Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(4): 53-62
Received: 23-05-2023
Accepted: 28-06-2023

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Antimicrobial properties of *Catha edulis* (Miraa) against selected bacterial and fungal pathogens, an *in-vitro* experimental study

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DOI: https://doi.org/10.22271/phyto.2023.v12.i4a.14693

Abstract
The increasing risk of the emergence of antimicrobial resistance can be addressed using plant-based botanicals as alternatives to antibiotics. In this *in vitro* study, the antimicrobial properties of aqueous and methanolic extracts of *Catha edulis* (Miraa) were tested on select pathogenic bacteria and fungi using the agar well diffusion method. The aqueous Miraa extracts at 1000 mg/ml, 100 mg. ml, and 10 mg/ml concentrations significantly inhibited the growth of all bacterial pathogens except *E. coli* but did not have an effect on *C. albicans*. The largest zones of inhibition for the aqueous extracts were observed at 1000 mg/ml against *S. pneumoniae* (28.41 mm), *S. pyogenes* (24.27 mm), and MRSA (21.86 mm). The largest zones of inhibition for the methanolic extracts were at 1000 mg/ml against *S. pneumoniae* (26.75 mm), *S. pyogenes* (25.38 mm), and *S. aureus* (19.71 mm). Thus, crude Miraa extracts have significant antimicrobial effects *in vitro* against the tested microorganisms.

Keywords: *Catha edulis*, Miraa, antimicrobial, extracts, bacteria

Introduction
The burden of infectious diseases remains a significant threat to health, especially in developing countries. Infections affect a large proportion of the world's population. For example, about 55 million people have a urinary tract infection at any given time [1]. Approximately 357 million people get sexually transmitted infections annually [2]. Respiratory, gastrointestinal, and wound infections, as well as bacteremia and sepsis, are also a significant causes of morbidity disability, and mortality.

Infectious diseases are still a substantial problem despite the rapidly increasing use of antibiotics. Between 2000 and 2020, the defined daily doses (DDD) of antibiotics consumed globally increased from 21.1 billion to 34.8 billion, which translates to a 65% increase [3]. The high rates of use of antibiotics are causing the emergence of antibiotic-resistant bacteria. Besides, synthetic antibiotics are associated with several negative features such as short half-life *in vivo*, toxicity, and high cost of synthesis [4].

There is a need to reduce the use of antibiotics in response to the problem of antimicrobial resistance (AMR) so that the currently available antibiotics stock may not get depleted [5]. Plant-based botanicals are a viable alternative to antibiotics in efforts to address AMR [6]. Their antimicrobial and chemo-sensitizer effects can be leveraged to optimize the use of the available antibiotics while reducing the pressure on them [7]. Investigating plants used in traditional medicine to treat infectious diseases for antimicrobial effects can provide evidence of their value in managing infectious diseases.

The varieties of *C. edulis* in Yemen, South Africa, Saudi Arabia, and Lebanon have been shown to have antibiotic effects against various bacteria in *in-vitro* studies [8-12]. Fatima, *et al.* [8] tested methanol, dimethyl sulfoxide (DMSO), and water extracts of *Catha edulis* Forsk in Saudi Arabia against *Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis*, and *Candida albicans* clinical isolates. The methanol and DMSO extracts had significant zones of inhibition in all the bacteria tested while the aqueous extract was only active against gram-positive organisms, particularly *Staphylococcus aureus* [8].

Siddiqui [11] tested methanolic crude extracts of *C. edulis* purchased from a shop in London against various laboratory-stocked microbes (*Escherichia coli, Bacillus magisterium, Brevundimonas diminuta*, and *Micrococcus luteus*) using the antibiotic disc diffusion assay.

The researcher established that the extracts had significant (breakpoint of 14 mm) antimicrobial effect against all the bacteria tested (zones of inhibition > 16 mm for *B. Magisterium*; > 19 mm for *M. luteus*) except *E. coli* (zone of inhibition < 11 mm) [11]. The variation in *E. coli* results in the studies by Fatima, *et al.* [8] and Siddiqui [11] imply that *Catha Edulis* grown in different geographical areas could be having varying antimicrobial effects.

Al-hebshi, Al-haroni, and Skaug [9] evaluated the antimicrobial effect of aqueous extracts of Yemen's *C. edulis* against organisms comprising oral microbiota. The extracts showed more effect on the gram-negative bacteria (*Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Prevotella intermedia*), which are mainly pathogenic in the mouth, compared to the gram-positive bacteria (Streptococci and Actinomyces), which are mostly the normal flora of the oral cavity [9]. Therefore, *Catha edulis* could be selectively active against pathogens by sparing normal flora.

Miraa, the common name for *Catha edulis* varieties cultivated in the Igembe region of Meru County in Kenya, is one of the plants used to treat infectious diseases in traditional medicine. The Miraa plant is a dicotyledonous shrub in the Celastraceous family whose twigs are harvested and commonly chewed for recreational purposes [13]. There are *C. edulis* varieties in other parts of the world including Yemen, Ethiopia, Saudi Arabia, South Africa, and Lebanon. According to an ethnobiology study by Kiunga, *et al.* [14], herbalists in Meru use decoctions of the leaves and roots of Miraa to treat oral, respiratory, diarrheal, and urogenital diseases. Miraa can be a suitable source of plant-based botanicals if its antimicrobial properties are established. Its availability is assured given that it is a cultivatable plant without scarcity challenges faced when wild plants are used as sources [15].

