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Original

Antimicrobial Effects of Aqueous Bark Extracts of *Khaya senegalensis*, *Azadirachta indica* and *Euclyptus camaldulensis* against Different Bacterial Pathogens

Efectos antimicrobianos de los extractos acuosos de corteza de *Khaya senegalensis*, *Azadirachta indica* y *Eucalyptus camaldulensis* contra diferentes patógenos bacterianos

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ABSTRACT

Introduction: aqueous bark preparations of *Khaya senegalensis*, *Azadirachta indica*, and *Eucalyptus camaldulensis* are widely employed in traditional medicine for the management of microbial infections in both humans and animals. The present work aimed to investigate the antibacterial potential of these plant extracts against selected Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella dublin*).
Method: bark materials were extracted with water to obtain concentrations equivalent to 20 g, 40 g, and 50 g per 100 mL. Antibacterial activity was evaluated using the agar well diffusion technique at the Microbiology Laboratory, Faculty of Veterinary Medicine, University of Khartoum. Bacterial susceptibility was determined by measuring inhibition zone diameters around the wells.
Results: all tested aqueous extracts demonstrated inhibitory effects against the examined bacterial strains at every tested concentration. Antibacterial activity increased with rising concentration, with the maximum effect observed at 0,5 mg/mL. At this level, inhibition zones against *Staphylococcus aureus* measured 3,7 ± 0,52 cm for *Khaya senegalensis*, 3,0 ± 0,24 cm for *Azadirachta indica*, and 2,8 ± 0,57 cm for *Eucalyptus camaldulensis*.
Conclusion: the findings indicate that bark-derived aqueous extracts from the three studied species possess notable antibacterial activity against both Gram-positive and Gram-negative pathogens, particularly at higher concentrations. These results support their traditional use and suggest the need for further investigations involving other plant organs—such as leaves, fruits, and roots—tested across a wider range of concentrations.

Keywords: Antimicrobial; Exotic Species; Aqueous Extract; Bark.

RESUMEN

Introducción: los extractos acuosos de corteza de *Khaya senegalensis*, *Azadirachta indica* y *Eucalyptus*

camaldulensis se utilizan tradicionalmente para tratar infecciones microbianas en humanos y animales. Este estudio evaluó la actividad antimicrobiana de dichos extractos frente a bacterias Gram positivas (*Staphylococcus aureus*, *Staphylococcus epidermidis*) y Gram negativas (*Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella dublin*).
Método: se prepararon extractos acuosos de corteza en concentraciones de 20 g, 40 g y 50 g por 100 mL. La actividad antimicrobiana se determinó mediante el método de difusión en agar en el Laboratorio de Microbiología de la Facultad de Medicina Veterinaria, Universidad de Jartum. Se midieron los diámetros de los halos de inhibición para evaluar la sensibilidad bacteriana.
Resultados: todos los extractos mostraron actividad inhibitoria contra las cepas bacterianas evaluadas en todas las concentraciones. La mayor inhibición se obtuvo con la concentración más alta (0,5 mg/mL). A esta concentración, *Staphylococcus aureus* presentó halos de inhibición de 3,7 ± 0,52 cm, 3,0 ± 0,24 cm y 2,8 ± 0,57 cm tras el tratamiento con extractos de *Khaya senegalensis*, *Azadirachta indica* y *Eucalyptus camaldulensis*, respectivamente.
Conclusión: los extractos acuosos de corteza de las tres especies demostraron efectos antibacterianos prometedores contra patógenos Gram positivos y Gram negativos, con la mayor actividad a 0,5 mg/mL. Se recomienda realizar estudios adicionales para evaluar la eficacia antimicrobiana de otras partes de las plantas, como hojas, frutos y raíces, en diferentes concentraciones.

Palabras clave: Antimicrobiano; Especies Exóticas; Extracto Acuoso; Corteza.

