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Antimicrobial Activity, Cytotoxicity, and Qualitative Phytochemistry of Leaf, stem bark, and root bark extracts from *Prunus africana* (Hook. F.) Kalkman

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ABSTRACT

The rise of resistant strains poses a significant public health risk, particularly, in sub-Saharan Africa, where over 50% of global infectious disease-associated deaths occur, highlighting the urgent need for novel, safe, affordable, and accessible antimicrobials. Accordingly, we investigated the antimicrobial activity, cytotoxicity, and qualitative phytochemistry of the aqueous, hydroethanolic, and acetonic leaf, stem bark, and root bark extracts of *Prunus africana* (Hook. F.) Kalkman, based on its ethnomedicinal information. The results showed the aqueous root bark and aqueous/acetonic stem bark extracts demonstrated significant ($p<0.05$) antimicrobial efficacy against *S. aureus* at 800 µg/ml, outperforming other extracts and the reference antibiotic. Growth inhibition zones for most extracts on *S. aureus* showed a concentration-dependent increase, though not significantly ($p>0.05$) different. The acetonic root bark extract, particularly at 800 µg/ml, exhibited superior inhibitory effects against *B. cereus* compared to other extracts ($p<0.05$), although the positive control antibiotic significantly ($p<0.05$) outperformed all plant extracts. Notably, none of the studied extracts affected *P. aeruginosa* and *E. coli*, while varying effects were observed against *C. albicans*. Further we observed that the hydroethanolic and aqueous stem bark extracts' exceptionally low Minimum Inhibitory and Bactericidal Concentrations (MICs and MBCs) against *S. aureus* (3.125 µg/ml). Conversely, the acetonic leaf extract showed higher MIC and MBC values against *S. aureus* (100 µg/ml). Cytotoxicity assessments using brine shrimp nauplii revealed the percentage mortalities caused by Vincristine and aqueous root/stem bark extracts at 1000 µg/ml, were significantly ($p<0.05$) higher than those caused by other extracts (Median lethal concentrations (LC_{50}) of 513 µg/ml to 24327.82 µg/ml). Qualitative phytochemistry identified alkaloids in root bark and stem bark extracts, flavonoids, phenols, quinones, steroids, and terpenoids across all samples, with saponins in acetonic root bark and all three leaf extracts, and glycosides in acetonic stem bark, hydroethanolic root bark, and acetonic leaf extracts. These findings highlight the diverse antimicrobial and cytotoxic properties of *P. africana* extracts, suggesting potential therapeutic applications and emphasise the need for further exploration.

Keywords: Disk Diffusion Technique, Minimum Inhibitory/Bactericidal/Fungicidal Concentrations, Brine shrimp lethality assay, Ethnomedicine.

INTRODUCTION

Antibiotic chemotherapy plays a pivotal role in managing the severity and transmission of infectious diseases; nevertheless, the indiscriminate utilization of antibiotics and the emergence of resistant strains pose significant health hazards [1]. Epidemiological evidence underscores the disproportionate burden borne by low- and medium-income countries, particularly those in sub-Saharan Africa, where pathogenic microbes are primary causative agents of illness [2,3]. This phenomenon is partly attributable to inadequate hygiene standards, notably prevalent in rural regions and informal settlements, coupled with limited access to quality healthcare services and the prohibitive cost of efficacious therapies. Consequently, vulnerable populations, such as those afflicted with diarrhoea, experience heightened morbidity rates [4]. For instance, a staggering proportion—exceeding 50%—of global deaths linked to infectious diseases transpire in developing nations across Africa and Asia due to the dearth of novel, cost-effective, and accessible antimicrobials [5]. Furthermore, conventional antimicrobial agents exhibit undesirable side effects, including nephrotoxicity, hepatoxicity, and gastrointestinal complications, thereby constraining their clinical utility [1,6,6–8]. Consequently, it is imperative to explore alternative strategies that effectively combat microbial infections, address antimicrobial resistance without engendering adverse effects, and alleviate the limitations associated with conventional chemotherapy.

The World Health Organisation (WHO) underscores that a substantial proportion of the global population utilises medicinal plants to promote human and animal health [9–12]. The widespread use of medicinal plants is pegged on their cultural acceptability, perceived safety, accessibility, affordability,

and potency continuum associated with them [10,13–15]. Research shows that medicinal plants actively synthesise phytochemical compounds with pharmacological efficacy, some of which have been successfully isolated and characterised [16,17]. Apart from serving as the plant's defence mechanism against biotic and abiotic stresses, these compounds offer dietary and pharmacological benefits to our bodies when consumed [18]. Further, the presence of antimicrobial phytochemicals, such as tannins and flavonoids, among others, in medicinal flora presents a promising reservoir for natural products-these could potentially function as viable substitutes for synthetic antibiotics [19–21]; however, there are limited empirical studies that are aimed at validating their pharmacological efficacy.

The absence of standardised methods and regulations for preparing, packaging, storing, establishing dosage regimes, and administering these plant preparations raises safety concerns [22]. Moreover, insufficient empirical data exists on herb-herb interactions with conventional drugs as well as their associated toxicity profiles [23,24]. Hence, a scientific investigation is imperative to scrutinise the safety and efficacy of medicinal plants. This process not only validates their usage but also furnishes empirical data for directing the isolation, characterisation, and development of alternative medicines that are safe, potent, accessible, and cost-effective [25] Accordingly, this study investigated the antimicrobial activity, cytotoxicity, and qualitative phytochemistry of the aqueous, hydroethanolic, and acetonic leaf, stem bark, and root bark extracts of *Prunus africana* (Hook. F.) Kalkman, based on its ethnomedicinal information.

Prunus africana (Hook. F.) Kalkman, a prominent member of the *Prunus* genus within the Rosaceae family, is a multifaceted tree with over 400 species [26]. Recognised by various names such as *Pygeum africanum*, African cherry, and red stinkwood, it is deeply ingrained in the cultural fabric of Kenyan communities, where it is locally known as '*Mumbaume*' among the Kamba, '*Tenduet*' among the Keiyo, '*Muiri*' among the Agikuyu, '*Kumutura*' among the Bukusu, and '*Olkojuk*' among the Maasai communities [27]. It is indigenous to Kenya but also thrives in the southern hemisphere, particularly in Africa's central, eastern, southern, and western regions, at altitudes ranging from 1500 to 2000 meters above sea level [27,28]. Its bark has been extensively utilised for medicinal purposes to treat ailments affecting various body systems, including gastrointestinal, respiratory, and renal conditions. Its extracts are used to treat constipation and infectious diseases like gonorrhoea, urinary tract infections, and malaria [29,30]. Noteworthy is its application in managing impotence, insanity, appetite disorders, cognitive enhancement, and benign prostatic hypertrophy, among others [26,31], which have enhanced its reputation in traditional medicine. A study by Mutuma et al. [32] among the Meru community in Kenya indicates its use in managing inflammatory conditions. However, there is a dearth of empirical information to validate its ethnomedicinal application as an antimicrobial remedy, hence this study.

