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Phytonutrient Screening and *In vitro* Antibacterial and Antifungal Properties of Polar and Nonpolar Extracts of *Albizia gummifera*, *Prunus africana*, and *Combretum molle* from Mount Elgon Region, Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SM and AWW designed the study and secured the funding. Author SPN managed the literature searches, performed the statistical analysis, and managed the analyses of the study. Authors SPN, JMM and AWW wrote the protocol and the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Globally, and particularly in less-developed countries, one of the principal factors associated with morbidity and mortality is infectious diseases. Over the years, the abuse and misuse of pharmaceutical products have caused an increase in resistant microbes, and consequently,

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today, the rate of infectious disease cases continues to increase to dangerously high levels as most medications have lost their efficacy. This indicates that there's a need for new effective medications and calls for active research in drug discovery to curb this dangerous trend.

Results: Obtained data demonstrated the presence of different bioactive compounds in the tested plant extracts such as glycosides, alkaloids, tannins, terpenoids, saponins, and phenols. Using the Kirby-Bauer disc diffusion method, *P. africana* methanol and ethyl acetate extracts showed significantly bigger inhibition zones compared to the rest against *S. aureus* (excluding controls). None of the tested extracts, however, were able to inhibit *C. albicans* and *E. coli*. The *P. africana* methanolic extract and the *A. gummifera* hexane, ethyl acetate, and methanolic extracts all inhibited the growth of *S. aureus* at the same minimum concentration of 31.25 mg/ml. The methanolic extract of *C. molle* exhibited the least activity against *S. aureus*, with an MIC of 250 mg/ml and mean zones of inhibition of 9.33±0.33 mm.

Conclusions: This study revealed the presence of various phytoconstituents in crude extracts of the selected medicinal plants, but also highlighted the resistance of *E. coli* and *C. albicans* to these phytochemicals. The *P. africana* methanolic extract showed strongest inhibitory effect against *S. aureus* compared to the other plant extracts. The highest susceptibility was demonstrated by *S. aureus*, while *E. coli* and *C. albicans* were resistant to all the extracts. These findings support the usage of *A. gummifera, P. africana,* and *C. molle* in folk medicine against infections caused by *S. aureus* and highlight them as potential sources of phytonutrients for the development of new drugs.

Keywords: Phytonutrients; In-vitro; antibacterial; antifungal; Albizia gummifera; Prunus Africana; Combretum molle.

ABBREVIATIONS

ANOVA : Analysis of Variance
CDC : Centers for Disease Control and Prevention
DMSO : Dimethyl Sulfoxide
MBC : Minimum Bactericidal Concentration
MFC : Minimum Fungicidal Concentration
MIC : Minimum Inhibitory Concentration
SPSS : Statistical Packages for Social Sciences
WHO : World Health Organization

1. BACKGROUND

Infectious diseases are among the major threats to human health [54]. Over the years, the abuse and misuse of pharmaceutical products have caused an increase in the number of microbes that are resistant to antimicrobials. Elevated rates of resistance against antibiotics usually used to treat common bacterial infections, such as sexually transmitted infections, sepsis, urinary tract infections, and some types of diarrhoea, have been observed globally, indicating that effective antibiotics are going out of stock. The CDC's report on antibiotic/antimicrobial resistance threats indicates that methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Candida* and carbapenem-resistant Enterobacterales, such as *E. coli,* are among the microorganisms that are serious and urgent threats to human health [9]. In many traditional cultures, medicinal plants play crucial roles in relieving health challenges. This is particularly eminent on the African continent, where approximately eighty percent of inhabitants utilize medicinal plants to cure illnesses and sustain good health [55]. Kenya abounds with medicinal plants that are helpful in the management of common infections and chronic diseases. More than seventy percent of the Kenyan population depends on folklore medicine as the main source of curative substances, while a greater percentage (approximately 90%) of the population utilizes medicinal plants at one moment or another [25]. Availability, efficacy, and affordability have been identified as factors that contribute to the partiality toward traditional medicines. Although previous in vivo studies revealed that most of these plants possess bioactive components at high concentrations, simultaneous consumption with other drugs and usage for long periods may have toxic effects [5]. Culturally, the use of traditional medicines is more approved in various communities [26]. To date, various studies have identified compounds present in medicinal plants that have effective antimicrobial properties [2]. This implies that plants can serve as potential raw materials for the manufacturing of new pharmaceutical products. However, issues such as scarcity of information concerning their active compounds and pharmacological properties considerably affect their usage in modern medicine [38]. Today, a censorious gap is left in research and

development, especially for antibacterial agents against gram-negative carbapenem-resistant bacteria [56]. Among the numerous medicinal plants employed for the management of diseases in Kenya, the most utilized include *A. gummifera, P. africana,* and *C. molle.*