INTRODUCTION

Medicinal plants have played a pivotal role in the discovery and development of therapeutic agents and continue to serve as a fundamental resource for pharmaceutical research worldwide.(1) Plant-based medicines are generally associated with a wide safety margin when compared with synthetic counterparts,(1) in addition to providing effective treatment options at relatively low cost. In recent years, medical research has shown a clear shift toward the utilization of natural products, particularly plant-derived remedies, as alternatives or complements to conventional chemotherapeutic drugs.(2)

The widespread and often uncontrolled use of antimicrobial agents has contributed markedly to the emergence of multidrug-resistant pathogenic microorganisms.(3) Antibiotic resistance has therefore become a global health challenge, prompting an urgent demand for novel, safe, and effective antimicrobial agents.(1) Infectious diseases remain among the most serious threats affecting both human and animal health worldwide.(4) A broad range of illnesses—including anthrax, diphtheria, rheumatic fever, pharyngitis, cholera, typhoid fever, shigellosis, salmonellosis, and whooping cough—are caused by Gram-positive and Gram-negative bacteria.(1)

Although synthetic antimicrobial drugs are generally effective against numerous pathogens, their clinical use is often limited by undesirable side effects.(1) Moreover, the remarkable genetic adaptability of many microorganisms enables them to rapidly develop resistance to available antimicrobial therapies,(4) reinforcing the need for continued research into new antimicrobial agents.(5) Salih and colleagues (2019) emphasized that microbial resistance necessitates the exploration of alternative sources—such as plants and algae—for the identification of new lead compounds.(4)

Traditional medicine relies heavily on plant-derived products for the treatment of various diseases.(6) It has been estimated that nearly 80 % of the global population depends on herbal remedies to meet primary healthcare needs, particularly in developing regions.(7,8) Plants are therefore recognized as important reservoirs of structurally diverse bioactive compounds that may serve as templates for new chemotherapeutic agents.(9) Many species are rich in secondary metabolites, including tannins, terpenoids, alkaloids, and flavonoids, which have demonstrated antimicrobial activity *in vitro*.(9)

An essential initial step in the discovery of plant-based antimicrobial agents is the evaluation of their effectiveness using *in vitro* bioassays.(9,10,11) Currently, the systematic exploration of natural resources for drug development has become a global research priority. Indeed, natural product-derived compounds account for more than 30 % of therapeutic agents presently used in clinical practice.(12) The rising incidence of resistance among human pathogens to commonly prescribed antibiotics has further reinforced interest in identifying antimicrobial compounds from alternative biological sources, particularly medicinal plants.(13)

In recognition of the importance of traditional medicine, the World Health Organization (WHO) initiated programs targeting diseases such as diarrhea, emphasizing the integration of traditional medical practices into disease prevention and management strategies.(14,15) Additionally, the cultural significance of plant species has gained increasing attention across medical, agricultural, pharmaceutical, and nutraceutical fields.(16) Ethnobotanical approaches are especially valuable in identifying regionally important medicinal plants that may provide sources of novel crude drugs.[17,18] Documentation of indigenous knowledge related to plant-based therapies has already contributed to the development of several modern medicines.[16,19]

In many African countries, including Sudan, traditional healers continue to rely extensively on medicinal plants for disease management.[20] Over recent decades, there has been growing scientific interest in documenting indigenous knowledge related to medicinal plant use.[20,21,22,23] Given Sudan's rich biodiversity and the cultural diversity of its ethnic groups, substantial traditional knowledge regarding medicinal plant species is expected.[16] Several forestry species have been reported to possess antimicrobial properties, including *Acacia nilotica*,[5] *Ziziphus* species,[4,24,25,26] as well as *Diospyros blancoi*, *Phoenix dactylifera*, and *Morus nigra*, which have shown inhibitory effects against pathogens associated with dental caries.[27]

In this context, the present study aimed to evaluate the antibacterial activity of aqueous bark extracts from *Khaya senegalensis* (Desr.) A. Juss., *Azadirachta indica* A. Juss. (family: Meliaceae), and *Eucalyptus camaldulensis* Dehnh. (family: Myrtaceae). These species were selected as representative exotic forestry trees commonly grown in Sudan, and their antimicrobial potential was assessed against selected bacterial pathogens of medical and veterinary importance.

METHOD

Collection and preparation of plants materials:

Bark samples from *Azadirachta indica* A. Juss. (locally "Neem"), *Khaya senegalensis* (Desr.) A. Juss. ("Mahogany"), and *Eucalyptus camaldulensis* Dehn. ("Khaor") were collected from mature trees growing in the Arboretum of the Faculty of Forestry, University of Khartoum, Sudan (15°39'23.48" N; 32°30'54.36" E). Samples were placed in clean envelopes, authenticated, washed thoroughly, and dried in sunlight for 7–10 days. The dried bark was then pulverized using a mortar and pestle in the Silviculture Laboratory, Faculty of Forestry, University of Khartoum.

