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Exploring the Antimicrobial Efficacy of Embelia Keniensis Leaf Extracts against Selected Microbial Strains

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The pursuit of novel bioactive compounds from natural sources has prompted an assessment of the antimicrobial properties and phytochemical composition of Embelia keniensis leaf extracts. Embelia keniensis is one of the five Myrsinaceae species endemic to Kenya. The interest in the scientific investigation of Embelia keniensis crude extracts is based on the claims of their effective use for the treatment of many diseases such as chest pains, common cold and stomach ailments. The plants used in this study were derived from Brackenhurst Botanical Garden Limuru sub-county, Kiambu County, central Kenya. The study was conducted from January to March 2023 at the Jomo Kenyatta University of Agriculture and Technology. The study aimed to evaluate the antimicrobial potential of crude extracts of this plant against Escherichia coli, Staphylococcus aureus, and Candida albicans. The antimicrobial activity was determined through the disc diffusion method. Both methanol and water were employed for extraction; however, neither demonstrated promising activities against the test organisms. Notably, the water extract exhibited greater potency compared to the methanolic extract against Escherichia coli. The water extract displayed a maximum inhibition

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zone of 9mm at a concentration of 1mg/ml. similar findings were observed for Staphylococcus aureus and Candida albicans with inhibition zones of 9mm and 7 mm, respectively, at the same concentration. Preliminary phytochemical screening identified the presence of bioactive agents such as saponins and flavonoids, while tannins and terpenoids were absent. Despite the observed zones of inhibition, the plant extract fell short of meeting the clinical and laboratory standards institute breakpoints for susceptibility and intermediate levels. Instead, they exhibited resistance breakpoints, rendering the plant extracts ineffective for antimicrobial activities. Nevertheless, this outcome does not negate the potential of the plant extract. Factors like mode of action and microorganism susceptibility could influence their effectiveness. Further evaluation is warranted to fully comprehend the potential of *Embelia keniensis* extracts. This study provides insight into their antimicrobial activity and a preliminary phytochemical profile.

Keywords: Embelia keniensis; Staphylococcus aureus; Escherichia coli; Candida albicans; Clinical and Laboratory Standards Institute (CLSI).

1. INTRODUCTION

The value of medicinal plants is based on various chemical constituents that bring about some concise physiological activities in the human body [1]. Phytochemistry is associated with the huge variety of organic components (primary and secondary metabolites.) that are developed in detail and amassed by plants and also deals with the complex structure of plant chemicals, their biological synthesis, metabolic turnover, metabolic pathways, their distribution in nature and their biological roles [2].

The treatment of infectious diseases with antimicrobial agents continues to present problems in modern-day medicine with many studies showing a significant increase in the incidence of bacterial and fungal resistance to several antibiotics [3].

Therefore, research into biologically active extracts and compounds from natural sources has been of great interest to scientists in their attempt to discover new sources for drugs that may be useful in combating infectious diseases [4].

Embelia keniensis, an endemic member of the myrsinaceae family within Kenya holds a position of ecological and medicinal significance. This rare and endangered species thrives exclusively in Brackenhurst Botanical Garden (in Limuru, Kiambu county) and Mount Kenya Forest. The family Myrsinaceae is renowned for its therapeutic potential, underscoring the importance of investigating *Embelia keniensis* [3].

The traditional medicinal applications of *Embelia keniensis* such as treating chest pains, colds, and stomach ailments call for a detailed scientific study of this species [5]. However, despite those historical uses, the plant remains relatively unexplored, particularly in terms of its antimicrobial activity. As antibiotic resistance looms, exploring alternative therapeutic options becomes imperative. The study of *Embelia keniensis* phytochemicals presents a potential avenue to address the challenge.

Investigating plants with intrinsic antimicrobial attributes, driven by secondary metabolites, is a key trend in pharmaceutical research. Understanding the interplay between these phytochemical constituents and their antimicrobial potential is fundamental. In this context, the research aims to contribute to our understanding of *Embelia keniensis* antimicrobial attributes, aligning with the broader scientific endeavor to harness natural resources for medicinal advancement.

Given the global challenges posed by infectious diseases and the rise of antibiotic-resistant strains, exploring alternative therapeutic strategies is crucial [4]. Evaluating the efficacy of *Embelia keniensis* phytochemicals presents a promising approach. By tapping into the natural properties of these compounds, the research may offer a solution to combat antibiotic resistance while promoting more sustainable and accessible healthcare practices [6].