Previous research shows that dichloromethane stem bark extracts of *P. africana* possess glycosides, tannins, alkaloids, saponins, and terpenoids, among other phytochemicals, demonstrating a broad spectrum of bioactivity [31,32]. Similarly, Rubegeta et al.[31] demonstrated that various extracts from *P. africana* possess triterpenes, carbohydrates, flavonoids, tannins, and saponins, among others. Further research on parts of *P. africana*, employing diverse solvent systems for comprehensive phytochemical exploration, remains necessary as these studies are far between and scanty. Therefore, engaging in such endeavours deepens our understanding of this botanical resource's therapeutic potential.

MATERIALS AND METHODS

Plant Materials

In September 2022, botanical specimens comprising fresh leaves, stem barks, and root barks of *P. africana* were meticulously gathered from Rwathia ward, Kangema SubCounty, Muranga County, Kenya (00°39'40.7"S 36°55'56.9"E), under the guidance of a local herbalist. The selection of this plant and its various components was informed by their recognized ethnomedicinal utility in the indigenous community, particularly as antimicrobial agents. Voucher specimens were diligently collected, processed, and subjected to taxonomic authentication at the Department of Land Resource Management and Agricultural Technology (LAMART-UON/HR/143) and the East African Herbarium (NMK/BOT/CTX/1/2). Upon acquisition, the selected plant materials were gently rinsed under running tap water to eliminate extraneous soil. Subsequently, they were finely chopped into small fragments and subjected to a drying period spanning 14 days within the controlled environment of the research laboratory housed at the Department of Public Health, Pharmacology, and Toxicology, College of Agriculture and Veterinary Sciences, Kabete Campus, University of Nairobi. During this process, the materials were exposed to ambient room temperature, shielded from direct exposure to sunlight to prevent degradation of active constituents. After that, the dried plant constituents were pulverized into powders utilizing an electric plant mill, ensuring uniformity and fine texture conducive to subsequent extraction procedures. The resultant powders were labelled and securely stored in zip-lock bags on laboratory shelves, awaiting further processing for extraction and subsequent analysis.

Extraction Procedures for the Plant Materials

The methodologies for extraction, initially delineated by Harborne [33] and subsequently refined by Moriasi et al. [34], were meticulously adhered to in this study. In the extraction of hydroethanolic and acetonic compounds, 500 g of each pulverized substance underwent separate maceration processes in 1L of ethanol-water mixture (1:1) and 1L of acetone, respectively, over a period of 72 hours, with intermittent agitation. The resulting solutions were meticulously decanted, subsequently filtered through Whatman filter paper (No. 1), and collected in suitably labelled conical flasks. Concentration of the solutions was achieved utilizing a rotary evaporator at 40 °C for acetonic extracts and 60 °C for hydroethanolic extracts. The hydroethanolic extracts were further subjected to lyophilization under vacuum using a freeze dryer, while the acetonic extracts were subjected to drying in a hot-air oven set at 35 °C until complete desiccation, a process requiring five days. Moreover, the aqueous extracts were obtained through a lyophilization technique wherein 500 g of each powdered material was soaked in 5000 ml of distilled water, heated to 60 °C for 10 minutes, cooled to ambient temperature, filtered through Whatman filter paper (No. 1), and subsequently subjected to freeze drying for a duration of 48 hours. All extracts were meticulously stored in appropriately labelled glass containers in a refrigerated environment maintained at 4 °C until further utilization.

Determination of In Vitro Antimicrobial Activity

Test Microorganisms

We investigated the antimicrobial efficacy of the extracts against a spectrum of microbial strains including *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), and *Candida albicans* (ATCC 10231), sourced from the repository of microbial cultures housed within the Department of Public Health, Pharmacology, and Toxicology, specifically the Microbiology Section of the venerable University of Nairobi.

Preparation Standardisation of Microbial Inoculums

In accordance with the prescribed protocols outlined by the Clinical Laboratory Standards Institute (CLSI) [35], microbial inocula were meticulously prepared by subculturing the bacterial strains in Mueller Hinton Agar medium, and the fungal strain, *C. albicans*, in Sabouraud Dextrose Agar medium. This cultivation process transpired over a duration of 24 hours, within a controlled environment set at 37°C. After incubation, colonies were carefully harvested and suspended in

normal saline solution. To ensure uniformity in microbial density, the absorbance of the suspensions was meticulously adjusted utilizing a spectrophotometer, to a turbidity commensurate with 0.5 on the McFarland scale.

Disk Diffusion Assay

In accordance with established guidelines [35–37], the present study employed the disk diffusion assay, a commonly utilized method for determining antimicrobial susceptibility. The procedure involved several steps: initially, each extract, quantified at 0.8 g, was dissolved in 10 ml of 1.4% dimethylsulphoxide (DMSO) and vigorously vortexed to attain a stock concentration of 800 µg/ml. Subsequently, serial two-fold dilutions were prepared to yield concentrations ranging from 800 µg/ml to 6.25 µg/ml. Thereafter, 20 µl of each extract concentration was meticulously dispensed onto sterile 6 mm diameter disks fabricated from Whatman No. 1 papers. These disks were then evenly distributed on petri dish plates previously inoculated with 1 ml of the respective microbial inoculums. Each experiment was conducted in triplicate, with DMSO serving as the negative control, and Gentamycin (10 µg) or Fluconazole (10 µg) utilized as positive controls. The plates were subsequently incubated at 37°C for a duration of 24 hours. Following incubation, the diameters of the resultant growth inhibition zones were measured and documented.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)/Minimum Fungicidal Concentration (MFC) followed an adapted methodology as delineated in prior literature [38]. Serial dilutions, employing a two-fold scheme, ranging from 800 µg/ml to 0.15625 µg/ml, were prepared for each extract within test tubes utilizing Mueller-Hinton Broth. Subsequently, microbial inoculums were separately introduced into these tubes at a density of 10^4 colony forming units (cfu) per milliliter, and the interactions were allowed to transpire at ambient temperature. The assemblies were then subjected to incubation at 35°C for a duration of 18 hours [35]. Post-incubation, the concentrations of the investigated extracts that entirely arrested microbial proliferation were identified as MICs, whereas those resulting in complete eradication of the test microorganism were designated as MBCs. Each experimental procedure was replicated thrice to ensure representative data, with 1.4% dimethyl sulphoxide serving as a negative control. Gentamycin (10 µg) was employed for cultures of *P. aeruginosa, E. coli, S. aureus*, and *B. cereus*, whereas Fluconazole (10 µg) was utilized for *C. albicans* cultures.