Extraction process and stock solutions preparation

Aqueous extraction followed the method described by Gibreel and Salih (2019).[28] For each species, 50 g of air-dried bark powder were weighed and mixed with 100 mL of distilled water in a sterile 250 mL conical flask. The mixture was agitated on a rotary shaker at room temperature (37 °C) for 48 hours. The resulting crude extract was filtered twice through Whatman filter paper, and the filtrate was left to dry at 37 °C for 24 hours.

The dried yields were 40 mg for *Khaya senegalensis*, 38 mg for *Azadirachta indica*, and 35 mg for *Eucalyptus camaldulensis*. Extracts were stored in labeled glass bottles (50 mL) at room temperature. Working concentrations of 0,2, 0,4, and 0,5 mg/mL were prepared by dissolving 0,2 mg (200 µg), 0,4 mg (400 µg), and 0,5 mg (500 µg) of dried extract, respectively, in 100 mL of distilled water.

Isolation, culturing and identification of bacteria

Enriched media (Blood agar and MacConkey agar) and culture preparations were carried out in the Microbiology Laboratory, Faculty of Veterinary Medicine, University of Khartoum.[28,29] Swabs collected from clinically infected animals were incubated for 24–48 hours at 37 °C. Colony smears were stained using Gram's Method to distinguish Gram-positive from Gram-negative organisms.

Pure colonies were sub-cultured on nutrient agar, and standard biochemical tests were performed to confirm genus and species identification following established procedures.[4,30]

Anti-bacterial sensitivity test

The antibacterial activity of the bark extracts was evaluated using the agar well diffusion technique on Mueller-Hinton agar plates. Five bacterial isolates— *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella dublin*— were tested.

The experiment followed a completely randomized design with three replicates. A 0,1 mL aliquot of each bacterial broth culture was uniformly spread on separate Mueller-Hinton agar plates. Under aseptic conditions, three wells (8 mm diameter) were punched using sterile blue plastic tubes. Each well was filled with 20 µL of the prepared aqueous bark extracts (0,2, 0,4, or 0,5 mg/mL for each species).

Plates were kept at 4 °C for two hours to allow diffusion of the extract into the agar, then incubated upright at 37 °C for 24–48 hours. After incubation, inhibition zones were measured in centimeters using a ruler under appropriate lighting. Plates were considered valid only if the positive control produced a clear inhibition zone. Diameters greater than 12 mm were interpreted as sensitive responses.

Statistical analysis

Data were analyzed using SAS statistical software (Version 9). Descriptive statistics were used to calculate the means and standard errors. One-way ANOVA was applied to compare inhibition zone diameters among the three extract concentrations. Duncan's multiple range test was used to separate means, and differences were considered significant at p < 0,05.

RESULTS

The findings of the study demonstrated that aqueous bark extracts of *Khaya senegalensis* (KS), *Azadirachta indica* (AZ), and *Eucalyptus camaldulensis* (EC)— prepared at concentrations of 0,2, 0,4, and 0,5 mg/mL— were capable of inhibiting the growth of all tested bacterial isolates (table 1; figures 1 and 2). The extracts produced inhibition zones of varying diameters against both Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative (*Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella dublin*) bacteria.

Overall, higher extract concentrations resulted in stronger antibacterial effects. The most pronounced inhibition occurred at 0,5 mg/mL. At this concentration, *Staphylococcus aureus* exhibited inhibition zones of 3,7 ± 0,52 cm, 3,0 ± 0,24 cm, and 2,8 ± 0,57 cm following treatment with extracts of *K. senegalensis*, *A. indica*, and *E. camaldulensis*, respectively.

In contrast, the smallest inhibition zones were recorded at the lowest concentration (0,2 mg/mL). *Eucalyptus camaldulensis* produced inhibition diameters of only 1,0 ± 0,33 cm against *K. pneumoniae* and 1,1 ± 0,33 cm against *S. dublin*, representing the lowest antibacterial activity observed in the study.

Analysis of variance revealed that extract concentration had a statistically significant effect (p = 0,0001) on inhibition zone diameter across all three species and all tested bacteria (table 2). Differences in antibacterial activity among the three plant species were also significant at each concentration level.