As the threat of antibiotic resistance continues to grow, the study of *Embelia keniensis* assumes paramount significance. This research endeavors to shed light on the potential of plant-derived compounds, not only to combat resistance but also to contribute to a more sustainable and effective approach to health care. By exploring the rich pharmacological resources of *Embelia*

keniensis, this study aligns with the global call for innovative solutions to address pressing healthcare challenges.

2. MATERIALS AND METHODS

Experimental site: This research was conducted at the Government of Kenya Biology Laboratory at Jomo Kenyatta University of Agriculture and Technology in Nairobi, Kenya from January to March 2023. *Embelia keniensis* was selected as plant material and obtained from Brackenhurst Botanical Garden in Limuru, sub-county, Kiambu County in Central Kenya.

Sample collection: *Embelia keniensis* leaves samples were collected from Brackenhurst Botanical Garden in Limuru, Kiambu county, Kenya. The plant samples were authenticated by Mr. Kamau Muchuku a taxonomist from the Department of Botany, Jomo Kenyatta University of Agriculture and Technology.

Sample preparations: The leaves were examined to be free from diseases. Only healthy plant leaves were used. Extraneous materials were also removed from the plant materials. The leaves were spread in a piece of newspaper and were allowed to air dry for 14 days. The dried leaves were ground into a fine powder using a mortar and pestle and preserved in plastic bags.

Phytochemical screening: Preliminary phytochemical tests were carried out first on the samples to establish the presence or otherwise of the chemical constituent using standard procedures by Kinghorn [7].

Extraction of leaf extract: To extract the compounds, 250ml of sterile water and 250ml of methanol were added to two different containers respectively. Then 50g of the grounded powder was added to the containers and covered using aluminum foil, they were left for 24 hours and placed in a water bath shaker to facilitate extraction. The resulting solution was filtered using a funnel with Whatman filter paper, yielding the methanolic and water extracts. The methanolic extract was concentrated using a rotary evaporator for 10 hours, resulting in a sticky residue that was then diluted using 30 ml of sterile distilled water. Different concentrations of the diluted extract were prepared through serial dilution, creating concentrations ranging from 10^0 to 10^4. The serial dilution procedure was carried out as outlined by James and Sally [8].

Inoculum preparations: For this research study to be conducted two different mediums were prepared which included the nutrient agar medium (for bacteria strains) and the potato dextrose agar medium (for fungal strains).

Nutrient agar medium preparation was carried out using the standard procedures as outlined by Baker [9]. while potato dextrose agar medium was carried out using the standard procedure as outlined by Juan and Manuel [10].

Microbe's selection: Microbes that were used included *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* which were obtained from the Government of Kenya laboratory at the Jomo Kenyatta University of Agriculture and Technology as pure cultures.

Sensitivity test: Agar plates were inoculated with a standardized inoculum of the test microbes. Then filter paper discs (6mm in diameter), containing the plant extract at the desired concentration, were placed on the agar surface. The Petri dishes were then incubated under different suitable conditions for both bacterial and fungal strains as outlined by Biemer [9].

To ensure that the method has worked well and to compare the activity of the crude extract, a positive control of the antibiotic gentamycin was added. The positive control was tested at the same concentration as the plant extracts.

Experimental design and statistical analysis: This work was set in a completely randomized design. The experimental design encompassed the investigation of extract concentrations against two bacterial species and a fungus. Five distinct extract concentrations (10^0, 10^1, 10^2, 10^3, 10^4, and 10^5) were employed for each organism, along with a crude plant extract. A control group was also included, involving sterile distilled water placed on the paper disc. This resulted in a total of five treatment groups for each test organism. To ensure robustness, all experimental conditions and treatments were replicated once.

The experimental results were presented in mean (+-) SD of the mean of five replicates. The sample means were compared using analysis of variance (ANOVA) to determine the level of significance. Differences in mean values were considered significant at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

Phytochemical screening of *Embelia keniensis* leaves: The results of the phytochemical screening of *Embelia keniensis* revealed the presence of saponins and flavonoids while tannins and terpenoids were not detected in any of the samples (Table 1).