In Vitro Cytotoxicity study

The brine shrimp lethality assay method [39] was employed to examine the cytotoxicity of the study extracts. In brief, 1 g of *Artemia salina* nauplii cysts was dispensed into an artificially prepared sea containing 3% marine salt in the dark compartment of a holding plastic container with two compartments. The light compartment was illuminated, and the setup was allowed to settle for 48 hours at 25-29 °C. After that, 10 nauplii were carefully transferred using a Pasteur pipette into test tubes containing 5 ml of serial concentrations (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. The percentage mortality in each tube was calculated, and the median lethal concentration (LC_{50}), defined as the concentration causing 50% mortality, was derived from a line of best-fit plots (simple linear regression) of percentage mortality against concentration.

The brine shrimp lethality assay [39] was implemented to evaluate the cytotoxic properties of the investigated extracts. In summary, 1 gram of Artemia salina nauplii cysts was introduced into a synthetic saline solution comprising 3% marine salt within the darkened compartment of a bifurcated containment vessel. The adjacent light-exposed compartment was illuminated, and the system was allowed to equilibrate for a duration of 48 hours at a temperature range of 25-29°C. Subsequently, 10 nauplii were meticulously transferred into test tubes, each containing 5 ml of incremental concentrations (10 µg/ml, 100 µg/ml, and 1000 µg/ml in 1% dimethyl sulfoxide [DMSO]) of the respective extracts, alongside vincristine sulphate serving as the positive control, with each concentration replicated fivefold. Following inoculation, the test tubes were maintained at ambient temperature for a duration of 24 hours. Post-incubation, the surviving organisms were enumerated. The mortality rate within each test tube was calculated as a percentage, and the median lethal concentration (LC_{50}), denoting the concentration causing 50% mortality, was ascertained through the generation of line-of-best-fit plots employing simple linear regression analysis, juxtaposing percentage mortality against concentration.

Qualitative Phytochemical Screening

The presence or absence of various phytochemicals was determined following established procedures by Harborne [33], Trease and Evans [40] , and Maina et al. [41].

Saponins: To ascertain the presence of saponins, approximately 1 g of plant were boiled in 5 ml of distilled water in well-labelled test tubes for five minutes and then allowed to cool to room temperature. Subsequently, the test tubes containing the extracts were vigorously shaken, and the persistence of foam or frothing for more than two minutes indicated the presence of saponins.

Alkaloids: About 100 mg of each extract was individually mixed with 5 ml of 1% hydrochloric acid (HCL), heated, and then filtered through Whatman paper No. 1. Meyer's reagent was introduced to a portion of the filtrate, and the formation of a cream-colored precipitate indicated the presence of alkaloids. Similarly, Dragendorff's reagent was added to another portion of the filtered extracts, forming a reddish-brown precipitate, which showed the presence of alkaloids.

Flavonoids: In this experiment, about 100 mg of each plant extract was mixed with 10 ml of 70% ethanol and warmed gently for three minutes in a water bath at 55 ˚C. Then, the mixtures were added five drops of concentrated hydrochloric acid (HCL) and heated gently for five minutes. The immediate development of a red colour indicated the presence of flavonoids. Two additional techniques were employed to confirm flavonoid presence: a 10 ml solution of each test extract was hydrolysed with 10% sulfuric acid, divided into two parts, and treated separately. The first portion was diluted with ammonia solution, and the appearance of a greenish yellow colour confirmed flavonoid presence. The remaining portion was treated with dilute sodium carbonate solution, resulting in a pale-yellow colouration as evidence of flavonoids.

Tannins: About 500 mg of each extract was mixed with 5 ml distilled water, heated for five minutes, and then then filtered through Whatman paper No.1. Three drops of a 0.1% ferric chloride ($FeCl_3$) solution were added to respective to the filtrates. The development of a blue precipitate indicates the presence of tannins.

Phenols: To detect the presence of phenols, 100 mg of each extract was combined with 10 ml of 70% ethanol and heated in a water bath for 5 minutes. After filtration, the supernatants were cooled under gently flowing tap water. Later, 5 drops of 5% ferric chloride were added to 2 ml of the filtrates, and the formation of a green precipitate signified the presence of phenols in the test samples.

Coumarins: Approximately 200 mg of the extracts were warmed with 2 ml of absolute ethanol for five minutes in a water bath (55 °C). The test tubes were covered with a Whatman filter paper soaked in 10% ammonium hydroxide solution (NH_4OH) for 5 minutes and then exposed to ultraviolet light (365 nm). The appearance of a yellow

fluorescence under UV light denoted the presence of coumarins in the samples.

Cardiac Glycosides: About 200 mg of the test extracts were combined with 5 ml of chloroform and evaporated to dryness. The resulting materials were supplemented with 0.5 ml of concentrated sulphuric acid and 0.4 ml of glacial acetic acid containing a few drops of $FeCl_3$. The appearance of a blue acetic layer marked presence of cardiac glycosides.

Phytosterols: A drop of Liebermann-Burchard reagent was added to 1 ml of the test plant extracts, and the development of a reddish-purple tint indicated the presence of steroids.

Anthraquinone: About 200 mg of each extract was mixed with 5 ml of benzene and filtered through Whatman paper No. 1. Subsequently, 5 ml of 10% ammonium hydroxide was added to the filtrates, thereby the formation of a violet colour in the ammoniacal layer confirmed the presence of anthraquinones.

Data analysis

Quantitative data derived from both the assessment of antimicrobial efficacy and cytotoxicity were meticulously collated using Microsoft 365 spreadsheet software, subsequently transposed into Minitab statistical software version 21.4 (State College, Pennsylvania) for comprehensive analysis. Descriptive statistical methodologies were applied, with findings delineated as the mean accompanied by the standard error of the mean (SEM) ($\bar{x}$±SEM). Following this, inferential statistical procedures, employing One-Way Analysis of Variance (ANOVA) with Tukey's *post hoc* analysis, were conducted at a significance level (α) of 0.05 to ascertain noteworthy disparities among means, and to facilitate pairwise comparisons and distinctions of means. The determination of LC_{50} values in the brine shrimp lethality assay involved the application of a simple linear regression analysis, correlating percentage mortality with concentration. Qualitative data gleaned from phytochemical screening endeavours were solely tabulated and described.