The illnesses that herbalists treat using Miraa could be caused by myriad pathogens. *S. aureus* is a common cause of respiratory, urinary tract, and gastrointestinal infections [16], which herbalists apply Miraa decoctions to treat. Methicillin-resistant *S. aureus* (MRSA) is rapidly spreading globally amidst the reduced antibiotic options to treat its infections [17]. *Streptococcus pyogenes* is a common cause of sore throat, one of the respiratory infections that herbalists treat using Miraa decoctions. Its infections have increased in the last three decades and it is commonly developing resistance to antibiotics [18]. *Streptococcus pneumoniae*, which causes respiratory infections, is a pathogen against which antibiotic resistance is rapidly emerging due to antibiotic selection pressure [19]. *Escherichia coli* is a common cause of urinary tract, gastrointestinal, and respiratory infections; it is common in outbreaks [20]. Hence, *E. coli* is a suitable representative of gram-negative bacteria in studies to identify plants that can be sources of antimicrobial botanicals. *Candida albicans* is a fungus that causes oropharyngeal and vulvovaginal candidiasis. Its multi-drug-resistant strains are rapidly emerging. Thus, it is an appropriate representative of pathogenic fungi in this study [21].

Our comprehensive review of the literature did not find any study investigating the antimicrobial properties of Miraa, the *C. Edulis* cultivated in Kenya. Studying Miraa to determine its antimicrobial effects was essential to generate information on whether it has antimicrobial effects like the varieties tested in other countries. This article reports the *in-vitro* antimicrobial effects of aqueous and methanolic crude Miraa extracts against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Candida albicans* clinical isolates, and *Streptococcus pyogenes* ATCC 19615 and methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300 standard strains.

Materials and Methods

Aim, Design and Setting

The aim of this study was to investigate the antimicrobial effects of Miraa in an effort to explore it as a potential source of plant-based antimicrobial botanicals. An *in-vitro* experimental study to test the antimicrobial effects of 1000 mg/ml, 100 mg/ml, and 10 mg/ml of Miraa's aqueous and methanolic crude extracts on *S. aureus*, *S. pneumoniae*, *E. coli*, and *C. albicans* clinical isolates, and *S. pyogenes* ATCC 19615 and MRSA ATCC 43300 standard strains was conducted. The experiments were done in the Drug Analysis and Research Unit (DARU) laboratories in the University of Nairobi, Kenya.

Plant Materials

Miraa twigs and leaves weighing one kilogram were plucked from Miraa plants in an organic farm in Ithanja village (°15'45.0" N 37°56'31.6" E) in Njia location, Igembe Central District, Meru County, Kenya. They were transported in a Cooler Ice Box at 2-8 ºC to the Drug Analysis and Research Unit on the same day and stored at -20 ºC until the day of the extraction. A specimen of the twigs and leaves was deposited in the University of Nairobi's School of Biological Science herbarium. It was assigned voucher number DK2020/001. The twigs and leaves were chopped into small pieces using a scissor and ground using a blender after removing debris. The weight of the ground material was 892.24 g.

Five-hundred grams of the ground material were put in a 5-litre conical flask and 99.8% AR/ACS methanol (meets the standard Macron Fine Chemicals™ grade of analytical reagents and the requirements of the American Chemical Society Committee on Analytical Reagents) was added and stirred at room temperature for 24 hours for methanolic extraction. Whatman filter papers with particle retention of > 11 µm and Welch 0.9 CFM Dry Fast 2 Head PTFE diaphragm vacuum pump were used to filter the extract. The filtrate in a 250 mL round-bottomed flask was reduced in vacuum using a rotary evaporator and fixed using dry ice to form a uniform coating on the walls of the flask. It was then freeze-dried using a Heto Power Dry R LL1500 Freeze Dryer. For the aqueous extraction, 300 g of the ground material was mixed with 900 mL of distilled water in a conical flask and the mixture was heated for 40 minutes. It was left overnight. It was then filtered, reduced, fixed, and freeze-dried for 24 hours like in the methanolic extraction process.

Microbial Strains

The *S. Aureus*, *S. Pneumoniae*, *E Coli*, and *C. Albicans* were obtained from the stock of clinical isolates in the University of Nairobi's microbiology laboratory. *S. Pyogenes* ATCC 19615 and MRSA ATCC 43300 were standard organisms obtained from the same laboratory. *S. aureus*, MRSA, and *E. coli* were provided while subcultured in nutrient agar. *S. Pyogenes* and *S. Pneumoniae* were provided while subcultured in blood agar. *C. albicans* was in Sabouraud's dextrose agar (SDA).

Antimicrobial Susceptibility Testing

The agar well diffusion method was used. Colonies from the subcultures were suspended in normal saline to attain an equivalent of McFarland's standard 0.5. Two milliliters of

each of *S. aureus*, MRSA, and *E. coli* suspensions were added to their respective 200 mL of sterile trypticase soy agar (TSA) in liquid form at 50^0C. Suspensions of *S. Pyogenes* and *S. Pneumoniae* were similarly inoculated but in TSA with 5% defibrinated sheep blood. *C. albicans* was also inoculated following the same procedure but in SDA. The mixtures of media and suspension of the organisms were poured into 20 mL culture media plates. Eight plates were prepared for *S. aureus*, eight for *E. coli*, and six for each of the other four organisms. They were left to cool to room temperature. The plates were divided into two equal sets, one for testing the aqueous extracts and the other for the methanolic extracts.

An 8-mm sterile cork borer was used to punch five uniformly-spaced wells in each of the culture plates. The wells labelled A, B, C, D, and E were for the negative control (A), the positive control (B), and 1000 mg/ml (C), 100 mg/ml (D), and 10 mg/ml (E) concentrations of the extracts. A hundred microliters of distilled water for the negative control, 50 µL of 0.3mg/ml gentamicin (anti-bacterial) or 0.3 mg/ml nystatin (anti-fungal) for the positive control, and 100 µL of each of the three doses of the aqueous and methanolic extracts were added in their respective wells. The plates were left at room temperature for one hour for the preparations to diffuse. The culture plates with *S. aureus*, MRSA *E. coli*, and *C. albicans* were incubated at room temperature for 18 hours. *S. Pyogenes* and *S. Pneumoniae* were incubated in 5% CO_2 at 37^0 C for 18 hours. Diameters of zones of inhibition were measured using a vernier caliper calibrated to give the measurements in a precision of two decimal points.

