for alternative and complementary stratagems is warranted.

Medicinal plants have across ages formed a critical component of healthcare, especially to persons of low income [11-14]. The World Health Organization (WHO) declared that over 80% of the world population, especially in Asia and Sub-Saharan Africa, depend on herbals for their health solutions [15,16]. The enormous utilization and high confidence in tradition medicines are attributable to their alleged high efficacies, safety, acceptability, extensive usage, easy availability, and accessibility compared with synthetic medicines [17-19]. Consequently, the viability of traditional medicine has been fostered by the fact that many potent medicines used in western medicine were derived from medicinal plants [20]. Despite the rich ethnomedical history and claimed potency of medical plants, only a handful has been scientifically investigated to validate their pharmacologic efficacies [21, 22]. Moreover, concerns regarding the safety and toxicity of medicinal plants have been raised [23], thus hindering their integration into universal healthcare systems [24]. The lack of policies regulating traditional medicine practice and insufficient research data supporting their healing claims, as well as their assumed low toxicities, have curtailed the advancement of traditional medicine. Therefore, it is essential to empirically study and document the safety, toxicity, and bioactivity of medicinal plants as they are viable sources of potent medicines.

Physalis peruviana L (*Solanaceae*) is a small herb belonging to the *Solanaceae* family and is widely distributed in temperate and tropical climate regions [25]. In French, it is called "Amour an Cage" ("love in a cage"). It is locally known as 'Nathi' by the Kikuyu of Murang' a County, Kenya [26]. It has a round and smooth berry (fruit), which resembles a miniature yellow tomato measuring 1.25–2 cm in diameter [27]. Traditionally, various parts of *P. peruviana* are used in the treatment of malaria, pneumonia, typhoid, among other health conditions [28].

Previous studies have demonstrated the anti-inflammatory, anti-hepatoma, antimicrobial activities in fruit and leaf extracts of *P. peruviana* [29-32]. Phytochemical analysis has revealed the presence of ergosterol, campesterol, stigmasterol, lanosterol, total sterols, withanolides, carotenoids, among other pharmacologically important phytochemicals [33-36]. However, antimicrobial, cytotoxicity, acute oral toxicity, and qualitative phytochemistry of the aqueous and methanolic stem bark extracts have not been evaluated, hence this study.

MATERIALS AND METHODS

Plant collection, identification and processing

PThe stem barks of *P. peruviana* plant were selected for this study based on their ethnomedical usage among the Agikuyu community of Murang' a County in the treatment and management of microbial infections [26]. The plant was first identified by its local name ('Nathi') with the help of a reputable herbalist, and later by a taxonomist at the East Africa Herbaria at the National Museums of Kenya under voucher specimen number: NMK/02/2019. The stem barks of the studied plant were then collected from its natural habitat 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. They were then evenly spread to dry at room temperature for two weeks, after which they were then 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 of the collected plant materials

The methanolic and aqueous stem bark extracts of *P. peruviana* were prepared according to the procedures described by Harborne [26]. The methanolic stem bark extract of *P. peruviana* was obtained by cold maceration method, whereby 250 g of the powdered material was soaked in a litre of analytical-grade methanol in a 2-litre conical flask. The flask containing the merc-menstruum mixture was gently shaken and covered with an aluminum foil paper. The mixture was shaken once daily for two days; after that, the mixture was decanted and filtered through Whatman filter paper (No.1). The filtrate was then concentrated in vacuo by using a rotary evaporator. The obtained extract was transferred into a glass bottle and further dried in a hot- air oven at 35°C for five days. For the aqueous extract, about 50 g of *P. peruviana* powder was macerated in 500 ml of distilled water and heated for ten minutes at 58 °C. The mixture was allowed to cool to room temperature and then filtered through a Whatman filter paper. The filtrate was transferred into freeze-drying flasks, at volumes of 200 ml, and fitted into a freeze-dryer, where they were lyophilized for 48 hrs. The dried extracts were weighed, and their respective percentage yields were determined. All the extracts were stored in a refrigerator (4°C) and only retrieved when required.

Determination of the antimicrobial effects of the aqueous and methanolic stem bark extracts of *P. peruviana* on selected microbes

In this study, *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 25923), and *Candida albicans* (ATCC 10231) were obtained from the Department of Public Health, Pharmacology and Toxicology of the College of Agriculture and Veterinary Sciences, University of Nairobi, Kabete Campus. These microbial strains were selected based on their clinical significance and availability. To investigate the effects of the studied plant extracts on the selected microbial strains, the disc diffusion and broth microdilution techniques described by the Clinical and Laboratory Standards Institute were followed [37].