Ethical Approval

The present inquiry garnered the imprimatur of ethical sanction from the Biosafety, Animal Care and Use Committee (FVM-BAUEC) at the esteemed University of Nairobi (Reference number FVM BAUEC/2023/425). Moreover, a research license indispensable for the pursuit of this investigation was duly conferred by the National Commission for Science, Technology, and Innovation (NACOSTI) (Permit number: NACOSTI/P/23/31004).

RESULTS

Percentage yield of the extracts

In this study, the yields of the obtained extracts were 17.5 % (aqueous root bark extract), 10.73 % (acetonic stem bark extract), 10.57 % (acetonic root bark extract), 10.00 % (aqueous and hydroethanolic stem bark extracts), 9.50 % (hydroethanolic root bark extract), 7.00 % (hydroethanolic leaf extract), and 3.72 % (acetonic leaf extract), respectively, in reducing order.

Antimicrobial activity of the study extracts

Growth inhibition zones of the studied plant extracts

The present investigation assessed the antimicrobial efficacy of diverse extracts derived from the foliage, stem bark, and root bark of *P. africana*, predicated on purported traditional antimicrobial properties, against prevalent pathogenic microorganisms encompassing *S. aureus, B. cereus, P. aeruginosa, E. coli,* and *C. albicans*. Remarkably, the aqueous root bark extract and both aqueous and acetonic stem bark extracts exhibited a notable ($p<0.05$) antimicrobial effect against *S. aureus* at a concentration of 800 µg/ml, exceeding the efficacy of alternative extracts and the reference antibiotic (Table 1). Conversely, the aqueous leaf extract, even at a concentration of 100 µg/ml, evinced no discernible inhibition of S. aureus growth (Table 1). Although the dimensions of growth inhibition zones for most extracts against S. aureus did not display significant disparities ($p>0.05$), they evinced a concentration-dependent augmentation, as shown in Table 1.

Furthermore, the acetonic root bark extract, notably at 800 µg/ml, demonstrated superior inhibitory effects against B. cereus relative to other extracts ($p<0.05$; Table 1). Nevertheless, the positive control antibiotic significantly surpassed all plant extracts against *B. cereus* ($P<0.05$; Table 1). The growth inhibition zones also exhibited significant augmentation with escalating extract concentrations ($p<0.05$), indicative of a dose-dependent correlation (Table 1). Intriguingly, none of the scrutinized plant extracts evinced observable inhibitory effects on plates inoculated with P. aeruginosa and *E. coli*, whereas the positive control antibiotic (Gentamycin) manifested significant inhibition ($p<0.05$), as depicted in Table 1. Regarding *C. albicans*, the inhibition zones manifested notable variation ($p<0.05$) contingent upon the extract type and concentration (Table 1). Particularly, the acetonic leaf extract at 100 µg/ml exhibited a significantly diminished inhibitory zone relative to other extracts and the positive control antibiotic ($p<0.05$; Table 1),

Table 1: Microbial growth inhibition zones produced by the studied plant extracts

Concentration	Extract	Growth inhibition zone diameter (mm)				
		S. aureus	***B. cereus***	***P. aeruginosa***	***E. coli***	***C. albicans***
100 µg/mL	LVACPA	17.17 ± 0.15^{f}	18.00 ± 0.10^{g}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	10.67 ± 0.06^{aa}
	LVAQPA	0.00 ± 0.00^{h}	14.07 ± 0.06^{i}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	11.07 ± 0.06^{n}
	LVHEPA	14.97 ± 0.15^{g}	15.00 ± 0.00^{h}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	18.00 ± 0.00^{kl}
	RBACPA	20.10 ± 0.10^{cd}	19.93 ± 0.21^{e}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	18.90 ± 0.00^{jk}
	RBAQPA	19.967 ± 0.15^{cd}	19.03 ± 0.21^{f}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	17.13 ± 0.06^{op}
	RBHEPA	20.00 ± 0.10^{cd}	19.00 ± 0.00^{f}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	17.10 ± 0.00^{opq}
	SBACPA	19.93 ± 0.12^{cd}	19.00 ± 0.10^{f}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	16.40 ± 0.00^{r}
	SBAQPA	20.03 ± 0.06^{cd}	19.10 ± 0.10^{f}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	16.93 ± 0.06^{q}
	SBHEPA	18.00 ± 0.10^{e}	18.03 ± 0.06^{g}	0.00 ± 0.00^{b}	$0.0\ 0\pm0.00^{b}$	14.40 ± 0.00^{X}
200 µg/mL	LVACPA	18.10 ± 0.17^{e}	18.17 ± 0.06^{g}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	11.70 ± 0.00^{Y}
	LVAQPA	15.10 ± 0.10^{g}	15.03 ± 0.06^{h}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	11.13 ± 0.12^{y}
	LVHEPA	18.13 ± 0.15^{e}	17.97 ± 0.06^{g}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	20.00 ± 0.00^{g}