The mean diameter of zones of inhibition (mDZOI) was calculated for each concentration of the extracts by obtaining the average of the three or four measurements taken from the replicates of the experiments. A mDZOI of 9.0 mm was used as the breakpoint, where < 9 mm was interpreted as resistance (R) and > 9 mm as susceptibility (S). The independent t-test was used to compare the mDZOI of each of the three dosages of Miraa extracts to the respective mDZOI of the respective negative control. One-way ANOVA was applied to determine whether at least one of the mDZOI was significantly different from the mean DZOI of other concentrations of Miraa extracts and the negative control. Tukey's HSD test was applied when ANOVA detected a significant difference so that it would be possible to know where the difference was lying. Paired t-test was used to compare the mDZOI of the aqueous Miraa extracts (aME) and the methanolic (mME). A 95% confidence level was used in all the stages of data analysis. A level of significance of 5% and power of 80% were applied in all the analyses.

Results

Extraction yields

The methanolic extraction process yielded 28.93 g from the 500 g ground material used, which was obtained from the Miraa leaves and twigs shown in Figure 1. The yield's weight was 5.8% of the weight of the material used. On the other hand, the aqueous extraction method produced 6.75 g of extract from 300 g of the ground material. Thus, the extract's weight was 2.3% of the weight of the material used.

Fig 1: Miraa leaves and twigs before preparation for extraction

Antibacterial Effects Against *S. aureus*

Both aqueous Miraa extracts (aME) and methanol Miraa extracts (mME) significantly inhibited the growth of *S. aureus* at the three doses tested. The DOI for the 1000 mg/ml, 100 mg/ml, and 10 mg/ ml concentrations of the aME and the corresponding positive and negative controls were as shown in Figure 2.

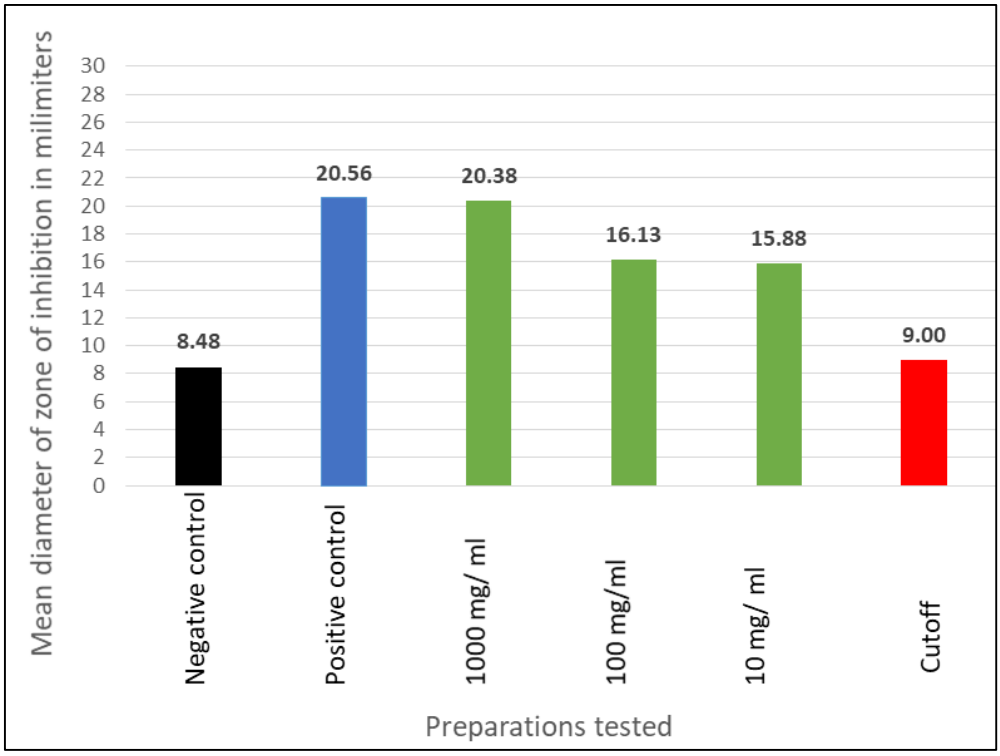


Fig 2: A graph showing the mean diameters of the zone of inhibition of aqueous Miraa extracts against S. aureus cultures

Independent t-test revealed the existence of a significant difference between the mDZOI of the 1000 mg/ml, 100 mg/ml, and 10 mg/ml, and the negative control (p<0.05 in all three comparisons). One-way ANOVA test showed that at least one of the mDZOIs for the three doses was significantly different (F=64.235, p=0.0001). Tukey HSD posthoc test identified the mDZOI of the 1000 mg/ml as the only one significantly different from the other two (p=0.0001) for both tests.

For the mME, each of the three doses had a significant antimicrobial effect against *S. aureus*. The mDZOIs were as shown in Figure 3. They were all significantly different from the mDZOI of the negative control (p=0.0001 for each of the concentrations). They were also significantly different from each other (F=438.44, p=0.0001; p=0.0001 for all sets of comparisons).