Preparation and standardization of microbial inoculum for experimentation

The fungal strain (*C. albicans*) was grown in Sabouraud Dextrose Agar (SDA; Oxoid) for 24 hrs according to the directions of the

M100-S23 document of the CLSI. After that, sterile normal saline was used to standardize the inoculum to achieve a 0.5 McFarland standard at 530 nm using a UV-Vis spectrophotometer. Optical density ranges of between 0.11 and 0.14 at 530nm were obtained. This was considered to be 1-5 × 10^6 cfu/ml. On the other hand, the bacterial strains (*E. coli*, S. typhimurium, *and S. aureus*) were grown in Mueller-Hinton agar as per the CLSI guidelines for 24 hrs. After that, the inocula were standardized to a turbidity equivalent to a 0.5 McFarland scale of approximately 1-2 × 10^8 cfu/ml [38].

The disc diffusion assay for antimicrobial susceptibility

In this assay, 1 g of each of the studied extracts was dissolved in 10 ml of 1.4% DMSO (in sterile water) in a 15 ml centrifuge tube and thoroughly vortexed to make stock solutions of containing 100 µg/ml. The stocks were then serially diluted two-fold to give 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml and 3.125 µg/ml respectively. Afterward, 20 µl were aspirated and carefully impregnated on sterile Whatman discs (6 mm diameter). The discs were gently pressed on the media containing 1 ml of the bacterial or fungal inocula to allow for proper drug-microbe contact. The assays were performed in triplicate with DMSO as negative control and streptomycin or ciprofloxacin or amphotericin B as positive controls. All the plates were incubated for 24 hrs at 37 °C, and then diameters of zones of inhibition of microbial growth measured in millimeters.

The broth microdilution technique for Minimum Inhibitory Concentration (MIC) determination

In this determination, the modified CLSI method described by Golus et al. [39] was adopted. Briefly, cultures were prepared and adjusted in Mueller-Hinton Broth media to 0.5 Mc Farland equivalent turbidity. Carefully, 10 µl of the previously prepared test extracts at a 10-fold concentration were transferred into Eppendorf tubes containing 90 µl of molten Mueller-Hinton agar in triplicate and gently vortexed. Two-fold microdilution was done in volumes of 100 µl in sterile 96-U-shaped multiwell plates. In each of the micro-titer plates, the growth, sterility control, and negative (1.4% DMSO) controls were included for each of the tested microbial strains.

All the multiwell plates could settle at room temperature for the agar to solidify. Then, 2 µl of freshly prepared inoculate at a concentration of 104 cfu/spot were dispensed into the wells using a multichannel micropipette and allowed to interact at room temperature. The wells at the sides were added sterile water, after which the plates were covered in zip-lock plastic bags and incubated at 35 °C for 18 hrs. The MIC was determined as the lowest concentration of the studied extracts, which could completely inhibit microbial growth as per the CLSI recommendations [38].

Evaluation of the effects of the aqueous and methanolic extracts of *P. peruviana* on brine shrimp *nauplii*

In this study, the brine shrimp lethality assay method described by Meyer et al. [40] was followed. Briefly, approximately 0.5 g of *Artemia salina* cysts (Sanders Great SaltLake, Brine Shrimp Company LC, USA.) was placed in an artificial sea containing 500 ml of brine water. They were incubated for two days to hatch into *nauplii* under continuous normal bulb illumination at 25–29 °C temperature and ample aeration. Thereafter, ten *nauplii* were transferred using Pasteur pipettes into three sets of sample vials containing either the studied plant extracts at concentrations of 0, 10, 100, and 1000 µg/ml or podophyllotoxin in 5 ml brine solutions in triplicate. The *nauplii* were then incubated for 24 hours after which the number of survivors in each test vial were counted and documented. The percentage lethality was determined as a ratio of surviving *nauplii* in the test groups to those in the control (vehicle treated) group. The Median lethal concentration (LC_{50}) values were derived from the line of best fit in a plot of percentage survival against concentration.

Evaluation of the acute oral toxicity effects of the aqueous and methanolic bark extracts of the studied plant extracts

In this study, the guidelines posited by the Organization for Economic Co-operation and Development (OECD) in protocol document number 425 were adopted [41]. Experimental female Winstar Rats weighing 150 ± 20 g were sourced from the Department of Public Health, Pharmacology, and Toxicology animal breeding unit and acclimatized for 72 hrs before dosing. The studied plant extracts were reconstituted in normal saline solution to achieve the appropriate dose for administration. On the experimentation day, the animals were fasted for 4 hrs and randomly assorted into groups of three rats. The experiment was initiated by administering a single dose of 175 mg/kg of the studied plant extracts orally to the first group and normal saline (10 ml/Kg bw) to the control group.