Table 1. phytochemicals screening of *Embelia keniensis* leaves

Phytochemical	Methanol extract
saponins	++
flavonoids	++
tannins	–
terpenoids	–

++: Present, -: Absent

To determine the antimicrobial effects of *Embelia keniensis* extracts against selected microbes: The methanolic extract exhibited the highest inhibition against *Staphylococcus aureus* with a diameter zone of inhibition of (8.8 ±0.8 mm) at a concentration of 0.001 ml/mg while the least with (6.5 ±0.7 mm) was recorded against *Escherichia coli* at the concentration of 1ml/mg. *Staphylococcus aureus* had a maximum inhibition zone of (8.8 ±0.8 mm) at 0.001mg/ml methanol extract concentration and a minimum inhibition zone of (6.5 ± 0.7 mm) at 0.00001 mg/ml methanol extract concentration. *Escherichia coli* had a maximum of (8 ± 0 mm at 0.0001 mg/ml) and a minimum of (6.5 ± 0.7 mm at 1 mg/ml). *Candida albicans* had a maximum of (7 ± 0 mm at 1 mg/ml) and a minimum of (6 ± 0 mm at 0.1 mg/ml) (Fig. 1). The diameter of the zone of inhibition for all the positive control treatments was greater than 10 mm for all the concentrations tested. This clearly indicated that the respective bacteria grew on media and inhibition of growth was expedited as appropriate giving confidence that our experimental set up worked successfully.

Fig. 2 represents the antibacterial activity of water extract of *Embelia keniensis* leaves at various concentrations.

The water extract showed the highest inhibition against *Escherichia coli* with a diameter zone of inhibition of (9 ±0) mm at a concentration of 1mg/ml while the least inhibition was recorded in *Candida albicans* at a diameter zone of inhibition of (6 ±0) mm at a concentration of 0.00001ml/mg. *Staphylococcus aureus* had a maximum inhibition zone of (8.5 ± 1.5 mm) at $10^{\wedge -2}$ water extract concentration and a minimum inhibition zone of (7 ± 1 mm) at 1 mg/ml water extract concentration. *Escherichia coli* had a maximum of (9 ± 0 mm at $10^{\wedge 0}$) and a minimum of (8 ± 0 mm at 0.1 mg/ml). *Candida albicans* had a maximum of (6.5 ± 0.7 mm at 1 mg/ml) and a minimum of (6 ± 0 mm at 0.00001mg/ml).

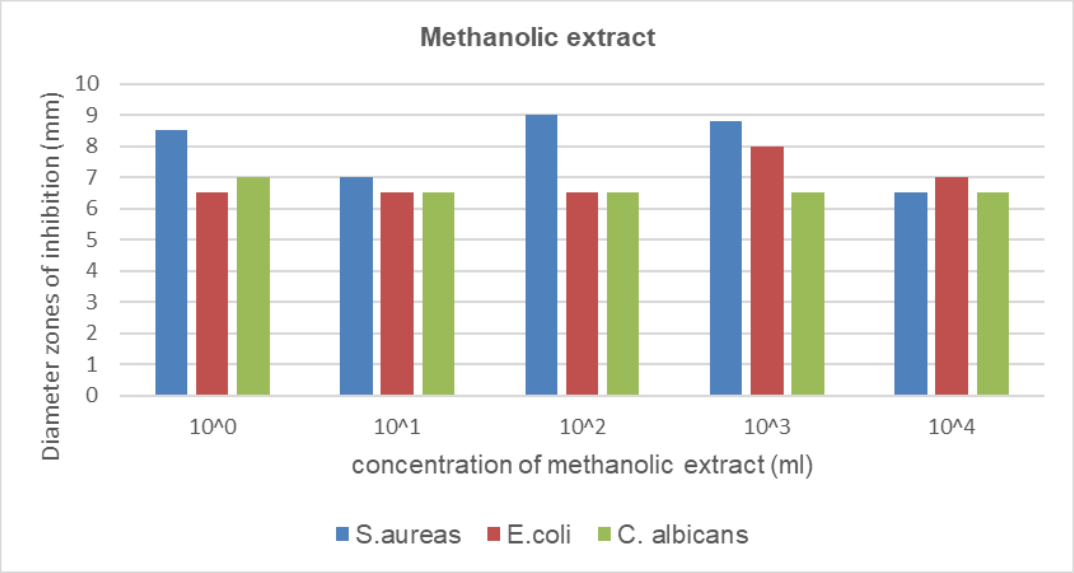

Fig. 1. Concentrations of methanolic extract against the zone of inhibitions in (mm)