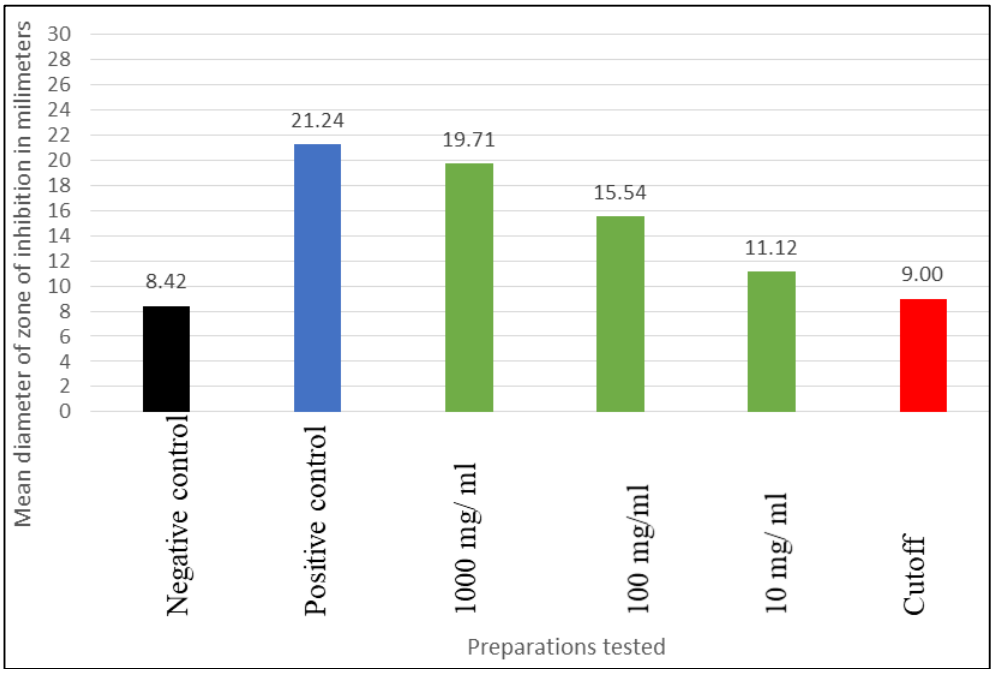


Fig 3: A graph showing the mean diameters of the zone of inhibition of methanolic Miraa extracts against S. aureus cultures

A paired t-test revealed that only the 10 mg/ml of the aME was different from the corresponding concentration of the mME (p=0.001). The 1000 mg/ml and 100 mg/ml of aME had no significantly different effect compared to their respective concentrations of the mME.

Antibacterial Effect of Miraa against MRSA ATCC 43300

Both aME and mME had antibacterial effects against MRSA. The three concentrations of aME significantly inhibited the growth of MRSA. The mDZOIs are as shown in Figure 4. They were all significantly different from the mDZOI of the

negative control (p<0.005 for all the comparisons). Additionally, each of the three concentrations had an effect that was substantially different from the others (F=400.387, P=0.0001; p=0.0001 for all the sets of comparisons).

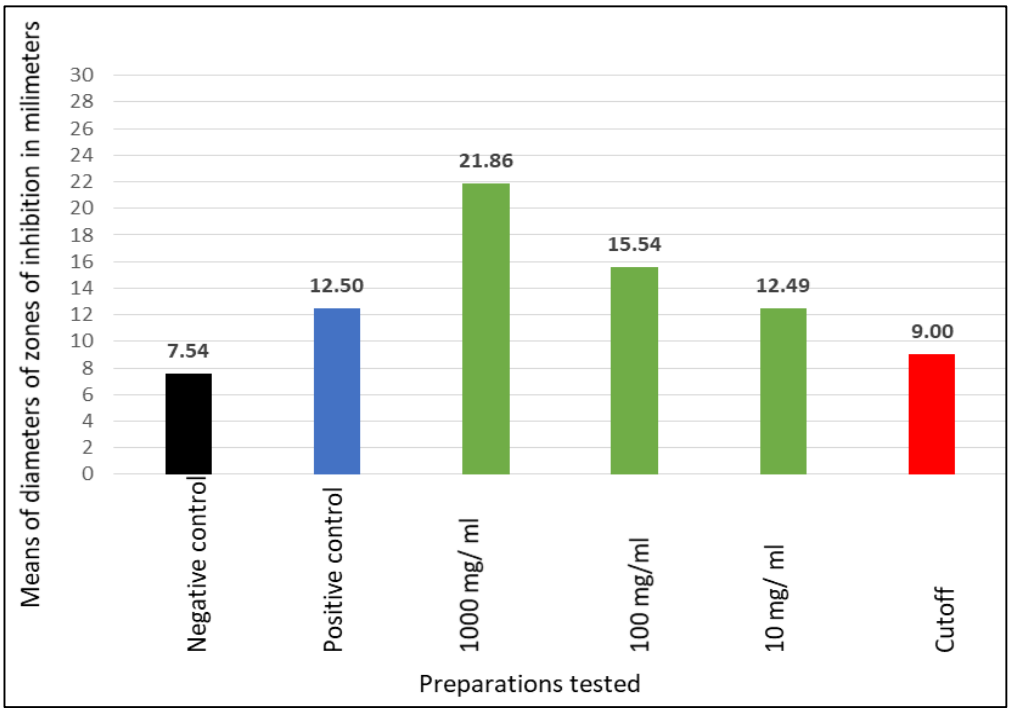


Fig 4: A graph showing the mean diameters of the zone of inhibition of aqueous Miraa extracts against MRSA cultures

Similarly, the three concentrations of mME exerted significant inhibition against MRSA. The mDZOIs are shown in Figure 5. Each of them had an mDZOI different from the negative control (p=0.0001). Their effects were significantly different from each other's (F=214.884, p=0.0001; p<0.003 for all the sets of comparisons). The aME exerted significantly greater effects compared to the mME at all three concentrations (p<0.003 for all of them).