Observations of wellness parameters (skin fur, eye colour, mucus membrane, salivation, lethargy, sleep, coma, convulsions, tremors, and diarrhoea) were recorded at intervals of 30 min., 4 h., 24 h., 48 h., one week, and two weeks for each individual rat. In the absence of observable signs of toxicity or mortality during the 14-day experimentation period, the next subsequent higher doses of 550 mg/kg and 2000 mg/kg, respectively, were administered into new groups of rats. All the experiments were done in triplicate [41]. At the end of the experiments, the experimental rats were euthanized and disposed of according to the set protocols.

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

In this study, the standard protocols for qualitative phytochemical screening described by Harborne (1998) were followed. The phytochemicals that were evaluated include alkaloids, flavonoids, tannins, Saponins, Anthraquinones, and phenols.

Test for alkaloids

Dragendorff test: About 0.1 g of the aqueous and methanolic extracts of *P. peruviana* were extracted by boiling with 10 ml of 1% hydrochloric acid in independent test tubes. The mixtures were filtered, and to about 2 ml of the filtrate, a few drops of the Dragendorff's reagent were added. The formation of the red precipitates in the respective tubes indicates the presence of alkaloids.

Mayer's test: To 2 ml of the remaining portion of the filtrate of the respective extracts in the Dragendorff test, a few drops of Mayer's reagent were added along the sides of the respective tubes. The formation of the creamy white precipitates in the respective tubes indicated the presence of alkaloids.

Test for flavonoids: To approximately 5 ml of ethanolic filtrates of the respective extracts, 2 ml of 2% sodium hydroxide were added. The formation of an intense colour that decolorizes the addition of a few drops of diluted hydrochloric acid indicates the presence of flavonoids.

Test for tannins (ferric chloride test): About 0.1 g of the aqueous and methanolic bark extracts of the studied plant was extracted by boiling with 20 ml of the distilled water. The mixtures were filtered through Whatman filter paper, and into 2 ml of the filtrate, a few drops of 5% ferric chloride were added. The appearance of the dark green colour indicates a positive test for tannins.

Test for phenols: About 0.1 g of the studied extracts were boiled in 10 ml of 70% of ethanol for 5 minutes in a water bath and then filtered while hot. The filtrates were cooled to room temperature, and 2 ml of it be transferred into a clean test-tube then followed by dropwise addition of 5% ferric chloride solution. The appearance of green precipitates will indicate the presence of phenols.

Test for glycosides: The aqueous and methanolic extracts (0.1 g) of the studied plant were re-extracted with 10 ml of chloroform. The mixtures were then be filtered, and the filtrates reduced by heating on a hot plate to dryness. Into the remaining filtrate after heating to dryness, 0.4 ml of the glacial acetic acid with a trace amount of ferric chloride were added, followed by 0.5 ml of the concentrated H_2SO_4 through the sides of respective test-tubes. The presence of blue colour in the acetic acid layer is a positive indication for cardiac glycosides' presence.

Test for saponins: About 0.5 g of the aqueous and methanolic extracts of the studied plant were dissolved in 5 ml of warm distilled water and vigorously shaken. The appearance of persistent frothing indicated the presence of saponins.

Test for anthraquinones: Approximately 0.1 g of each of the studied extract were warmed 1 ml of chloroform in a water bath for 5 minutes. Afterward, they were filtered through Whatman filter paper and allowed to cool to room temperature before adding equivalent volumes of 10% ammonia. The mixtures were then shaken, and the presence of pink coloration on the upper layer indicates the presence of anthraquinones.

Data management and analysis

Quantitative data from antimicrobial and brine shrimp lethality experiments were tabulated on an Excel spreadsheet (Microsoft 365) and exported to Minitab version 19.2 statistical software. Descriptive statistics were performed, and values were expressed. One-Way ANOVA was used to determine differences among means followed by Tukey's post hoc test for pairwise comparisons and separations of means. Means that showed p values <0.05 were considered statistically significant. Acute oral toxicity results were treated according to the OECD (2008) guidelines. Qualitative data on wellness parameters in the acute oral toxicity and qualitative phytochemical screening studies were only tabulated. The obtained findings were presented in tables.