A. gummifera is a native African tree species that is a member of the Fabaceae family [41]. It is known as “Seet” by the Nandi community in Kenya and is used to cure a variety of illnesses. The tree's pod extract is used to treat stomach illnesses, its root is ground into a paste to treat skin conditions, and its bark is used to make a decoction to treat malaria [39]. Previous investigations have demonstrated that extracts from several *A. gummifera* sections have antibacterial properties [34][35]. Spermine alkaloids, oleanane saponins, and triterpenes have been associated with the plant’s anticancer, antibacterial, antiplasmodial, and antitrypanosomal characteristics [51][46].

P. africana, also referred to as African cherry or Pygeum, is a member of the Rosaceae family. It can be found in West Africa, Comoros, Madagascar, and central Africa (Katanga, Congo) and is indigenous to the highland tropical woods that are 1500 meters above sea level in Madagascar and Sub-Saharan Africa. It is widely spread throughout many Kenyan regions, including that of Mt. Elgon, and can be found throughout the mountainous forests of Africa and underlying islands in 22 countries [16]. Its indigenous names are “Muiri” and “Orkujuk” in the Kikuyu and Maasai communities of Kenya, respectively. Extracts from the roots and stem bark contain compounds that have antiviral, anticancer, and anti-inflammatory properties [22]. The plant is used in traditional Kenyan medicine to treat fever, malaria, and chest pain [29]. Allergies, kidney problems, prostate gland illness, and diarrhea are some of its additional traditional applications [20]. According to a study by Bii *et al.,* 2010, flavonoids and terpenes were the main secondary metabolites found in the stem bark of this plant [8].

C. molle is a member of the Combretaceae family. It differs from various species of *Combretum* by having a larger, straighter trunk, dense crown, and rougher bark. It can be found in places with a predominance of forests and wooded grasslands throughout tropical Africa and the Arabian Peninsula, frequently creating pure stands on hillsides [23]. “Muama” and “Kiama” are some of its indigenous names by the Kamba community in Kenya. In Africa, *C. molle* is frequently used to treat a variety of illnesses, including HIV and malaria [44]. It is used in Kenya by the Kamba community to alleviate dysentery and stomach-aches [30]. Secondary metabolites such as flavonoids, steroids, alkaloids, essential oils, coumarins, and terpenoids are reportedly abundant in various parts of this plant [7][14].

In this study, *A. gummifera, P. africana,* and *C. molle* stem barks commonly used in folk medicine against bacterial and fungal infections were collected from the Mount Elgon region in Kenya, where they are naturally found. Using solvents with different polarities, various extracts of each medicinal plant were obtained. Crude extracts were used to screen for major bioactive compounds, while the yielded polar and nonpolar extracts were tested for antibacterial and antifungal activities in vitro against *E. coli, S. aureus* and *C. albicans*.

2. MATERIALS AND METHODS

2.1 Plant Materials

Stem barks of *A. gummifera, P. africana,* and *C. molle* were randomly collected in dense areas of the Mt. Elgon region. Harvesting took place in the month of May, which is the beginning of raining season in most parts of the country. A plant taxonomist from the National Museum of Kenya, Nairobi, together with the local herbalists, helped in the identification of collected plant species. Voucher samples (AWW-JKUATBH/*Ag*/003/2022, AWW-JKUATBH/*Pa*/002/2022, and AWW-JKUATBH/*Cm*/006/2022 respectively) were kept at the herbarium of the Plant Sciences Department, Jomo Kenyatta University of Agriculture and Technology.

2.2 Microorganisms

One gram-positive strain (*S. aureus* ATCC 25923), one gram-negative strain (*E. coli* ATTC 25922) and a yeast strain (*C. albicans* ATTC 10231) were used in this study. All test microorganisms were obtained from the microbiology laboratory at Kenyatta University, Kenya.