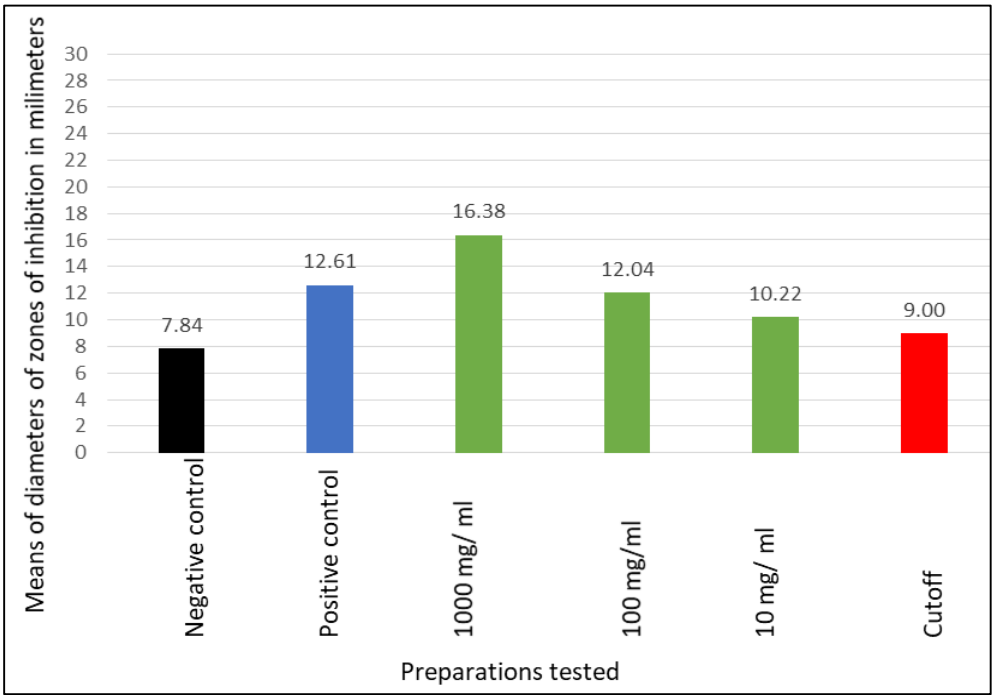


Fig 5: A graph showing the mean diameters of the zone of inhibition caused by methanolic Miraa extracts in MRSA cultures

Antibacterial Effects of Miraa Extracts against *S. Pneumoniae*

Both the aME and mME at 1000 mg/ml, 100 mg/ml, and 10 mg/ml substantially inhibited the growth of the *S. pneumoniae*. For both the aME and mME, there were significant differences between the mDZOIs of each of the concentrations of extracts and the respective negative control as shown in Figure 6 and Figure 7 (p=0.0001 for all comparisons). There were also significant differences between the mDZOI of the three doses of aME (F=1885.88, p=0.0001; p=0.0001 for all sets of comparisons).

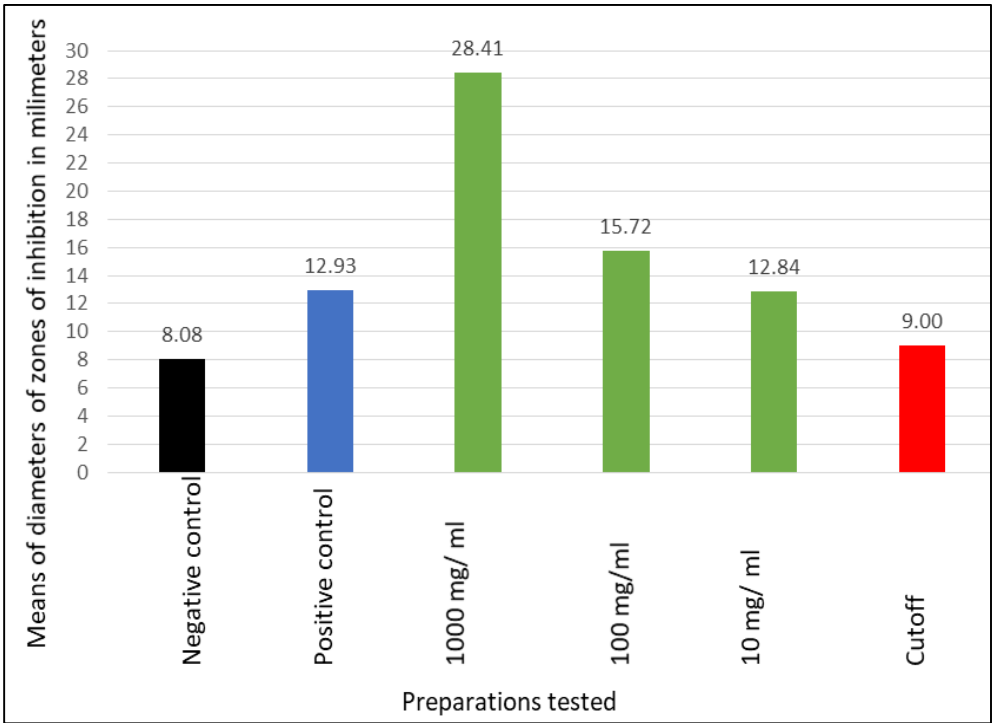


Fig 6: A graph showing the mean diameters of the zone of inhibition caused by aqueous Miraa Extracts in Streptococcus pneumoniae cultures

Similarly, the mDZOI of the three doses of mME were significantly different (F=2714.72, P=0.0001; p=0.0001 for all sets of comparisons). A comparison of the corresponding concentrations of aME and mME showed that only the mDZOIs of the 10 mg/ml doses were significantly different (p=0.01).