RESULTS

Antimicrobial activities of the aqueous and methanolic extracts of Physalis peruviana on selected microorganisms

Antimicrobial effects of the aqueous bark extract of *P. peruviana* on selected microbial strains: The obtained results showed that the zones of inhibition produced by the aqueous bark extract of *P. peruviana* on E. coli ranged from 6.50 ± 0.50 mm at a concentration of 3.125 µg/ml to 9.00 ± 0.58 mm at a concentration of 100 µg/ml (Table 1). Compared with the negative control, the zones of inhibition produced by this extract at all concentrations except at 3.125 µg/ml and 6.25 µg/ml were significantly higher (p<0.05) (Table 1). However, the zone of inhibition caused by the positive control was significantly higher than the zones of inhibition recorded by all the other experimental treatments (p<0.05). Besides, the results showed no significant differences in zones of inhibition of E. coli when the methanolic bark extract of *P. peruviana* was applied at concentrations of 50 µg/ml and 100 µg/ml (p>0.05) (Table 1). Also, the zones of inhibition of E. coli by the methanolic bark extract of *P. peruviana* at concentrations of 6.25 µg/ml , 12.5 µg/ml , and 25 mg/ml were not significantly different (p>0.05; (Table 1). However, the zone of inhibition produced by the positive control on E. coli was significantly larger than the zones in all the other incubations (p<0.05) (Table 1).The *S. typhimurium* was not susceptible to the aqueous bark extract of *P. peruviana* at concentrations of 3.125 µg/ml, 6.25 µg/ml, 12.5 µg/ml and 25 µg/ml respectively, and the respective zones of inhibition were not significantly different from that of the negative control (p>0.05; (Table 1). However, the zones of inhibition recorded by this extract at concentrations of 50 µg/ml and 100 µg/ml were significantly higher than those recorded at the other extract concentrations and the negative control. The standard antibiotic produced a significantly larger zone of inhibition than those of the rest of the treatments (p<0.05; Table 1). On the other hand S. typimurium strain was not susceptible to the methanolic extract of *P. peruviana* up to the concentration of 50 µg/ml , as the zones of inhibition were like that of the negative control(p>0.05; Table 1). However, at 100 µg/ml of the methanolic extract of *P. peruviana*, the observed zone of inhibition was significantly larger than those observed at the rest of the extract concentrations (p<0.05) (Table 1). The standard antibiotic recorded the largest zone of inhibition of *S. typhimurium* compared to all the other incubations (p<0.05) (Table 1).

Table 1: **Antimicrobial effects of the aqueous and methanolic bark extract of P*hysalis peruviana* on selected microbial strains.**

Concentration (µg/ml)	Zone of inhibition (mm)						
	E. coli		*S. typhimurium*		*S. aureus*		*C. albicans*
	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract
3.125	6.50 ± 0.50^{ef}	6.90 ± 0.30^{cd}	6.00 ± 00^{d}	6.00 ± 0.00^{c}	8.67 ± 1.33^{bc}	7.83 ± 1.17^{cd}	11.67 ± 0.88^{b}
6.25	7.50 ± 0.29^{de}	8.33 ± 1.36^{bcd}	6.00 ± 00^{d}	6.00 ± 0.00^{c}	9.00 ± 1.15^{bc}	8.67 ± 0.88^{bc}	11.67 ± 0.33^{b}
12.5	8.33 ± 0.88^{cd}	9.17 ± 0.44^{bcd}	6.00 ± 00^{d}	6.00 ± 0.00^{c}	9.00 ± 0.57^{bc}	9.67 ± 0.88^{bc}	12.67 ± 0.33^{ab}
25	8.83 ± 0.72^{bcd}	10.17 ± 1.59^{bc}	6.00 ± 00^{d}	6.00 ± 0.00^{c}	10.17 ± 1.36^{b}	9.67 ± 0.88^{bc}	12.67 ± 0.67^{ab}
50	9.83 ± 0.17^{b}	10.67 ± 0.33^{b}	7.33 ± 0.88^{c}	6.00 ± 0.00^{c}	10.67 ± 1.86^{b}	10.00 ± 0.00^{b}	12.83 ± 0.44^{ab}
100	9.00 ± 0.58^{bc}	11.83 ± 2.68^{b}	8.67 ± 0.33^{b}	7.33 ± 0.33^{b}	11.33 ± 0.33^{b}	10.67 ± 0.3^{b}	14.00 ± 0.58^{a}
-ve Control	6.00 ± 0.00^{f}	6.00 ± 0.00^{d}	6.00 ± 00^{d}	6.00 ± 0.00^{c}	6.00 ± 00^{c}	6.00 ± 0.00^{d}	6.00 ± 0.00^{c}
+ve Control	28.00 ± 0.00^{a}	30.00 ± 0.00^{a}	27.00 ± 0.00^{a}	27.00 ± 0.00^{a}	24.16 ± 0.16^{a}	23.67 ± 0.33^{a}	6.00 ± 0.00^{c}
MIC (µg/ml) [Extracts]	50	50	100	>100	25	100	6.3
MIC (µg/ml)[+ve Control]	0.3		0.3		0.62		>100

Values are expressed as $\tilde{x}$ ± SEM; Means which do not share a lowercase alphabet superscript within the same column are significantly different (One-Way ANOVA followed by Tukey's test (p<0.05); Positive control: For E. coli and S. typhimurium it was Ciprofloxacin (10 µg); For S. aureus it was Streptomycin (µg) and for C. albicans it was Amphotericin B (µg); Negative control: DMSO (1.4 %).