	RBACPA	19.97±0.15^{cd}	20.03±0.15^{e}	0.00±0.00^{b}	0.00±0.00^{b}	19.80±0.00^{h}
	RBAQPA	20.13±0.15^{cd}	20.00±0.00^{e}	0.00±0.00^{b}	0.00±0.00^{b}	18.67±0.06^{lm}
	RBHEPA	19.97±0.06^{cd}	20.033±0.06^{e}	0.00±0.00^{b}	0.00±0.00^{b}	18.70±0.00^{lm}
	SBACPA	20.07±0.12^{cd}	19.97±0.06^{e}	0.00±0.00^{b}	0.00±0.00^{b}	18.60±0.00^{m}
	SBAQPA	21.03±0.13^{b}	19.00±0.00^{f}	0.00±0.00^{b}	0.00±0.00^{b}	17.27±0.06^{0}
	SBHEPA	20.97±0.15^{b}	19.00±0.00^{f}	0.00±0.00^{b}	0.00±0.00^{b}	14.80±0.00^{w}
400 µg/mL	LVACPA	20.03±0.15^{cd}	19.20±0.10^{f}	0.00±0.00^{b}	0.00±0.00^{b}	15.47±0.06^{tu}
	LVAQPA	17.07±0.12^{f}	18.00±0.17^{g}	0.00±0.00^{b}	0.00±0.00^{b}	15.50±0.00^{t}
	LVHEPA	17.97±0.15^{f}	18.03±0.06^{g}	0.00±0.00^{b}	0.00±0.00^{b}	22.03±0.06^{c}
	RBACPA	21.00±0.00^{b}	22.07±0.15^{c}	0.00±0.00^{b}	0.00±0.00^{b}	19.20±0.00^{i}
	RBAQPA	20.00±0.10^{cd}	21.00±0.00^{d}	0.00±0.00^{b}	0.00±0.00^{b}	19.80±0.00^{h}
	RBHEPA	20.03±0.06^{cd}	19.00±0.00^{f}	0.00±0.00^{b}	0.00±0.00^{b}	19.00±0.00^{j}
	SBACPA	21.03±0.06^{b}	20.97±0.15^{d}	0.00±0.00^{b}	0.00±0.00^{b}	19.07±0.06^{ij}
	SBAQPA	21.00±0.00^{b}	19.03±0.06^{f}	0.00±0.00^{b}	0.00±0.00^{b}	15.30±0.10^{v}
	SBHEPA	20.00±0.20^{cd}	19.03±0.06^{f}	0.00±0.00^{b}	0.00±0.00^{b}	18.80±0.10^{kl}
800 µg/mL	LVACPA	21.07±0.12^{b}	22.10±0.10^{c}	0.00±0.00^{b}	0.00±0.00^{b}	15.83±0.06^{s}
	LVAQPA	18.00±0.10^{e}	19.03±0.06^{f}	0.00±0.00^{b}	0.00±0.00^{b}	17.00±0.00^{pq}
	LVHEPA	18.07±0.21^{e}	19.03±0.06^{f}	0.00±0.00^{b}	0.00±0.00^{b}	24.03±0.06^{b}
	RBACPA	21.13±0.12^{b}	22.97±0.06^{b}	0.00±0.00^{b}	0.00±0.00^{b}	21.83±0.06^{d}
	RBAQPA	22.03±0.06^{a}	21.03±0.15^{d}	0.00±0.00^{b}	0.00±0.00^{b}	20.30±0.10^{f}
	RBHEPA	20.00±0.20^{cd}	22.13±0.06^{c}	0.00±0.00^{b}	0.00±0.00^{b}	21.93±0.06^{cd}
	SBACPA	22.03±0.06^{a}	20.97±0.06^{d}	0.00±0.00^{b}	0.00±0.00^{b}	21.37±0.06^{e}
	SBAQPA	21.97±0.15^{a}	21.03±0.06^{d}	0.00±0.00^{b}	0.00±0.00^{b}	20.07±0.06^{g}
	SBHEPA	21.00±0.10^{b}	21.00±0.00^{d}	0.00±0.00^{b}	0.00±0.00^{b}	20.07±0.06^{g}
Positive Control		20.37±0.06^{c}	26.87±0.05^{a}	29.17±0.06^{a}	25.57±1.16^{a}	27.67±0.06^{a}

Values are expressed as $\bar{x} \pm SD$ for five (5) replicates. Means with different superscript alphabets within the same column are significantly different ($p<0.05$), while those with similar superscript alphabets within the same column are not significantly different ($p>0.05$) by One-way ANOVA and Tukey's *post hoc*. ND: Not determined; LVACPA: Acetonic leaf extract of *P. africana*; LVAQPA: Aqueous leaf extract of *P. africana*; LVHEPA; Hydroethanolic leaf extract of *P. africana*; RBACPA: Acetonic root bark extract of *P. africana*; RBAQPA: Aqueous root bark extract of *P. africana*; RBHEPA: Hydroethanolic root bark extract of *P. africana*; SBACPA: Acetonic stem bark extract of *P. africana*; SBAQPA: Aqueous stem bark extract of *P. africana*; SBHEPA: Hydroethanolic stem bark extract of *P. africana*. Positive control: Gentamycin(10µg)-Bacterial strains; Fluconazole (10µg): Fungal strain.

Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC)

This investigation undertook an evaluation of the antimicrobial potency inherent in P. Africana extracts, delineating their minimum inhibitory concentrations (MIC) and minimum bactericidal/fungicidal (MBC/MFC) values against selected microbial strains (Table 2). Noteworthy findings emerged from the study, elucidating the significantly low MICs and MBCs (3.125 µg/ml) exhibited by both aqueous and hydroethanolic stem bark extracts, as well as the hydroethanolic root bark extract of *P. africana*, in relation to their activity against *S. aureus*. Conversely, the acetonic leaf extract demonstrated higher MIC and MBC values (100 µg/ml) vis-à-vis *S. aureus*. A notable disparity was observed when juxtaposing the tested plant extracts with the positive control antibiotic, Gentamycin, which displayed the lowest MIC (0.625 µg/ml) and MBC (1.25 µg/ml) values (Table 2).

Additionally, discernible variations were noted among the MICs and MBCs of aqueous and acetonic leaf extracts of *P. africana* against *B. cereus* when compared with other extracts. The hydroethanolic leaf extract manifested a noteworthy efficacy with low MIC and MBC values (3.125 µg/ml), albeit being surpassed by the standard antibiotic with MIC and MBC values of 0.3125 µg/ml against *B. cereus*. Notably, none of the scrutinized plant extracts exhibited antimicrobial activity against *P. aeruginosa* and *E. coli* (Table 2). Regarding *C. albicans*, the aqueous leaf extract and the aqueous root bark extract registered the highest MIC and MFC values of 200 µg/ml, while other extracts presented substantially lower MIC and MFC values of 25 µg/ml. In contrast, the standard antifungal drug (Fluconazole) demonstrated the lowest MIC and MFC against C. albicans (12.5µg/ml). These observations are detailed in Table 2, providing a comprehensive overview of the comparative efficacy of the tested plant extracts and the standard antibiotics against the microbial strains under investigation.