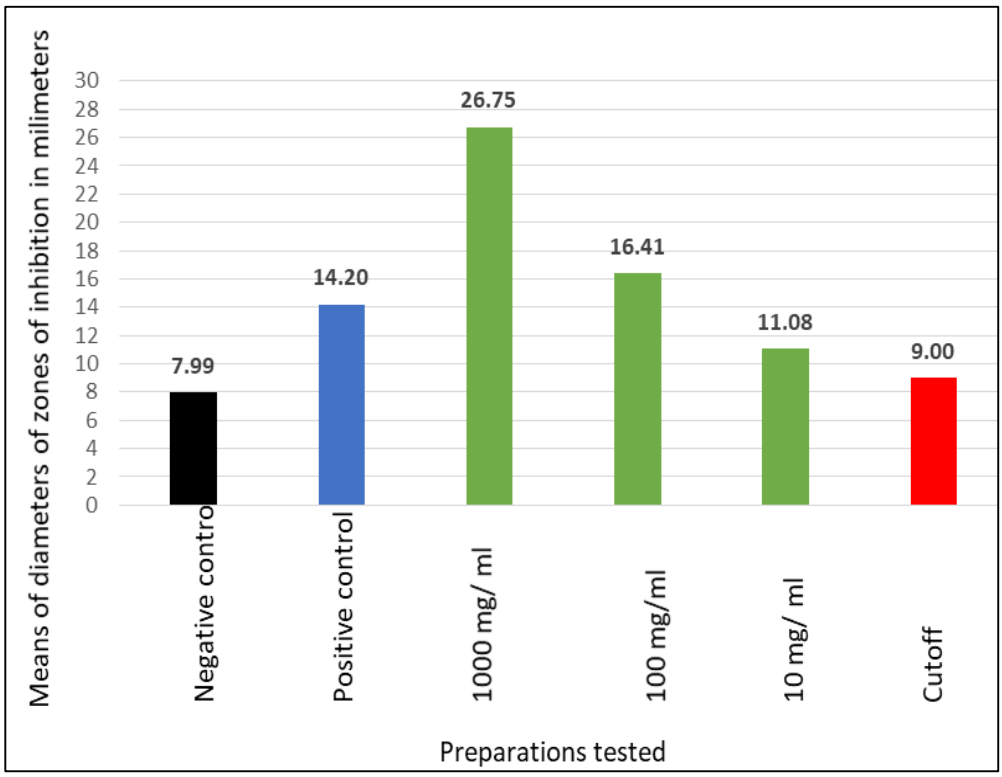


Fig 7: A graph showing the mean diameters of the zone of inhibition caused by methanolic Miraa Extracts in Streptococcus pneumoniae cultures

Antibacterial Effect of Miraa Extracts against S. Pyogenes

Both aME and mME significantly inhibited the growth of *S. pyogenes*. The mDZOI of each of the concentrations of aME and mME were as shown in Figures 8 and 9 respectively. They were significantly different from the mDZOIs of the respective negative controls for both the aME and mME (p=0.0001).

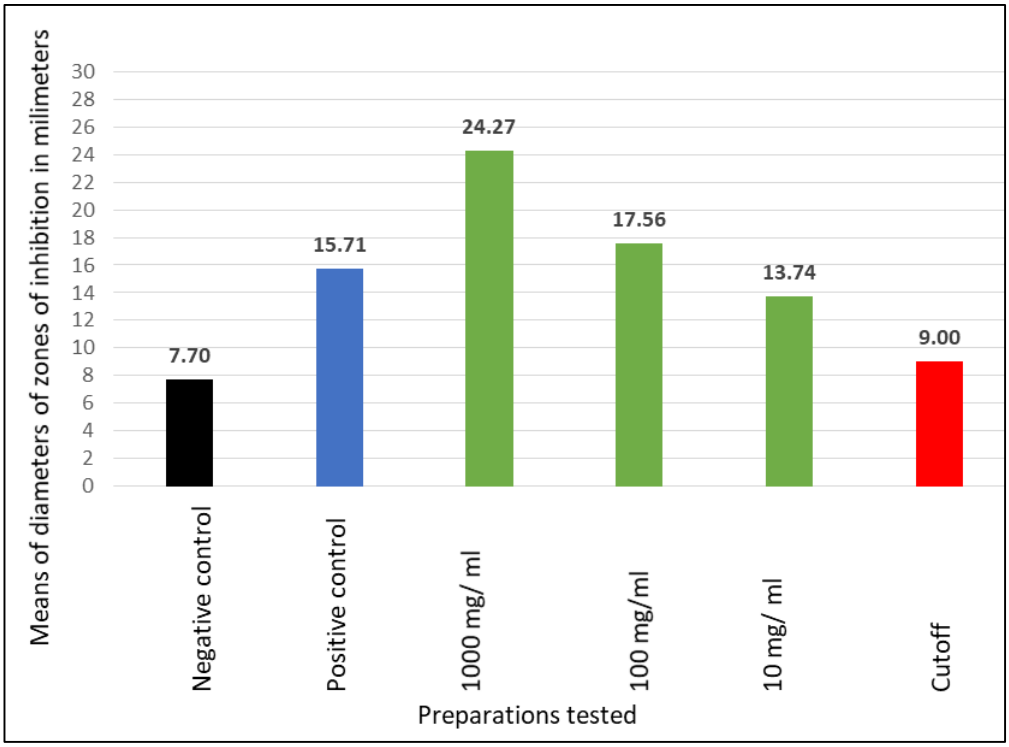
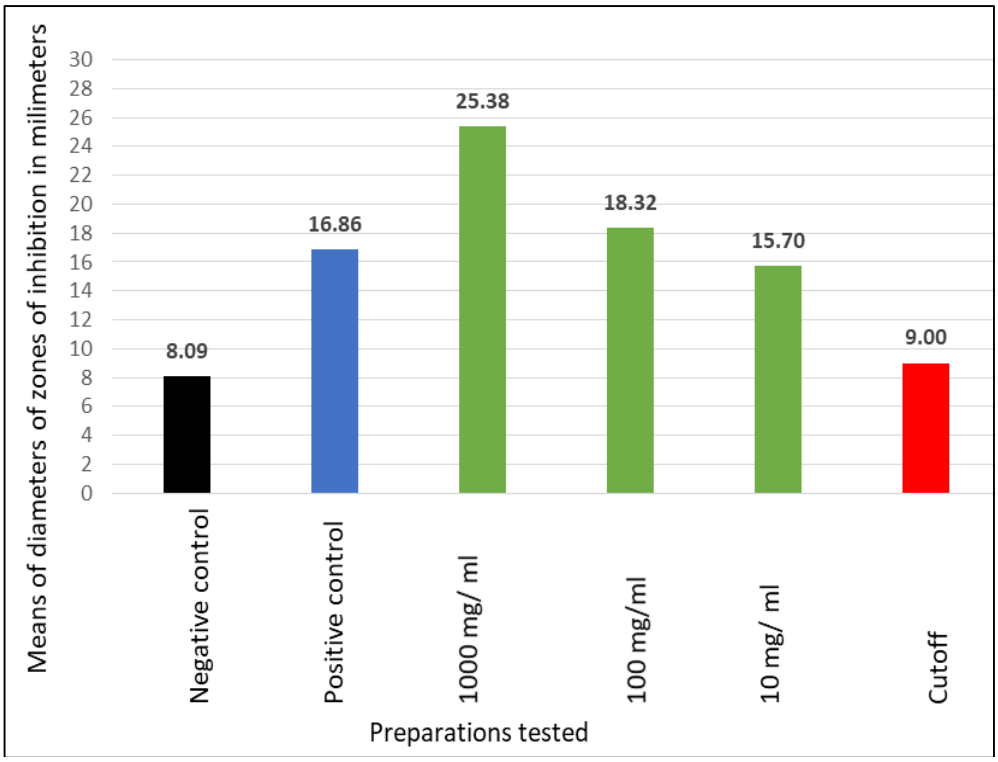