No significant differences among average zones of inhibitions at concentrations of 25 μg/ml, 50 μg/ml, and 100 μg/ml of P. peruviana were recorded against *S. aureus* bacterial strain. Similarly, the zones of inhibition recorded at extract concentrations of 3.125 μg/ml, 6.25 μg/ml and 12.5 μg/ml were not significantly different ($p>0.05$). However, the zones of inhibition recorded for the aqueous bark extract of P. peruviana at all the studied concentrations were significantly larger than that recorded for the negative control ($p<0.05$). Overall, the reference antibiotic had the highest zone of inhibition that was significant at α 0.05 (Table 1). Moreover, on *S. aureus*, the zones of inhibition produced by the methanolic extract of *P. peruviana* at concentrations of 6.25 μg/ml, 12.5 μg/ml, and 25 μg/ml were not significantly different ($p>0.05$; Table 1). Likewise, at 50 μg/ml and 100 μg/ml extract concentrations, the zones of inhibition of *S. aureus* were not significantly different ($p>0.05$; Table 1). In this case, the standard antibiotic exhibited a significantly larger zone of inhibition compared with the zones in all the other extract concentrations ($p<0.05$) (Table 1).

The effects of the aqueous extract of *P. peruviana* on *C. albicans* fungal strain were also determined. In this study, no significant difference in zones of inhibition recorded at concentrations 3.125 μg/ml and 6.25 μg/ml were observed ($p>0.05$) (Table 1). Likewise, the zones of inhibition recorded at extract concentrations of between 12.5 μg/ml and 50 μg/ml were not significantly different ($p>0.05$). However, at a concentration of 100 μg/ml, the obtained zone of inhibition was significantly higher than those recorded at all the other treatments ($p>0.05$). Notably, *C. albicans* was not susceptible to the reference drug ($p>0.05$) (Table 1). The effects of the methanolic bark extract of *P. peruviana* on *C. albicans* were also investigated (Table 1). In this study, no significant difference in zones of inhibition of *C. albicans* were observed among extract concentrations of 3.125 μg/ml , 6.25 μg/ml , and 12.5 μg/ml , and, between 25 μg/ml and 50 μg/ml ($p>0.05$) (Table 1). Notably, at the extract concentration of 100 μg/ml , the observed zone of inhibition was significantly larger than those recorded in all the other extract concentrations and controls ($p<0.05$; Table 1).

Furthermore MICs of the studied plant extracts were determined in this study. The results revealed that the MICs of the aqueous bark extract of *P. peruviana* ranged from 6.25 μg/ml on *C. albicans* fungal strain to 100 μg/ml on *S. typhimurium* bacterial strain (Table 1). On E. coli and *S. aureus* bacterial strains, the MICs of *P. peruviana* were 50 μg/ml and 25 μg/ml, respectively (Table 1). On the other hand, the MICs of the methanolic bark extract of *P. peruviana* were 100 μg/ml on both *S. aureus* and *C. albicans* and 50 μg/ml on E. coli. However, the MIC of the methanolic extract of *P. peruviana* on *S. typhimurium* was >100 μg/ml (Table 1).

Cytotoxic effects of the aqueous and methanolic extracts of the studied plants of *Physalis peruviana* on brine shrimp *nauplii*

The effects of the studied plant extracts on brine shrimp *nauplii* were also investigated in this study. The concentrations of the aqueous and methanolic extracts of *P. peruviana* that could kill 50% of the exposed brine shrimp *nauplii* were determined and considered as median lethal concentration (LC_{50}). The results are presented in Table 2.

The effects of the studied plant extracts on brine shrimp *nauplii* were also investigated in this study. The concentrations of the aqueous and methanolic extracts of *P. peruviana* that could kill 50% of the exposed brine shrimp *nauplii* were determined and considered as median lethal concentration (LC_{50}). The results are presented in Table 2. Generally, the LC_{50} values ranged from 10 μg/ml for the positive control (cyclophosphamide) to >1000 μg/ml for the aqueous bark extract of *P. peruviana* (Table 2). The LC_{50} value obtained for the methanolic extract of *P. peruviana* was significantly higher than that of the positive control drug ($p<0.05$) (Table 2).