Pretreatment of plant materials and crude extract preparation: Collected stem barks of *A. gummifera, P. africana,* and *C. molle* were brought to the microbiology laboratory, Kenyatta University, thoroughly washed with running

water, rinsed with distilled water, air-dried under shade for approximately 2-3 weeks, and finally ground into coarse powder using a grinding mill machine. Approximately 300 g was then macerated in 1500 mL of laboratory methanol for 48 h at room temperature, with occasional swirling. The filtrates were separated from residues using Whatman number 1 filter papers and a vacuum pump. Liquids obtained were concentrated using a rotary evaporator at 64-65°C and 120 rpm and then allowed to air-dry at room temperature. The obtained dry methanolic extract (crude extract) was weighed and stored at low temperatures (~5°C) for future use in the study [15].

Preparation of extracts: Prior to partitioning, the obtained crude extracts were solubilized in 50 mL of distilled water. Using separating funnels, different extracts were obtained via sequential solvent–solvent partitioning in a polarity-increasing sequence by hexane, dichloromethane, ethyl acetate, and methanol. The resulting liquid extracts were concentrated using a rotary evaporator at low temperature and allowed to air-dry at room temperature [15].

Standard inocula preparation: Few distinct colonies of *E. coli, S. aureus* and *C. albicans* were picked with the help of an inoculating loop (sterile). In test tubes, each microorganism was thoroughly suspended in 2 mL of sterile 0.9% saline solution. Suspensions' turbidities were then regulated up to a 0.5 McFarland standard (this corresponds to a bacteria concentration of approximately 10^8 CFU/mL and 10^7 CFU/mL for yeasts) [21].

Preparation of susceptibility test discs: Whatman No. 1 filter papers were punched and used to make discs with a diameter of 6 mm. The obtained paper discs were placed into universal bottles and sterilized by autoclaving at 121°C for 15 to 20 mins. The sterile discs were then impregnated with prepared 500 mg/ml stock solutions of *A. gummifera, P. africana, and C. molle* by gradually infusing 20 µl of each extract into the discs using a micropipette. The discs were allowed to fully absorb each extract and were allowed to dry in sterile petri dishes for approximately 30 minutes. Dried impregnated discs were later used to test for antimicrobial activity against *E. coli, S. aureus* and *C. albicans*.

Qualitative phytochemical screening: The screening of phytochemicals was performed to detect the presence or absence of major phytoconstituents, including alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids and phenols, using standard methods with some modifications.

a) **Alkaloids:** Approximately 0.05 g of crude methanolic extract was mixed with 1 mL of 1% HCl and warmed. Two to three drops of Mayer's reagent (mercuric chloride mixed with potassium iodide dissolved in water) was then added. The appearance of a cream-colored precipitate indicated the presence of alkaloids [13][47].

b) **Flavonoids (Shinoda test):** Approximately 0.05 g of extract was dissolved in 1 mL of methanol and warmed. Two milliliters of 1% HCl was then added, followed by 3 pieces of magnesium ribbon. The formation of a pink/red color confirmed the presence of flavonoids [52].

c) **Tannins:** Approximately 0.05 g of extract was dissolved in 1 mL of distilled water. A few drops of 1% ferric chloride solution were added and observed. Blue–black, blue, blue–green, or green coloration implied that tannins were present [52].

d) **Saponins (Frothing test):** Approximately 0.05 g of methanolic crude extract of each plant was dissolved in 2 mL of distilled water, warmed using a hot water bath and then allowed to cool. The resulting mixture was then shaken vigorously. The presence of saponins was confirmed by the formation of a stable foam [13][47].

e) **Glycosides:** In a test tube, approximately 0.5 ml of extract was mixed with 2 ml of chloroform and shaken. Concentrated sulfuric acid (a few drops) was added to the mixture and observed. The appearance of a reddish-brown steroid ring confirmed the presence of glycoside [53].

f) **Terpenoids (Salkowski test):** Approximately 5 mL of extract was mixed with 2 mL of chloroform and then 3 mL of concentrated sulfuric acid. The formation of a reddish-brown coloration at the interface of the formed layer was indicative of the presence of terpenoids [17][48].

g) **Phenols:** Approximately 0.05 g of plant extract was dissolved in 1 mL of methanol. A few drops of 10% lead acetate solution

were then added to the mixture and observed. The appearance of white precipitates was evidence of the presence of phenolic compounds [28].