Table 1. Showing types of bacterial strains, forestry species and aqueous bark extracts concentration (mg/mL), and the diameter of inhibitory zone (cm)

Species	Aqueous bark extract Concentration (mg/mL)	Bacterial Pathogens and inhibitory zone (cm)				
		Gram-positive bacteria		Gram-negative bacteria		
		S. aureus	*S. epidermidis*	*K. pneumonia*	*E. coli*	*S. dublin*
K. senegalensis "KS"	0,2	2,1±0,24	1,6±0,44	1,7±0,24	1,6±0,24	1,9±0,24
	0,4	2,9±0,53	1,8±0,24	1,9±0,54	1,9±0,54	2,5±0,54
	0,5	3,7±0,52	2,2±0,32	2,5±0,22	2,3±0,22	3,5±0,22
A. indica "AZ"	0,2	1,9±0,49	1,4±0,44	1,5±0,47	1,5±0,34	1,2±0,44
	0,4	2,6±0,28	1,7±0,48	1,8±0,55	1,7±0,44	2,4±0,25
	0,5	3,0±0,24	2,2±0,34	2,0±0,44	2,1±0,24	2,9±0,64
E. camaldulensis "EC"	0,2	2,1±0,39	1,3±0,42	1,0±0,33	1,3±0,22	1,1±0,33
	0,4	2,5±0,84	1,5±0,56	1,2±0,64	1,4±0,54	1,5±0,54
	0,5	2,8±0,57	1,8±0,67	1,4±0,53	1,9±0,27	2,4±0,52

Table 2. One-Way ANOVA for disc diffusion results for comparison between the three concentrations of aqueous plant's bark extracts (0,2, 0,4 and 0,5 mg/mL) among the three exotic forestry species.

Aqueous bark extracts concentration (mg/mL)	Species	Bacterial Pathogens and inhibitory zone (cm)				
		Gram-positive bacteria		Gram-negative bacteria		
		S. aureus	*S. epidermidis*	*K. pneumonia*	*E. coli*	*S. dublin*
0,2	KS	2,1±0,24a	1,6±0,44a	1,7±0,24a	1,6±0,24a	1,9±0,24a
	AZ	1,9±0,49b	1,4±0,44a	1,5±0,47a	1,5±0,34a	1,2±0,44b
	EC	2,1±0,39a	1,3±0,42a	1,0±0,33b	1,3±0,22b	1,1±0,33b
0,4	KS	2,6±0,28a	1,7±0,48a	1,8±0,55a	1,7±0,44a	2,4±0,25a
	AZ	2,9±0,53a	1,8±0,24a	1,9±0,54a	1,9±0,54a	2,5±0,54a
	EC	2,5±0,84a	1,5±0,56a	1,2±0,64b	1,4±0,54b	1,5±0,54b
0,5	KS	3,7±0,52a	2,2±0,32a	2,5±0,22a	2,3±0,22a	3,5±0,22a
	AZ	3,0±0,24b	2,2±0,34a	2,0±0,44a	2,1±0,24a	2,9±0,64b
	EC	2,8±0,57c	1,8±0,67b	1,4±0,53b	1,9±0,27b	2,4±0,52c

Note: Means (± standard diviation) with the same letter (a, b or c) along the column are not significantly different at 0,5 level of significant; KS: *Khaya senegalensis*; AZ: *Azadirachta indica*; EC: *Eucalyptus camaldulensis*.