Acute oral toxicity effects of the aqueous and methanolic extracts of *Physalis peruviana* on a rat model

In this study, the acute oral toxicity effects of the aqueous and methanolic stem bark extracts of the studied plants in laboratory rats were evaluated. Various wellness parameters were monitored throughout the 14-day experiment period, and the findings were recorded.

The results are presented in Table 3. The results showed that, at all the orally administered doses (175 mg/Kg bw, 550 mg/Kg bw and 2000 mg/Kg bw) of the aqueous and methanolic bark extracts of *P. peruviana*, there were no observable signs of toxicity recorded in all the experimental rats (Table 3). Since the wellness parameters were not interfered by the studied plant extracts, at all dose levels up to the limit dose of 2000 mg/Kg bw, the L.D. 50 values of all the studied plant extracts were considered to be >2000 mg/Kg bw according to the OECD/OCDE document No. 425 guidelines.

Qualitative phytochemical composition of aqueous and methanolic bark extracts of *Physalis peruviana*

The results showed the presence of alkaloids, saponins, tannins, glycosides, flavonoids, and phenols in the aqueous and methanolic extracts of *P. peruviana* (Table 4). However, anthraquinones were absent in the extracts (Table 4).

Table 2: Effects of the studied aqueous and methanolic plant extracts of *Physalis peruviana* on brine shrimp nauplii

Plant extract	LC50 (μg/ml)
P. peruviana (aq)	>1000
P. peruviana (met)	687.50 ± 5.94^{a}
+ control	10.00 ± 1.31^{b}

Values are presented as $\tilde{x} \pm$ SEM; Means with different lowercase alphabet superscript are significantly (Unpaired student t-test; $p<0.05$); aq: aqueous extract; met: Methanolic extract; + control; cyclophosphamide.

Table 3: Acute Oral Toxicity effects of the aqueous and methanolic bark extracts of the studied plants of Physalis peruviana in experimental rats.

Wellness parameter	Observation after											
	30 minutes		4 hours		24 hours		48 hours		7 days		14 days	
	EGR	CGR	EGR	CGR	EGR	CGR	EGR	CGR	EGR	CGR	EGR	CGR
Skin and Fur appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Faecal matter consistency	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Urination and urine appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Itching	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Convulsions and tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Breathing	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Somatomotor activity	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Aggression	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Grooming	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Teeth	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mortality/Death	None	None	None	None	None	None	None	None	None	None	None	None

EGR: Experimental group Rats (Administered with the studied plant extracts); CGR: Control group Rats (Administered with Normal saline only).

Table 4: Qualitative phytochemical composition of the aqueous and methanolic bark extracts of *P. peruviana*.

Phytochemical	***P. peruviana***	
	Aqueousextract	Methanolic extract
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Glycosides	+	+
Flavonoids	+	+
Anthraquinones	-	-
Phenols	+	+

+: Present; -: Absent

DISCUSSION

The resurgences of multidrug-resistant microbial strains have rendered the management, and treatment of infectious diseases a challenging endeavor, leading to increased morbidity and mortality [42]. It is estimated that annually, over 2 million persons are diagnosed with deadly infections, which are exacerbated by resistance, and of the diagnosed cases, over twenty thousand patients succumb as a result of therapeutic failure [43]. Globally, research has shown that antimicrobial resistance could cause over 10 million deaths by the year 2050 if not arrested early enough. Due to the inadequacy of therapeutic drugs to thwart microbial infections, there is an urgent need for the search for alternative and complementary strategies to curb these infections [42-44]. As a result, medicinal plants have proved to be a viable alternative with a high propensity for potent antimicrobials [45]. Since antiquity, humans have utilized herbals and herbal-derived products to fight infections and promote their health [46,47]. Throughout the world, more than 80% of the human population, mostly from low-income countries, depends on traditional medicine to meet their primary healthcare needs. The rich ethnomedical history of medicinal plants, their presumed efficacies, and low toxicity profiles has accelerated their exploitation as alternative therapies [48]. Furthermore, the easy accessibility and affordability of traditional medicines, as well as the deficiencies of conventional medicines, have elevated traditional medicine [14,15, 49]. Medicinal plants have significantly contributed to conventional medicine as their products have offered lead molecules for drug development [13,50,51]. For instance, the potent drugs currently used to manage cancer, malaria, among other conditions, are derived from medicinal plants [17]. Additionally, research has revealed that various plant extracts and plant-derived metabolites are very potent against non-resistant and resistant microbial strains [4,50,52]. Because less than 20% of the world medicinal plants have been scientifically explored for their therapeutic value, empirical ethnomedical exploration interest has been reinvented [20,53]. Therefore, medicinal plant bioprospecting, especially in the Kenyan context, due to the rich indigenous flora, is an impetus towards the search for affordable, accessible, efficacious, and safe antimicrobial agents [12,54]. Despite the prominent utilization of local herbals and herbal-derived products to manage microbial infections and associated maladies among the traditional Kenyan practitioners, there is insufficient empirical data to validate the claimed healing properties. As a result, the current study was designed to investigate the antimicrobial, cytotoxicity, and acute oral toxicity effects of the aqueous and methanolic stem bark extracts of *P. peruviana*. Since these plants are used traditionally to fight microbial infections, their scientific exploration serves as a guide towards the discovery of lead compounds for antimicrobial chemotherapy [26,55].