Antimicrobial bioassay

a) **Kirby-Bauer disc diffusion method:** A 0.5 McFarland standard suspension of each test microorganism was prepared in normal saline. Approximately 0.5 g of each extract was dissolved in 1000 μL of sterile dimethyl sulfoxide solution (DMSO; 5% in water) to prepare stock solutions (500 mg/mL) [6]. A few dried extract-impregnated discs were aseptically placed on the surface of Mueller Hinton plates that had previously been loaded with a bacterial inoculum and on PDA plates that had been loaded with a *C. albicans* inoculum. Diameters of zones of inhibition were measured after 24 h of incubation and noted in millimeters. Each extract was tested in triplicate. The positive controls used were ciprofloxacin (30 mcg) for bacterial pathogens and fluconazole (25 mcg) for fungal microbes. Dried paper discs impregnated with sterile 5% DMSO solution served as negative controls. Effectiveness was only conferred to extracts that inhibited microbial growth with a mean zone of inhibition equal to or greater than 10 mm [3][43].

Minimum inhibitory concentration: Determination of MICs was performed only for extracts that produced a mean zone of inhibition of at least 10 mm from the disc diffusion assay. Two hundred microliters (200 µl) of each crude extract (500 mg/ml) were dispensed in the 1st wells of a 96-well microtiter plate, and 100 µl of 5% DMSO solution was poured into all the other wells. Using a micropipette, 100 µl of crude extract from each 1st well was drawn and transferred into the 2nd wells containing 100 µl of 5% DMSO solution. A twofold serial dilution was then made up to the 8th well with concentrations ranging from 500 mg/ml to 3.91 mg/ml as described in the modified procedure of Wiegand and the CLSI guidelines [57]. The 9th wells served as growth control wells, in which no extract was added. Sterilized paper discs, 6 mm in diameter, were impregnated with 20 µl of the content of each well. A 0.5 McFarland broth inoculum was prepared and inoculated onto sterile media (MHA for bacteria and PDA for *Candida*). Impregnated discs were then placed on the surface of petri dishes containing the pure fungal/bacterial lawn and incubated for 24 hours at 37°C for bacteria and 24-72 hours at 37°C for *Candida*. Each test was performed in triplicate. MIC values were then obtained by matching the minimum diameter of the zone of inhibition with the lowest concentration of the extracts at which microbial growth was suppressed [1].

Minimum bactericidal/fungicidal concentration: The contents of the last wells (impregnated on sterile paper discs) that produced observable diameters of inhibition zones similar to those of negative growth control wells were aseptically placed on culture plates previously inoculated with a 0.5 McFarland broth inoculum of test microorganisms. The concentration of each extract that gave no observable growth after incubation for 24 h at 37°C was noted as MBC or MFC [19].

2.3 Data Analysis

The data collected were transferred to Microsoft Excel sheets. SPSS software, version 22, was used to analyze diameter readings of zones of inhibition and concentration values, where descriptive statistics were carried out to obtain their mean values. The results are given as the mean and standard error of the mean (mean ± SEM). One-way ANOVA was then utilized to compare the mean MIC of each extract against test microorganisms. Significant differences between the concentration values and mean MICs of the various extracts were ascertained using post hoc analysis (Tukey's HSD test) [24]. P value < 0.05 was considered significant [58].

3. RESULTS

a) **Qualitative phytochemical screening:** The results obtained from the qualitative phytochemical screening of *A. gummifera, P. africana*, and *C. molle* were recorded as shown in Table 1. *A. gummifera* is the only plant that demonstrated the presence of all tested bioactive compounds. No glycosides were detected in extracts of either *P. africana* or *C. molle.* In addition, *C. molle* was also found to lack alkaloids (Table 1).

Table 1. Phytochemical screening of *A. gummifera, P. africana, and C. molle* stem bark

Phytoconstituents	Plant Samples		
	P. africana	***C. molle***	***A. gummifera***
Saponins	+	+	+
Phenols	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Glycosides	-	-	+
Alkaloids	+	-	+
Tannins	+	+	+

Key: *(+) Indicates detected, (-) Indicates Not detected*

Antibacterial and antifungal activities: Each plant was partitioned using 4 solvents; thus, a total of 12 plant extracts with a concentration of 500 mg/ml were impregnated on sterile paper discs and tested for antimicrobial activities against standard strains of *E. coli, S. aureus,* and *C. albicans* using the Kirby-Bauer disc diffusion method. The inhibitory effects of these extracts are shown in Table 2.