Fig 8: A graph showing the mean diameters of the zone of inhibition caused by aqueous extracts of Miraa in Streptococcus pyogenes cultures

The three doses of the aME had significantly different mDZOI (F=1568.6, P=0.0001; p=0.0001 for all sets of comparisons). Similarly, the mME doses had mDZOI is significantly different from each other (F=926.76, p=0.0001; p=0.0001 for all sets of comparisons. Comparison of the aME and mME revealed significant differences between the 100 mg/ml (p=0.009) and 10 mg/ml (p=0.013) doses.



Neither the aME nor mME inhibited the growth of *E. coli* and *C. albicans*.

Fig 9: A graph showing the mean diameters of the zone of inhibition caused by methanolic extracts of Miraa in Streptococcus pyogenes cultures

Discussion

Few antimicrobial studies [8-12] have been done to investigate the antimicrobial properties of *C. edulis*. However, none of them indicated studying Miraa, the *C. edulis* cultivated in Kenya. The studies were conducted using *C. edulis* in Saudi Arabia [8], Yemen [9], the United Kingdom [11], South Africa [12], and Lebanon [22]. The study by Al-hebshi [9], reported that different cultivars of *C. edulis* can have varying antimicrobial effects. Hence, it was critical to test whether Miraa, the *C. edulis* cultivars grown in Kenya, have antimicrobial effects like the cultivars tested in other countries.

In the current study, the methanolic extraction process was more efficient than aqueous extraction. The yield from the methanolic extraction was 5.8% w/w, which was more than double the 2.3% w/w yield of the aqueous extraction. None of the previous studies has reported comparisons of extraction yields; hence this finding could be novel.

Our findings on the antibacterial effects of Miraa against *S. aureus* are consistent with the ethnobiology findings by Kiunga, *et al.* [14]. *S. aureus* is involved in multiple infections including the urogenital and gastrointestinal infections allegedly treated using Miraa in traditional medicine [14, 16]. The results also agree with the study by Fatima, *et al.* [8] which found both aqueous and methanolic extracts of Saudi Arabia's *C.edulis* to inhibit the growth of *S. aureus* clinical isolate at 0.125-1 mg/ml. Further, McGaw, *et al.* [12] found ethanol crude extracts of *C. Edulis* in South Africa to have antimicrobial effects against *S. aureus* clinical isolate with a minimum inhibitory concentration of 0.012 mg/ml. On the other hand, our findings contradict those of Al-hebshi's who found no growth inhibition with 1.25-20 mg/ml aqueous *C. edulis* in Yemen against *S. aureus* ATCC 6538 [9].

The difference in the activity of aME and mME at 10 mg/ml, where the aME exhibited a stronger effect, could be due to the difference in the composition of the extraction yields. Fatima *et al.* [8] demonstrated that aqueous and methanolic extracts of *C. edulis* have different constituent compounds. The antimicrobial effect against MRSA observed in the current study further points to the potential value of *C. edulis* in the search for alternatives to treat *S. aureus* infections. Possibly, the antimicrobial effect is due to 22b-hydroxytingenone and tinge none among other phytochemicals in the plant. Elhag, *et al.* [10] extracted them from *C. edulis* callus cultures and found them to have an antimicrobial effect against *S. aureus* at a MIC of 0.6 µg/ml.

The results of the current study showing significant antimicrobial effects of Miraa against *S. pyogenes* are consistent with the findings by Kiunga *et al.* [14] that Miraa treats sore throats in traditional medicine. *S. pyogenes* cause sore throats and related sequelae [18]. The findings are also in agreement with those of Al-hebshi *et al.* [9], who found aqueous extracts of Yemen's *C. edulis* to have antimicrobial effects against *S. pyogenes* clinical isolate, MIC of 10-20 mg/ml. The absence of effect observed by Fatima *et al.* [8] could be due to the low concentrations they tested, 0.125-1 mg/ml. Al-hebshi *et al.* [9] also noted that at 1.25 mg/ml, the aqueous extracts only stopped the hemolytic effect but not the growth of S. pyogenes. Therefore, the MIC of aqueous crude extracts of *C. edulis* against *S. pyogenes* could be between 1.25 mg/ml and 10 mg/ml.