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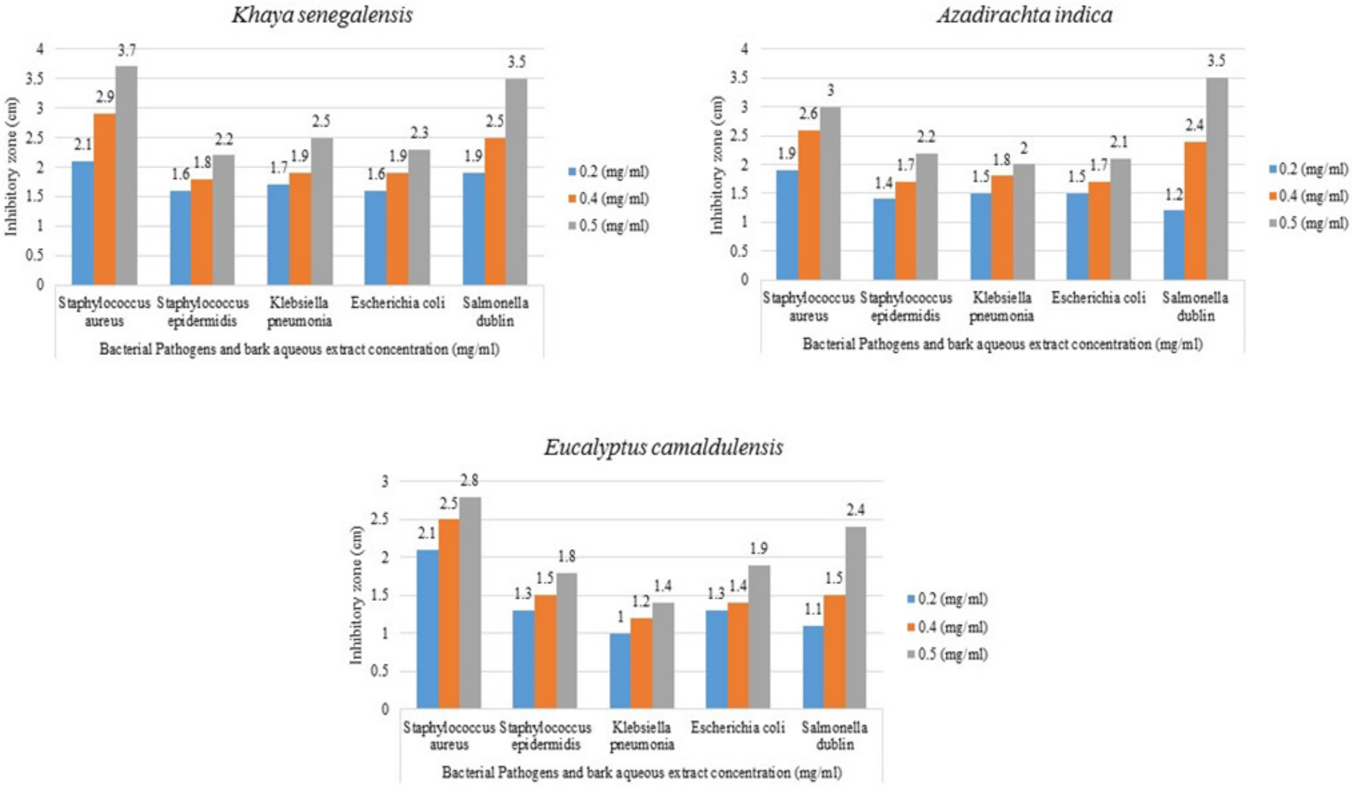

Figure 1. Sensitivity effect of bark aqueous extract (0,2, 0,4 and 0,5 mg/mL) of the three forestry tree species against different bacterial pathogens