Table 2: Minimum inhibitory concentrations (MIC) and Minimum bactericidal/Fungicidal concentrations (MBC/MFC)

Sample	*S. aureus*		*B. cereus*		*P. aeruginosa*		*E. coli*		*C. albicans*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MFC**
LVACPA	100	100	25	50	ND	ND	ND	ND	ND	ND
SBACPA	3.125	3.125	3.125	6.25	ND	ND	ND	ND	25	50
RBACPA	25	25	3.125	3.125	ND	ND	ND	ND	25	50
LVAQPA	50	50	50	50	ND	ND	ND	ND	200	200
SBAQPA	3.125	6.25	ND	ND	ND	ND	ND	ND	25	50

RBAQPA	12.5	12.5	3.125	3.125	ND	ND	ND	ND	100	100
LVHEPA	25	25	6.25	12.5	ND	ND	ND	ND	ND	ND
SBHEPA	3.125	3.125	3.125	3.125	ND	ND	ND	ND	25	50
RBHEPA	3.125	3.125	3.125	3.125	ND	ND	ND	ND	25	50
Gentamycin	0.625	1.25	0.3125	0.3125	0.3125	0.3125	0.3125	0.3125	-	-
Fluconazole	-	-	-	-	-	-	-	-	12.5	25

MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; MFC: Minimum Fungicidal Concentration; ND: Not determined; Acetonic leaf extract of *P. africana*; LVAQPA: Aqueous leaf extract of *P. africana*; LVHEPA; Hydroethanolic leaf extract of *P. africana*; RBACPA: Acetonic root bark extract of *P. africana*; RBAQPA: Aqueous root bark extract of *P. africana*; RBHEPA: Hydroethanolic root bark extract of *P. africana*; SBACPA: Acetonic stem bark extract of *P. africana*; SBAQPA: Aqueous stem bark extract of *P. africana*; SBHEPA: Hydroethanolic stem bark extract of *P. Africana.*

Cytotoxic effects of the studied plant extracts

The investigation into the cytotoxic properties of diverse plant extracts involved the assessment of brine shrimp nauplii mortality rates as a measure of safety. Notably, comparable mortality rates were observed among brine shrimp nauplii exposed to Vincristine at concentrations of 100 µg/ml and 1000 µg/ml, as well as the aqueous root bark and stem bark extracts of *P. africana* at 1000 µg/ml ($p>0.05$). These values were notably higher compared to those resulting from exposure to other extracts ($p<0.05$; Table 3). Conversely, the acetonic leaf and stem bark extracts, alongside aqueous and hydroethanolic root bark extracts at concentrations of 10 µg/ml and 100 µg/ml, demonstrated no discernible mortalities in brine shrimp nauplii (Table 3). Similarly, aqueous and hydroethanolic leaf extracts exhibited no mortalities at all concentrations, while hydroethanolic and aqueous stem bark extracts at 10 µg/ml showed no toxic effects (Table 3). To gauge the cytotoxic potency of the plant extracts, their median lethal concentrations (LC_{50}) were determined. The recorded LC_{50} values ranged from 513 µg/ml for the aqueous stem bark extract to 24327.82 µg/ml for the acetonic stem bark extract of *P. africana* (Table 3). Remarkably, Vincristine displayed a lower LC_{50} value compared to all tested plant extracts (Table 3).

Table 3: Cytotoxic effects of the studied plant extracts against brine shrimp nauplii

Sample	**Concentration**	**Percentage mortality**	**LC_{50} (**µg/ml)
Vincristine	10 µg/ml	72.00 ± 8.37^{C}	6.94
	100 µg/ml	100.00 ± 0.00^{a}	
	1000 µg/ml	100.00 ± 0.00^{A}	
LVACPA	10 µg/ml	0.00 ± 0.00^{G}	1384.19
	100 µg/ml	0.00 ± 0.00^{G}	
	1000 µg/ml	36.00 ± 15.17^{D}	
LVAQPA	10 µg/ml	0.00 ± 0.00^{G}	ND
	100 µg/ml	0.00 ± 0.00^{G}	
	1000 µg/ml	0.00 ± 0.00^{G}	
LVHEPA	10 µg/ml	0.00 ± 0.00^{G}	ND
	100 µg/ml	0.00 ± 0.00^{G}	
	1000 µg/ml	0.00 ± 0.00^{G}	
RBACPA	10 µg/ml	0.00 ± 0.00^{G}	1485.78
	100 µg/ml	14.00 ± 5.48^{F}	
	1000 µg/ml	34.00 ± 21.91^{DE}	
RBAQPA	10 µg/ml	0.00 ± 0.00^{G}	530.35
	100 µg/ml	0.00 ± 0.00^{G}	
	1000 µg/ml	98.00 ± 4.47^{AB}	
RBHEPA	10 µg/ml	0.00 ± 0.00^{G}	691.14
	100 µg/ml	0.00 ± 0.00^{G}	
	1000 µg/ml	74.00 ± 11.4^{C}	
SBACPA	10 µg/ml	0.00 ± 0.00^{G}	24327.82
	100 µg/ml	0.00 ± 0.00^{G}	
	1000 µg/ml	2.00 ± 4.47^{G}	
SBAQPA	10 µg/ml	0.00 ± 0.00^{G}	513.03
	100 µg/ml	4.00 ± 5.48^{G}	
	1000 µg/ml	100.00 ± 0.00^{A}	
SBHEPA	10 µg/ml	0.00 ± 0.00^{G}	572.39
	100 µg/ml	20.00 ± 12.25^{EF}	
	1000 µg/ml	84.00 ± 11.40^{BC}	

Values are expressed as $\bar{x} \pm SD$ for five (5) replicates. Means with different superscript alphabets within the same column are significantly different (P<0.05), while those with similar superscript alphabets within the same column are not significantly different (p>0.05) by One-way ANOVA and Tukey's *post hoc*. ND: Not determined; Acetonic leaf extract of *P. africana*; LVAQPA: Aqueous leaf extract of *P. africana*; LVHEPA; Hydroethanolic leaf extract of *P. africana*; RBACPA: Acetonic root bark extract of *P. africana*; RBAQPA: Aqueous root bark extract of *P. africana*; RBHEPA: Hydroethanolic root bark extract of *P. africana*; SBACPA: Acetonic stem bark extract of *P. africana*; SBAQPA: Aqueous stem bark extract of *P. africana*; SBHEPA: Hydroethanolic stem bark extract of *P. africana*. LD_{50}: Median Lethal concentration.

Qualitative phytochemistry

We assessed the acetonic, hydroethanolic, and aqueous extracts from the leaves, stem bark, and root bark of *P. africana* to determine their pharmacologically significant phytochemicals' presence. Our results consistently showed the presence of alkaloids in the root bark and stem bark extracts; however, alkaloids were absent in leaf extracts (Table 4). Besides, all investigated samples contained flavonoids, phenols, quinones, steroids, and terpenoids (Table 4). Moreover, saponins were detected in the acetonic root bark, and all three leaf extracts of *P. africana* and glycosides were present in the acetonic stem bark, hydroethanolic root bark, and acetonic leaf extracts (Table 4).