In the study by Fatima, *et al.* [8], methanolic extracts of *C. edulis* inhibited *S. pyogenes* at concentrations that aqueous extracts could not (0.125-1 mg/ml). The superior strength of the methanolic extracts against *S. pyogenes* was also observed in our study. The antimicrobial effect of the extracts is consistent despite the variability of the source of *C. edulis* cultivars (Kenya, Yemen, and Saudi Arabia) tested in the various research. The types of *S. pyogenes* used in the multiple studies are also diverse: Standard organism, clinical isolate, and multi-drug resistant clinical isolate in the current study, the research by Al-hebshi *et al.* [9], and the study by Fatima *et al.* [8] respectively.

The finding that Miraa extracts have substantial antimicrobial properties against *S. pneumoniae* is consistent with the folklore assertion that Miraa treats respiratory diseases [14]. *S. pneumonia* is a common cause of respiratory diseases [23]. This finding could be novel; we could not identify a publication of a previous study that reported the antimicrobial effects of *C. edulis* against *S. pneumoniae*. Given the rapidly-developing antimicrobial resistance against *S. pneumoniae* [19], our findings are valuable as they may aid the identification of sources of plant-based botanicals to use as alternatives to conventional antibiotics in treating *S. pneumoniae* infections.

Our findings of the absence of an antimicrobial effect of *C. edulis* against *E. coli* agree with the results of the study by Siddiqui, *et al.* [11], in which methanolic extracts did not show inhibitory effects against the growth of *E. coli* K1 strain RS218 clinical isolate. Similarly, McGaw, *et al.* [12] found ethanol extracts of *C. edulis* to have no antimicrobial effects against *E. coli*. Elhag, *et al.* [10] also found no effect when they tested 22b-hydroxytingenone and tinge none compounds extracted from *C. edulis* callus culture against *E. coli*. Perhaps the urinary tract infections and gastrointestinal infections allegedly treated using Miraa in traditional medicine are caused by other pathogens such as *S. aureus*, but not *E. coli* [14].

In Contrasta, Fatima, *et al.* [8] Found 1 mg/ml of *C. edulis* extracts to inhibit the growth of *E. coli* clinical isolate. However, the fact that 2.5% methanolic solution was used to dilute their extracts instead of water as in the other studies may explain the variance in the results. Methanol is known to be antibacterial, but Fatima *et al.* [8] justified its use by indicating that is not inhibitory at low concentrations.

Our findings that Miraa extracts have no antimicrobial effects against *C. albicans* are consistent with the findings by Al-Hebshi, *et al.* [9] and Elhag, *et al.* [10]. It is unlikely that the oral and urogenital diseases treated by Miraa in traditional medicine are caused by *C. albicans.* Notably, just as in *E. coli*'s scenario, our findings on *C. albicans* differ from those of Fatima *et al.* [8], who found the extracts to inhibit the growth of *C. albicans.*

This research was derailed by a few limitations. First, scarcity of resources did not allow the purchase of standard organisms or characterization of the clinical isolates used. The use of available standard organisms and laboratory-stocked clinical isolates partly addressed the challenge. The inadequacy of resources also limited the number of pathogens included in the study and the varieties of extraction methods used. It was also not possible to use a quantitative method for the determination of MICs. The comparison of the effects of *C. edulis* from various countries was also done with caution because there is no study showing the variability of the genetic makeups of the *C. edulis* cultivars grown in various countries across the world.

Conclusion

In conclusion, the increasing emergence of antimicrobial resistance, the slowed-down discovery of new antibiotics, and the continued indiscriminate use of antibiotics pose a serious global challenge. The challenge can be partly addressed by exploring alternatives to conventional antibiotics for use in treating infectious diseases. Plant-based botanicals with antimicrobial properties are a suitable alternative. Investigating the antimicrobial effects of cultivated plants such as Miraa as potential plant-based botanicals is necessary because they show promise of consistent supply. This study showed that aqueous and methanolic crude Miraa extracts have antimicrobial effects against *S. aureus*, MRSA, *S. pyogenes*, and *S. pneumoniae* but not against *E. coli* and *C. albicans*. The revealed *in-vitro* antimicrobial effects provide

the basis for testing the crude extracts in *in vivo* animal studies to help predict whether the crude extracts can have the observed antimicrobial effects in humans. Future studies should also identify the specific compounds in Miraa imparting the antimicrobial effect and assess the resistance-modifying effects of both the crude extracts and the isolated compounds.

Acknowledgements
I acknowledge the University of Nairobi for support with laboratory facilities and microorganisms for conducting the experiments.
I recognize my mother Sabina Nkatha, the herbalist who explained how she uses Miraa to treat some oral and genitourinary diseases.
I also acknowledge Gideon Thananga, Joseph Mwenda, and Stanley Mwenda for their support in identifying the organic farm to purchase the Miraa needed for this study.

Declarations
Consent for publication: Not applicable.

Availability of data and materials
The dataset used and analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests
The authors declare that they have no competing interests

Funding
The research cost was supported through the Centre of Materials, product development & Nanotechnology (MAPRONANO)'s Master's Research Scholarship (Ksh. 200,000).

Author's Contributions
DK designed the study, conducted the experiments, analyzed the data, and wrote the main draft of the manuscript.
SM was a major contributor in refining the manuscript.
SN was a major contributor in selecting the extraction methods and procedures
HN was integral in performing the extraction procedures and the antimicrobial susceptibility testing.
JO provided substantial input in refining the research idea, selecting the microorganisms for the experiments, and choosing the antimicrobial susceptibility testing method and procedure.
All authors read and approved the final manuscript.

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