Against *S. aureus*, *P. africana* ethyl acetate and methanolic extracts showed significantly larger zones of inhibition compared to all other tested extracts. The zones of inhibition produced by the *C. molle* methanolic extract and the *A. gummifera* ethyl acetate and methanolic extracts were all significantly similar (Table 2). The inhibitory effects exhibited by *A. gummifera* hexane were noted to be comparable to those of both *A. gummifera* and *P. africana* ethyl acetate extracts (Table 2). The positive control (ciprofloxacin), however, had the highest antibacterial activity against *S. aureus*, with an inhibition zone of 32.33±0.33 mm (Table 2). The negative control (DMSO) did not show any activity and produced zones of growth inhibition significantly commensurate with those of *A. gummifera* and *P. africana* DCM extracts, *C. molle* and *P. africana* hexane extracts and *C. molle* ethyl acetate extract (Table 2). These extracts were thus disregarded in subsequent tests.

For active extracts that showed considerable antibacterial activity against *S. aureus* (Zone of inhibition ≥ 10 mm), MICs were determined using the broth dilution method, and the results were recorded as displayed in Table 3.

The antibacterial activity of the *P. africana* methanolic extract against *S. aureus* at a concentration of 500 mg/ml was significantly like that observed at 250 mg/ml, which in turn was higher than those of subsequent dilutions (Table 3). It was also noted that at concentrations of 125 and 62.5 mg/ml, the extract had a significantly commensurate inhibitory ability against *S. aureus*. The positive control (ciprofloxacin), however, caused a significantly larger zone of inhibition in comparison with all tested concentrations of *P. africana* methanolic extract, and the negative control (DMSO) caused no inhibitory action, similar to the extract at concentrations of 15.62, 7.81, and 3.91 mg/ml (Table 3).

The *P. africana* ethyl acetate extract showed antibacterial activity against *S. aureus* up to a concentration of 125 mg/ml, with a larger zone of inhibition of 11.67±0.33 noted at 500 mg/ml (Table 3). At concentrations of 62.5, 31.25, 15.62, 7.81, and 3.91 mg/ml, the extract demonstrated no antibacterial potential and produced zones of inhibition significantly similar to that of the negative control (Table 3). Compared to the positive control (ciprofloxacin), the effects of all tested concentrations of *P. africana* ethyl acetate were significantly lower (Table 3).

The antibacterial activity exhibited by the *A. gummifera* methanolic extract against *S. aureus* was higher at concentrations of 500 and 250 mg/ml, both exhibiting significantly similar zones of inhibition, as shown in Table 3. However, the highest inhibitory effect was caused by the positive control (ciprofloxacin), with an average zone of inhibition of 32.33±0.33. The negative control (DMSO) had no activity against *S. aureus* and effected a zone of inhibition significantly comparable to that of the *A. gummifera* methanolic extract at concentrations of 15.62, 7.81, and 3.91 mg/ml (Table 3).

Table 2. Antibacterial and Antifungal Activities of Hexane, DCM, Ethyl acetate, and Methanolic Extracts of *A. gummifera, P. africana,* and *C. molle* against *E. coli, S. aureus,* and *C. albicans*

Medicinal Plants	Plant Extracts	Inhibition/mm ± SE Mean		
		S. aureus	***E. coli***	***C. albicans***
A. gummifera	Dichloromethane	6.33 ± 0.33^{gh}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
	Ethyl acetate	**12.33 ± 0.33^{de}**	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
	Hexane	**13.33 ± 0.33^{cd}**	6.67 ± 0.00^{b}	6.00 ± 0.00^{c}
	Methanol	**11.67 ± 0.33^{e}**	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
C. molle	Dichloromethane	7.67 ± 0.33^{fg}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
	Ethyl acetate	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
	Hexane	6.33 ± 0.33^{gh}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
	Methanol	**11.67 ± 0.33^{e}**	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
P. africana	Dichloromethane	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
	Ethyl acetate	**14.67 ± 0.33^{bc}**	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
	Hexane	6.00 ± 0.00^{h}	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
	Methanol	**15.33 ± 0.33^{b}**	6.00 ± 0.00^{c}	6.00 ± 0.00^{c}
Negative Control	5% DMSO solution	6.00±0.00	6.00±0.00	6.00±0.00
Positive control (Bacteria)	Ciprofloxacin	32.33±0.33	31.00±0.58	NA
Positive Control (Fungus)	Fluconazole	NA	NA	22.33±0.33

Values with similar lowercase superscript letters are not significantly different column wise using one way ANOVA and Tukey's multiple comparison (p>0.05).