Table 4: Qualitative phytochemical composition of the studied plant extracts

Phytochemicals	Stem bark			Root bark			Leaves		
	H/E	Ac	Aq	H/E	Ac	Aq	H/E	Ac	Aq
Alkaloids	+	+	+	+	+	+	-	-	-
Glycosides	-	+	-	+	-	-	-	+	-
Flavonoids	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	-	+	-
Steroids	+	+	+	+	+	+	+	+	+
Quinones	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	+	-	+	+	+

+: Present; -: Absent; H/E: Hydroethanolic extract; Ac: Acetonic extract; Aq: Aqueous extract.

DISCUSSION

The high morbidity and mortality rates due to microbial infections, the rapidly increasing antimicrobial resistance burden, coupled with the inefficiencies of currently utilised antimicrobial drugs prompt the need for alternative therapies and strategies [42,43]. Accordingly, we investigated the antimicrobial activity, cytotoxicity, and qualitative phytochemistry of *P. africana* extracts based on its ethnomedicinal background as an antimicrobial remedy.

Prior research demonstrates that proper extraction of bioactive compounds from medicinal plants or their products is a crucial step in drug development endeavours; thus, the choice of appropriate extraction methods yields phytocompounds with desired activities [44]. Notably, polar solvents, such as water, ethanol, and methanol, and acetone, isolate polar phytochemicals with diverse biological activities, especially antioxidant efficacy. In addition, the percentage yields of the extracts are dependent on the percentages depend on compound concentrations in the plant material. Higher yields suggest a greater extractive value of the solvent, resulting in higher concentrations of extracted phytocompounds [45]. This study used standard phytochemical methods [33] to prepare aqueous, hydroethanolic, and acetonic extracts from leaves, stem barks, and root barks of *P. africana* based on their ethnomedicinal applications as antimicrobial remedies. The high percentage yield for the extracts except for the acetonic and hydroethanolic leaf extracts in this study indicate high extractive values of the respective solvents, denoting a relatively higher concentration of soluble phytochemical compounds.

The extraction method employed significantly influences the quantity and quality of extracts obtained, as emphasised by Dhanani et al. [46]. The results support the suitability of the extraction methods for obtaining the studied plant extracts. Existing research demonstrates that water, acetone, and ethanol, when used as extraction solvents, isolate antioxidant phytochemicals, including phenols, tannins, coumarins, and flavonoids, among others, which exhibit broad-spectrum bioactivity, including antimicrobial efficacy both *in vitro* and *in vivo* [17,44]. Therefore, it is reasonable to infer that the obtained plant extracts in this study comprised these bioactive phytochemical compounds.

The investigation into the antimicrobial properties of the examined extracts was conducted utilizing a previously described disk diffusion method [35,37]. Assessment was undertaken following the guidelines outlined by Mwitari et al. [30], which categorizes the efficacy of antimicrobial activity based on the diameters of growth inhibition zones. Specifically, diameters ranging from 6 to 8 mm denote slight antimicrobial activity, while 9 to 12 mm indicate moderate activity, 13 to 15 mm signify high activity, 16 to 19 mm denote very high activity, and diameters above 20 mm indicate remarkable antimicrobial activity, respectively. Employing this criterion, it was observed that all investigated plant extracts exhibited very high to remarkable antimicrobial activity against strains of *S. aureus* and *B. cereus*, and moderate to remarkable activity against *C. albicans*, contingent upon the extract type and concentration. However, none of the studied extracts demonstrated activity against *P. aeruginosa* and *E. coli*. Disparities in antimicrobial activity among the extracts may be attributed to variations in the type and concentration of bioactive constituents present in each extract. These findings corroborate previously reported data [47].

Furthermore, the antimicrobial efficacy was evaluated based on the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) values. Literature suggests that plant extracts exhibiting MIC and MBC/MFC values lower than 1000 µg/ml may serve as potential sources of antimicrobial lead compounds [48,49], with values below 100 µg/ml holding greater promise [37,49]. In accordance with this assessment, most of the tested extracts derived from *P. africana* demonstrated potential antimicrobial efficacy against *S. aureus*, *B. cereus*, and *C. albicans* to varying degrees. Nonetheless, none of the studied extracts exhibited activity against *P. aeruginosa* and *E. coli*. These findings partially align with those of previous researchers in the field. Notably, previous studies have reported antimicrobial activity of stem bark extracts of *P. africana* against *E. coli* and *P. aeruginosa*, thus presenting a contradiction to the current study's findings. These disparities may be attributed to differences in agroecological conditions, harvesting

seasons, and variations in the type and concentration of phytochemical compounds associated with antimicrobial efficacy [24,50]. Nevertheless, the significant antimicrobial efficacy demonstrated by the investigated plant extracts against other tested microbes holds substantial importance in the context of escalating antimicrobial resistance, suggesting their potential as sources of new alternative antimicrobial compounds [4,51–53]. Given the high cost and mounting concerns regarding the implications of resistance associated with synthetic antimicrobials, further exploration of the active plant extracts may represent a viable strategy for the discovery and optimization of potent antimicrobial agents.

Numerous studies have illustrated that diverse phytochemicals confer the antimicrobial effectiveness of plant extracts, either independently or in combination, preventing microbial growth or survival [21,54,55]. Tannins, flavonoids, phenols, and other phytochemicals contribute to the antimicrobial activity of various plants [56]. The observed antimicrobial effects, particularly at higher concentrations, are ascribed to diverse antimicrobial-related phytochemicals in the extracts. Lower concentrations may result in the extracts' limited efficacy against some microbes, such as *P. aeruginosa*, due to the absence or low levels of responsible compounds [26]. Varied antimicrobial activities may be linked to differences in phytochemical composition, collectively influencing the potency of drug agents [48,57]. Further research elucidating the mechanisms by which these plant extracts exert antimicrobial efficacy could uncover alternative antimicrobials to address antimicrobial resistance. While the study provides valuable insights into the antimicrobial potential of *P. africana* extracts, the challenges in achieving efficacy comparable to synthetic antibiotics, especially against Gram-negative bacteria, call for concerted efforts in research, development, and policy formulation as emphasised by the World Health Organisation (WHO) [58,59]. Balancing traditional knowledge with rigorous scientific investigation will be essential in harnessing the therapeutic potential of plant-based antimicrobials in the era of antimicrobial resistance.