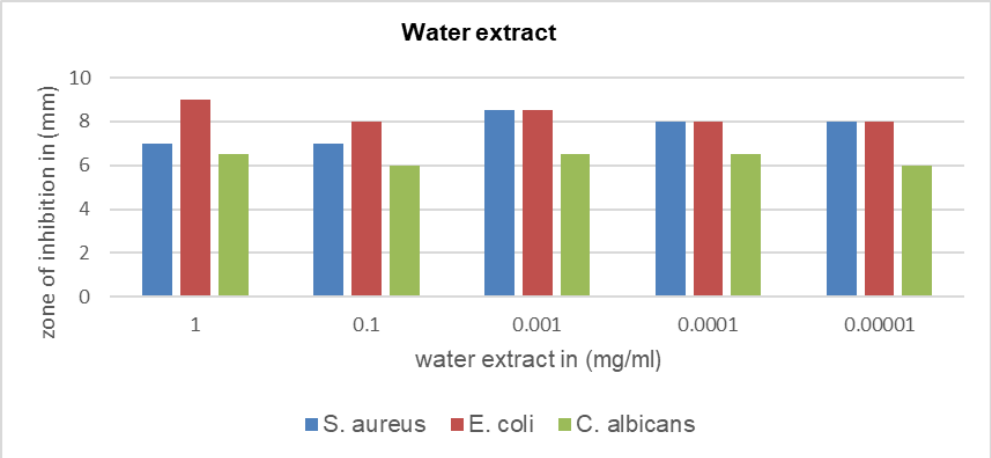

Fig. 2. concentration of water extract against zone of inhibitions (mm)

3.2 Discussion

The outcomes of the phytochemical analysis demonstrated the presence of secondary metabolites, specifically saponins, and flavonoids, while tannins and terpenoids were notably absent. The constituents identified in this investigation were consistent with those reported by (Manguro et al., 2005) [5]. Although flavonoids are typically synthesized by plants in response to microbial threats, it was unexpected that they did not exhibit efficacy against the tested microorganisms in vitro. Conversely, saponins have been acknowledged for their antifungal and antibacterial attributes (Mandal et al., 2005), which are thought to fortify plants against pathogens and support plant well-being [11].

For the extraction, methanol and water solvents were employed; however, both extracts showed limited activity against the test organisms. The water extract displayed more pronounced activity against *Escherichia coli*, with a maximum inhibition zone of (9 ± 0) mm at a concentration of 1mg/ml. This was followed by *Staphylococcus aureus* and *Candida albicans*, with inhibition zones of (8.5 ± 1.5) mm and (7 ± 0) mm, respectively, at the 1mg/ml concentration. Variations in solvent polarity could account for the differences in solubility and activity of the plant's active compounds.

Despite the observed zones of inhibition, the plant extracts did not meet the Clinical and Laboratory Standards Institute (CLSI) breakpoint guidelines for susceptibility or intermediate categories [12]; however, they did exhibit resistance breakpoints below 10mm. This suggests that the plant extracts lacked significant antimicrobial activity against the tested microorganisms at the assessed concentrations. Discrepancies between the observed inhibition zones and the CLSI breakpoints might arise from differences in microbial growth medium composition, incubation conditions, or antimicrobial agent application methods [13]. Consequently, the plant extract's ineffectiveness against the tested microorganisms was established.

The minimum inhibitory concentration results indicated that both methanolic and water extracts were ineffective in inhibiting microbial growth, as the microbes continued to proliferate even at the highest extract concentrations of (10^0) concentration. This was evidenced by visible microbial growth despite the various concentrations of plant extracts. Potential reasons for the lack of minimum inhibitory concentration efficacy include inherent microbial resistance mechanisms, such as efflux pumps or alterations in cell membranes. Additionally, experimental conditions might not have been optimal for detecting the antimicrobial potential of the agent, including pH, temperature, or nutrient conditions [14]. Previous research on antimicrobial efficacy of crude extracts has indicated diverse modes of actions. One major possible mechanism of action is damage to the bacterial cell membrane [15].

4. CONCLUSION

In conclusion, the phytochemical analysis conducted in this study unveiled the presence of noteworthy bioactive compounds within the leaf extract of *Embelia keniensis.* However, our investigation indicated that this extract did not

display substantial antimicrobial efficacy against the chosen microorganisms. Nevertheless, it is important to acknowledge the potential for different concentrations or combinations of the plant extract to harbor antimicrobial properties against these microorganisms. As such, further exploration is warranted to determine the optimal conditions for leveraging the plant extract as a potential antimicrobial agent.

The current findings emphasize the need for extended investigations to unravel the potential therapeutic applications of this extract in human ailments treatment. Additionally, future endeavors should focus on the isolation and meticulous characterization of the bioactive constituents present in the extracts, accompanied by a comprehensive understanding of their underlying mechanisms of action. By delving deeper into these aspects, we can unlock a more profound comprehension of *Embelia keniensis'* bioactive potential and its potential contributions to medicinal advancements. From the foregoing narrative, it is crucial to document ethnomedicinal plants before they are no longer available and go extinct [16]. This shall form a basis for their conservation and further research in diverse areas of plant sciences.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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