Key: *mm= Millimeters, SE Mean= Standard error of mean, NA= Not applicable*

Table 3. Minimum Inhibitory Concentration Average Zones of Inhibition against *S. aureus*

Concentration */* mg/ml	Zone of Inhibition/mm ± SE Mean					
	P.a MeOH	*P.a* EA	*A.g* MeOH	*A.g* Hex	*A.g* EA	*C.m* MeOH
500	12.33±0.33^{b}	11.67±0.33^{b}	12.33±0.33^{b}	12.33±0.33^{b}	12.67±0.33^{b}	10.67±0.33^{b}
250	12.33±0.33^{b}	10.33±0.33^{c}	11.67±0.33^{b}	11.00±0.58^{b}	11.67±0.33^{b}	**9.33±0.33^{c}**
125	10.33±0.33^{c}	**8.33±0.33^{d}**	10.67±0.33^{c}	10.67±0.33^{c}	10.67±0.33^{c}	6.67±0.33^{d}
62.5	9.33±0.33^{c}	6.67±0.33^{e}	9.33±0.33^{d}	9.33±0.33^{de}	10.33±0.33^{c}	6.00±0.00^{d}
31.25	**8.00±0.00^{d}**	6.00±0.00^{e}	**8.00±0.00^{e}**	**8.67±0.33^{ef}**	**8.33±0.33^{d}**	6.00±0.00^{d}
15.62	6.00±0.00^{e}	6.00±0.00^{e}	7.00±0.00^{ef}	7.33±0.33^{fg}	6.33±0.33^{e}	6.00±0.00^{d}
7.81	6.00±0.00^{e}	6.00±0.00^{e}	6.33±0.33^{f}	6.00±0.00^{g}	6.00±0.00^{e}	6.00±0.00^{d}
3.91	6.00±0.00^{e}	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{g}	6.00±0.00^{e}	6.00±0.00^{d}
Negative Control	6.00±0.00^{e}	6.00±0.00^{e}	6.00±0.00^{f}	6.00±0.00^{g}	6.00±0.00^{e}	6.00±0.00^{d}
Positive Control	32.33±0.33^{a}	32.33±0.33^{a}	32.33±0.33^{a}	32.33±0.33^{a}	32.33±0.33^{a}	32.33±0.33^{a}

Values with similar lowercase superscript letters are not significantly different column wise using one way ANOVA and Tukey's multiple comparison (p>0.05).

Key: *W.u= W. ugadensis, P.a= P. africana, A.g= A. gummifera, C.m= C. molle, DCM= dichloromethane, EA= ethyl acetate, Hex= hexane, MeOH= methanol, mm= millimetre, SE Mean= standard error of mean, Superscripts= Grouping Information using the Tukey Method and 95% Confidence*

At concentrations of both 500 and 250 mg/ml, the hexane extract of *A. gummifera* exhibited significantly similar activity against *S. aureus*. The zones of inhibition produced by the extract at concentrations of 125 and 62.5 mg/ml were also significantly the same (Table 3). However, compared to all tested concentrations, the positive control (ciprofloxacin) exhibited the highest antimicrobial activity (Table 3). Extract concentrations of 15.62, 7.81, and 3.91 mg/ml had no effect against *S. aureus* and produced zones of inhibition significantly comparable to that of the negative control (DMSO) (Table 3).

Table 4. Minimum Bactericidal Concentrations of Selected Plant Extracts against *S. aureus*

Medicinal Plant	Plant Extracts	MBC (mg/ml)
		S. aureus
P. africana	Methanol	125
	Ethyl acetate	500
A. gummifera	Methanol	250
	Hexane	250
	Ethyl acetate	125
C. molle	Methanol	500

Comparing all tested dilutions of *A. gummifera* ethyl acetate extract, higher antibacterial potential against *S. aureus* was achieved at a concentration of 500 mg/ml, which was significantly similar to the effect observed at 250 mg/ml. Zones of inhibition recorded at concentrations of 250, 125, and 62.5 mg/ml were all significantly comparable to one another. Again, all tested concentrations of *A. gummifera* ethyl acetate demonstrated a significantly lower activity compared to the positive control (ciprofloxacin), and the negative control (DMSO) had no activity, with an average zone of inhibition significantly similar to those of the extract at concentrations of 15.62, 7.81, and 3.91 mg/ml (Table 3).