The historical efficacy of herbal preparations in managing microbial infections has been acknowledged, albeit concerns regarding their safety profiles persist [60]. These apprehensions stem from inadequate data pertaining to various aspects including preparation procedures, storage, labelling, marketing, dosage regimens for different diseases, contraindications, potential interactions with synthetic drugs, herb-herb effects, and the absence of comprehensive legislation governing the practice of herbal medicine in many jurisdictions [22]. Such uncertainties underscore significant public safety concerns, as the inappropriate utilization of herbal preparations can potentially lead to life-threatening consequences [61]. Thus, a thorough assessment of the toxicity and safety of herbal preparations is imperative, as it not only provides essential data to empirically validate efficacy but also aids in mitigating potential toxicity risks and guiding further research endeavours [23,25]. Accordingly, this study endeavoured to investigate the cytotoxicity of aqueous, hydroethanolic, and acetonic extracts derived from the leaves, stem bark, and root bark of *P. africana*, drawing upon its ethnomedicinal usage among the Agikuyu people of Murang'a County for managing microbial infections [27,30].

Employing the brine shrimp lethality assay method elucidated by Meyer et al. [39], the cytotoxic effects of these various extracts were evaluated. This method serves as a valuable preliminary screening tool for assessing potential bioactivity and toxicity within plant extracts [62]. The findings not only contribute to understanding the safety profiles of these extracts but also shed light on their potential applications, particularly within the realm of anticancer research [24]. Our results indicate that the mortality percentages observed in brine shrimp nauplii exposed to Vincristine at varying concentrations, along with the aqueous extracts derived from the root bark and stem bark of *P. africana*, exhibited comparable cytotoxic effects. This concentration-dependent cytotoxicity underscores the potential of certain plant extracts to manifest toxicity levels akin to those observed with the reference cytotoxic drug Vincristine. Notably, these specific extracts elicited significantly higher mortality rates compared to other tested extracts, warranting further investigation into the compounds responsible for such observed cytotoxicity and their potential utility as antimicrobial and anticancer agents. Furthermore, the acetonic extracts from the leaf and stem bark, in addition to aqueous and hydroethanolic extracts from the root bark, at lower concentrations, including all concentrations of their leaf counterparts, did not induce mortality in brine shrimp nauplii. While this observation suggests a potential range for safe usage, it also underscores the necessity of exploring selective toxicity associated with these extracts [39,50]. The absence of observed mortalities at lower concentrations may indicate a favourable safety profile, emphasizing the need for comprehensive toxicity studies across an expanded spectrum of concentration ranges [62].

This study determined the median lethal concentrations (LC_{50}), offering a quantifiable gauge of cytotoxic effectiveness that enhances our understanding regarding the concentration causing 50% mortality. The plant extracts in this investigation presented varying LC_{50} values: from 513 µg/ml for the aqueous stem bark extract to an elevated level of 24327.82 µg/ml in the case of acetonic stem bark extract. It is worth noting, however, that Vincristine demonstrated an even lower LC_{50} value than all examined plant materials, thus underlining its acknowledged potent cytotoxic effects [62]. It is noteworthy that integrating these age-old traditional medicine practices into contemporary healthcare demands a meticulous balance: navigating potential risks and benefits is crucial. We will guarantee patient safety and honour our rich heritage by fostering collaborative efforts among traditional healers, scientists, and healthcare professionals.

Research findings highlight the pivotal role of phytochemicals in endowing medicinal plants with therapeutic attributes for combating diverse ailments [18,41]. Environmental stressors, both biotic and abiotic in nature, stimulate the biosynthesis of these secondary metabolites, thereby conferring protective functions upon indigenous flora [63]. Noteworthy among these bioactive compounds are antioxidant phytocompounds such as flavonoids, phenols, tannins, and coumarins, renowned for their broad spectrum of pharmacological effects, including antimicrobial properties [18,55,56]. Analysis of aqueous, hydroethanolic, and acetonic extracts from the leaves, stem bark, and root bark of *P. africana* revealed the presence of compounds linked to antimicrobial activity, reinforcing the correlation between observed antimicrobial effects and the bioactivity associated with specific phytochemicals [63–66]. Geographical factors and the age of the plant contribute to variations in phytochemical composition, emphasizing the necessity for a nuanced approach in drug development initiatives [67]. However, certain phytochemicals like alkaloids, anthraquinones, and specific glycosides have been associated with toxic effects, necessitating caution in their utilization [41,68,69]. The concentration-dependent toxicity observed in brine shrimp nauplii exposed to plant extracts underscores the influence of compound concentration on their toxicological effects [70]. Despite the generally low cytotoxicity of the tested extracts, further investigations employing diverse model organisms are warranted to elucidate their safety profiles comprehensively. Consequently, standardization of extraction methodologies and implementation of stringent quality control measures are imperative to ensure the consistency, potency, and therapeutic efficacy of plant-derived products while mitigating potential risks associated with toxicity [46,71,72].

This study actively contributes to the ever-evolving landscape of natural product research and its integration into global healthcare practices; such contributions are particularly significant as the world grapples with challenges like antimicrobial resistance.

CONCLUSIONS AND RECOMMENDATIONS

Based on the study findings, it was concluded that the aqueous, hydroethanolic, and acetonic extracts from the leaf, stem bark, and root bark of *P. africana* exhibit significant antimicrobial efficacy against *S. aureus*, *B. cereus*, and *C. albicans*, while showing no activity against *P. aeruginosa* and *E. coli*. These extracts are generally

safe at concentrations below 100µg/ml for brine shrimp nauplii, suggesting their potential as safe sources of lead antimicrobial compounds. The plant extracts contain various antimicrobial-associated phytochemicals, indicating potential for drug development. The study recommends further antimicrobial investigations using different solvents and against diverse pathogens. Additionally, extensive cytotoxicity and in vivo toxicity studies are advised to ascertain safety profiles and optimize therapeutic potency. Quantitative phytochemical assessment, isolation, and characterization of active antimicrobial phytocompounds through a bioactivity assay-guided approach are recommended for a comprehensive understanding of their medicinal value.

Availability of data and materials

The manuscript comprehensively presents all pertinent data. Nonetheless, authors remain amenable to furnishing supplementary information upon request, within the bounds of reasonability.

Competing interests

The authors herein assert the absence of any competing interests or conflicts of interest regarding the present publication.

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Authors' contributions

The research idea and experimental design were conceptualized by James Ndung'u, Joseph Nguta, and Isaac Mapenay. James Ndung'u executed the experiments, conducted data analysis, and wrote the draft manuscript under the guidance of Gervason Moriasi. The final draft for submission and publication was reviewed and approved by all authors.

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