To investigate the antimicrobial activities of the studied plant extracts of *Physalis peruviana*, on the selected microbial strains,

the most recommended antimicrobial susceptibility methodology described by the NCCLS was employed [38]. In this study, the standard disc diffusion and broth microdilution techniques were followed do determine the effects of the studied plant extracts on microbial growth. The zones of inhibition and the minimum inhibitory concentrations were considered indicators of antimicrobial activity.

Previous studies have shown that plant extracts exhibiting a zone of inhibition of above 6 mm on selected microbial strains have antimicrobial activity [22,52,56,57]. Plant extracts that show a zone of inhibition of between 6 mm and 9 mm are deemed to possess slight antimicrobial activity; those showing zones of between 9 mm and 12 mm have moderate activity, while those exhibiting inhibition zones of 13-16 mm are considered to have high antimicrobial activity. Additionally, plant extracts that have inhibition zones ranging from 16-19 mm have very high antimicrobial activity, while those exhibiting zones of inhibition with diameters of 20 mm or above on selected strains are considered to have remarkable antibiotic potency [56-58]. In this study, the results revealed that the aqueous extracts of *P. peruviana* exhibited slight to moderate antimicrobial effects against *E. coli* and *S. aureus* bacterial strains in a dose-dependent manner. On the other hand, this extract exhibited moderate to high activity on *C. albicans*, demonstrating its antifungal effects. Against *S. typhimurium*, the aqueous extract of *P. peruviana* demonstrated slight activity at 50 μg/ml and 100 μg/ml [57]. The methanolic bark extract of *P. peruviana* demonstrated slight to moderate antimicrobial effects against *E. coli*, *S. aureus*, and C. *albicans* based on the criteria described earlier. However, a slight antimicrobial effect by this extract was observed on the *S. typhimurium* strain in this study. The results imply that these extracts have both antibacterial and antifungal properties, with the antifungal effects being more pronounced. These results are consistent with those earlier reported by Göztok and Zengin [59] on fruit extracts of *P. peruviana* and Higaki et al. [60], who reported corroborating results on fruit fractions of the same plant. Moreover, research has shown that plant extracts which have MIC values that are less than 1 μg/ml (1000 μg/ml) have antimicrobial activity with the potential of offering potent antibiotics [19, 61]. In this study, the studied plant extracts exhibited low MIC values on selected microbes. Since the MIC values were much lower, it is anticipated that the studied plant extracts can be strong antibiotics.