The *C. molle* methanolic extract only showed activity against *S. aureus* up to the first dilution (250 mg/ml), with a higher antibacterial effect observed at a concentration of 500 mg/ml. The reference drug ciprofloxacin (30 mcg) produced the highest inhibitory activity compared to those of the extract at every concentration (Table 3). Dilutions with concentrations of 125, 62.5, 31.25, 15.62, 7.81, and 3.91 mg/ml showed no effect against *S. aureus* and exhibited zones of inhibition significantly like that of the negative control (DMSO) (Table 3).

Table 4 outlines the minimum bactericidal concentration of each tested extract, wherein *A. gummifera* ethyl acetate and *P. africana* methanolic extracts both showed bactericidal activity at a concentration of 125 mg/ml. Similarly, hexane and methanolic extracts of *A. gummifera* both demonstrated bactericidal effects at 250 mg/ml, and it was at their initial concentrations (500 mg/ml) that extracts of *P. africana* ethyl acetate and *C. molle* methanol caused complete death of *S. aureus.*

4. DISCUSSION

The rapid spread of resistance genes among different microbial populations and the global rise in antimicrobial resistance of commonly used and available pharmaceutical products has led to an imperative need for new and effective drugs. It is impossible to overstate the significance of medicinal plants in traditional medicines, as they are utilized extensively not just in Kenya but also around the world for a wide range of medical applications [33]. *A. gummifera, P. africana,* and *C. molle* are popular medicinal plants, particularly in Africa, used for the treatment and management of various ailments. Nonetheless, the scarcity of research investigating their bioactive compounds and antimicrobial effects using different solvents has hindered their recognition as potential drug sources. This study thus qualitatively examined the phytochemical constituents of *A. gummifera, P. africana,* and *C. molle* and examined their antibacterial and antifungal properties in various extraction solvents against standard strains of *E. coli, S. aureus*, and *C. albicans.*

To unravel the source of the medicinal properties of *A. gummifera, P. africana*, and *C. molle*, phytochemical screening of each crude extract was performed. Table 1 shows the type of bioactive compounds present in these plant stem barks, which probably played some roles in their antimicrobial effects. Tannins are a class of specific phytochemicals with a wide range of medicinal uses, including anti-inflammatory, antiviral, antiulcer, and antiparasitic applications [4][32][31]. According to Soine (1964), they are recognized to have antibacterial properties [49] and have been shown to be effective against microorganisms that cause diarrhea [12]. Moreover, numerous naturally occurring triterpenoids, which have been isolated from various plant sections, have been found to possess fungicidal, bactericidal, anticancer, antiviral, cytotoxic, anti-inflammatory, analgesic, and antiallergic properties [42]. Flavonoids have also been found to have cytotoxic, anti-inflammatory, and antiviral properties [10]. Alkaloids, on the other hand, have been proven to have antibacterial, antimalarial, analgesic, and

antiseptic properties, whereas most of the biological impacts on cell development and division that occur in humans are caused by saponins, which also have an inhibitory influence on inflammation [27]. The results revealed that *A. gummifera* stem bark typically contains all screened phytochemicals. These findings are like those found in leaf extracts of *A. gummifera* in a study conducted by Oloruntola *et al.* (2021) [40]. Similarly, *P. africana* was observed to contain all screened metabolites apart from glycosides (Table 1). These results are supported by previous studies that demonstrated the absence of this type of compound in *P. africana* stem bark [36]. *C. molle* extract indicated the presence of saponins, phenols, flavonoids, terpenoids, and tannins, which are similar to components found in a study on the phytochemical screening of *C. molle* by Koevi *et al.* (2015) [27]. These factors may have accounted for their antimicrobial activities against *C. albicans, E. coli,* and *S. aureus*.

The antimicrobial activity of *A. gummifera, P. africana, and C. molle* extracts varied between each tested microorganism. Table 2 shows that *E. coli* had the lowest susceptibility among the three tested microorganisms, whereas *S. aureus* had the highest susceptibility to the various extracts.