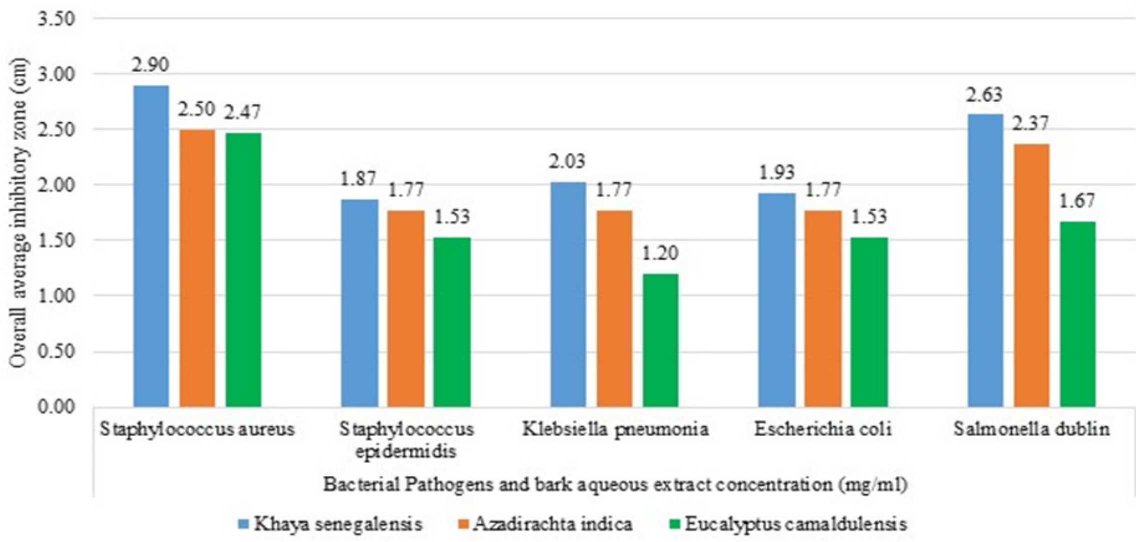

Figure 2. Variation in inhibitory zone (cm) obtained as affected by average concentration of bark aqueous extract (0,2, 0,4 and 0,5 mg/mL) of three forestry tree species against different bacterial pathogens

DISCUSSION

The antimicrobial activity observed in this study is consistent with the well-established role of plant secondary metabolites— such as tannins, flavonoids, alkaloids, terpenoids, saponins, and related phytochemicals— in suppressing microbial growth.[31,32] These compounds are known to possess diverse biological properties, many of which have been harnessed in the development of therapeutic agents.[6] Numerous plant organs have been documented as rich sources of such metabolites, including bark, leaves, roots, and fruits.[33]

Previous research on *Ziziphus* species highlighted the presence of alkaloids, tannins, saponins, flavonoids, glycosides, and terpenoids as contributors to antibacterial activity.[4] In line with this, the current work assessed

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the ability of aqueous bark extracts from three forestry species— *Khaya senegalensis* (KS), *Azadirachta indica* (AZ), and *Eucalyptus camaldulensis* (EC)— to inhibit pathogenic bacteria using an *in vitro* agar well diffusion method. The results confirmed that all extracts demonstrated inhibitory action, with the magnitude of the response increasing proportionally with extract concentration. The most substantial zones of inhibition were recorded at 0,5 mg/mL, particularly against *Staphylococcus aureus*, with diameters of 3,7 ± 0,52 cm for *K. senegalensis*, 3,0 ± 0,24 cm for *A. indica*, and 2,8 ± 0,57 cm for *E. camaldulensis*.

The strong antibacterial effect of *A. indica* may be attributed to its diverse phytochemical profile, which includes substances with antiviral, antibacterial, antimalarial, anti-ulcer, and contraceptive activities.[34,35] Its bark contains tannin-like compounds that are known to exert bacteriostatic or bactericidal properties.[36] Similarly, the antimicrobial potential of *Eucalyptus camaldulensis* has been demonstrated in earlier studies, supporting the results obtained here.[37]

The activity of *Khaya senegalensis* observed in this research aligns with findings reported by Dougnon et al.[15], who documented its effectiveness against certain Gram-negative bacteria.[15] Taken together, the present findings reinforce the growing body of evidence supporting the antibacterial potential of these forestry species and highlight their value as promising natural sources for future antimicrobial agent development.

Although the present findings provide valuable evidence of the antibacterial potential of the tested bark aqueous extracts, the study has several limitations that should be addressed in future work. The current investigation focused solely on measuring inhibition zones using the agar diffusion method, which does not reveal the specific phytochemical constituents responsible for the observed activity. Future studies should therefore include qualitative phytochemical screening of the most active aqueous extracts to obviously identify the groups of compounds contributing to the antimicrobial effects. In addition, determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) will be essential to better quantify the potency of these extracts and to establish more precise comparisons among the studied species. Incorporating these approaches will strengthen the scientific basis of our findings and provide deeper insights into the therapeutic potential of these forestry species which are traditionally used in treatment of various bacterial infections.

CONCLUSION

This study provides an initial assessment of the antibacterial properties of aqueous bark extracts from three forestry species tested at different concentrations against five clinically relevant bacterial pathogens affecting humans and animals. The extracts demonstrated clear inhibitory effects, with antimicrobial activity increasing progressively at higher concentrations. These findings support the traditional use of these species as natural remedies and indicate their potential as sources of biologically active compounds. Further investigations are recommended to evaluate extracts from additional plant parts—such as leaves, fruits, and roots—and to explore a broader range of concentrations for both Gram-positive and Gram-negative bacteria.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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