Medicinal plants are a host of various bioactive compounds with a broad spectrum of pharmacologic efficacies [37]. Research has shown that tannins, phenols, flavonoids, terpenoids, which are antioxidants [62], among other phytocompounds, are responsible for the antimicrobial activity of plants [63-65]. Therefore, bioactivities reported in this study are attributable to these phytochemicals, which work either solely or in synergy with others to cause the pharmacologic effects. Since the studied plant extracts exhibited varied antimicrobial activities depending on the microbial strain, and the type of extract, it is thus, suggestive that the phytochemicals which affect one strain may not affect another strain. Additionally, since various phytochemicals are solubilized and extracted with different solvents, the relative abundance of antimicrobial compounds may not be the same [21,66]. Moreover, even though water and methanol are both polar, their polarities are different, with water being more polar than methanol [67]. Therefore, water concentrated very polar compounds than did methanol, thereby accounting for the differences in the antimicrobial activities of the studied plant extract. Despite the long-standing utilization of herbals and their products for the management of various health conditions, serious concerns regarding their safety have been raised [23]. Various factors that affect the therapeutic potency of herbal medicines are generally not considered. There is a lack of standard procedures and regulations governing the preparation, labelling, marketing, and dispensing of herbal medicines [68]. This has led to an emergence of unscrupulous practitioners of herbal medicine, thereby raising safety concerns. There are no dosage guidelines, clearly outlines contraindications, conventional drug- herbal drug interactions, and toxicity profiles of herbal preparations [69, 70]. As a result, improper use of these medicines could cause life-threatening effects considering the insufficiency of scientific and clinical data. Since the studied plants have, for an extended period, been used in traditional medicine to manage infections among other diseases without clear scientific backup [71,72], the current study sought to determine the cytotoxicity and acute oral toxicity effects of the aqueous and methanolic extracts of the studied plants. Assessment of safety is critical to assure the safe use of herbal medicines in traditional medicine and to offer scientific data that can guide further development. The brine shrimp lethality assay technique is a rapid method widely used to screen the cytotoxic effects of plant extracts and chemicals thought of therapeutic value [40]. This technique was adopted to assess the cytotoxic/safety effects of the aqueous and methanolic stem bark extracts of *P. peruviana* in exposed shrimp *nauplii*. The concentration of the plant extracts able to kill 50% of *nauplii* following exposure is considered as the LC_{50}. Research has shown that a plant extract with an LC50 value that is <30 μg/ml is very cytotoxic. Furthermore, plant extracts exhibiting LC_{50} values that are over 100 μg/ml are considered to be of low toxicity or safe [73,74]. Given the described criteria [73,74], both the aqueous and methanolic extracts of P. peruviana proved to be non-toxic to brine shrimp *nauplii*, and thus safe as their LC_{50} values were >100 μg/ml. Notably, the aqueous extract of *P. peruviana* was the safest among the tested extracts with an LC_{50} value of >1000 μg/ml . Since most of the herbal medicines are administered orally, the acute oral toxicity effects of the studied plant extracts were investigated in rat models. In this study, the acute oral toxicity study top-down procedure described the OECD document number 425 was adopted [41]. The results showed that all the studied plant extracts were non-toxic at oral doses and, therefore, safe. Considering these results, the studied plant extracts are safe for use in traditional medicine.

The medicinal value of plants is attributed to the secondary metabolites which they synthesize [75]. The secondary metabolites (phytochemicals) are produced in response to stress, which is associated with the pathogenic attack, environmental stress, and oxidative stress [76]. Research has shown that each of these phytocompounds is pharmacologically active with various effects in biological systems [77]. Of the phytoactive compounds present in medicinal plants, antioxidant amalgams possess the widest spectra of pharmacologic activities. Flavonoids, phenols, and tannins have been demonstrated to confer antioxidant and antimicrobial activities [28]. Besides, alkaloids and anthraquinones possess cytotoxic effects. From the findings of this study, it is therefore arguable that the antimicrobial effects of the aqueous and methanolic extracts of *P. peruviana* are due to the presence of phenols, flavonoids, among other antimicrobial- associated phytochemicals. The safety of the studied plant extracts reported in this study could be attributed to low concentration or absence of toxicity associated phytochemical compounds [78]. Furthermore, the antimicrobial bioactive compounds anticipated to be present in the studied plant extracts in varying degrees do not cause observable signs of toxicity [79].

These findings indicate that the studied plant extracts can be good alternative sources of safe antimicrobial compounds. Therefore, this study supports the traditional use of the studied plant extracts for the management of the claimed conditions.

CONCLUSION AND RECOMMENDATIONS

It was concluded that the aqueous bark extract of *P. peruviana* has slight activity against *S. typhimurium* and E. coli, slight to moderate activity against *S. aureus*, and moderate to high activity against *C. albicans*. On the other hand, The methanolic bark extract of *P. peruviana* has slight antimicrobial activity against *S. typhimurium* and slight to moderate activities against *E.coli*, *S. aureus*, and *C. albicans* microbial strains. Moreover, both of the studied plant extracts do not cause acute oral toxicity effects in Winstar rats, and cytotoxicity in brine shrimp *nauplii*. Furthermore, the studied plant extracts contain antimicrobial-associated phytochemicals.

This study recommends quantitative phytochemical analysis, isolation, characterization, and development of antimicrobial compounds from the studied plant extracts. The specific mode(s) through which the studied plant extracts confer antimicrobial activity should be established. Furthermore, investigation of the antimicrobial efficacy of the studied plant extracts on other microbial strains of clinical significance is encouraged. Additionally, extensive toxicity and safety evaluation of the studied plant extracts on other experimental models should be done.

DATA AVAILABILITY

All data in this study are included within the manuscript; however, any additional information is available from authors upon request.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest whatsoever regarding this study.

AUTHOR CONTRIBUTIONS

Joseph Kathare conceived the research idea and performed the experiments under the close supervision of James Mbaria and Joseph Nguta. Gervason Moriasi designed, guided the experiments, and assisted with data analysis and interpretation. All authors reviewed and approved the final manuscript for publication.

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