Against *S. aureus* (ATCC 25923), three extracts (hexane, ethyl acetate, and methanol) of *A. gummifera* showed activity, two *P. africana* extracts (ethyl acetate and methanol) also demonstrated antibacterial effects, and only the *C. molle* methanolic extract was able to inhibit *S. aureus*. None of the tested extracts of *A. gummifera*, *P. africana, or C. molle* demonstrated antibacterial or antifungal activity against *E. coli* (ATTC 25922) or *C. albicans* (ATTC 10231). These observations align with findings from studies on medicinal plants conducted by Cheruiyot *et al.* (2009) [11] and Yibeltal *et al.* (2013) [59], who reported that when compared to *E. coli*, *S. aureus* is the most sensitive to plant extracts regardless of plant parts, extraction method, and solvent used. Additionally, due to the morphological differences between gram-positive and gram-negative microorganisms, plant extracts are usually more efficient against gram-positive (*S. aureus*) than gram-negative (*E. coli*) bacteria [50]. This may thus explain the variability in the antibacterial activity of the extracts noted in this study. In this research, *P. africana* extracts caused the highest antibacterial effects compared to all other medicinal plants against *S. aureus* (Table 2). However, the *P. africana* methanolic extract was found to be more potent than its ethyl acetate counterpart, as affirmed by a lower MIC (table 3). In a study conducted by Mwitari *et al.* (2013) [37], similar observations were made, whereby while the ethyl acetate fraction of *P. africana* demonstrated only modest efficacy against *S. aureus*, the methanol extract had good activity. This is supported by evidence that suggests that methanolic extracts have a high extraction capacity because of their strong polarity, which increases the availability of phytochemicals associated with antibacterial and antioxidant properties [18][45]. All tested extracts of *A. gummifera* inhibited the growth of S. aureus at the minimal concentration of 31.25 mg/ml (Table 3). Again, *P. africana* and *A. gummifera* methanolic extracts both exhibited significant antibacterial effects against *S. aureus*, with an MIC value of 31.25 mg/ml and mean inhibition zones of 8.00±0.00 mm (table 3). These findings correlate with those of Bii *et al.* (2010) [8] in a study on the possible uses of *P. africana.* This result demonstrated the strong efficacy of *P. africana* methanol extracts against bacterial strains. On the other hand, *A. gummifera* ethyl acetate was noted to have higher antibacterial activity against *S. aureus* compared to the ethyl acetate portion of *P. africana*. This was demonstrated by MIC values of 31.25 and 125 mg/ml, respectively (table 3). The *C. molle* methanolic extract exhibited the least activity against *S. aureus* compared to other plant extracts, with an MIC of 250 mg/ml and mean zones of inhibition of 9.33±0.33 mm (table 3). These variations in how microorganisms responded to the different extracts, however, further raise the question of how these bioactive extracts work.

5. CONCLUSION

Phytochemical screening revealed that medicinal plants involved in this study abound in bioactive compounds. These compounds could be associated with the antibacterial effect observed against *S. aureus* and can therefore be potentially looked upon in the development of new pharmaceutical products. However, despite the presence of these phytochemicals, notable resistance was observed in *E. coli* and *C. albicans*, suggesting developed resistance in these strains. Variations in the response of these microorganisms to the different extracts, further raise questions on the mechanism of action of these phytoconstituents and on their quantitative value in the plant parts. There is therefore a need

to quantitatively screen for the phytochemicals present in these plants and to identify specific bioactive compound(s) responsible for the observed antimicrobial activity as well as their mechanisms of action.

This research demonstrated the antimicrobial potential of methanolic extracts of *P. africana, A. gummifera,* and *C. molle*; hexane extract of *A. gummifera*; and ethyl acetate extracts of *P. africana* and *A. gummifera* against *S. aureus*. The *P. africana* methanolic extract showed the highest antibacterial effect. *S. aureus* demonstrated the highest susceptibility, while *E. coli* and *C. albicans* showed resistance to the tested extracts. These findings lay a foundation for future tests to validate and develop these extracts as potential sources or substitute treatments in the management of diseases or infections caused by *S. aureus*, thus promotes the sustainable use and conservation of all active plant species. Again, this work highlights the presence of resistance genes among microbial populations, a significant public health threat in this era.

AVAILABILITY OF DATE AND MATERIALS

The authors declare that all the data supporting the findings of this study are provided within the manuscript.

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ETHICAL APPROVAL

The National Commission for Science, Technology, and Innovation (NACOSTI) approved this research, and all operations were conducted in accordance with the Clinical & Laboratory Standards Institute (CLSI) recommendations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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