FEDERAL UNIVERSITY WUKARI INTERNATIONAL JOURNAL OF BIOSCIENCES (FUWIJOB)
pISSN: 3121-9799, eISSN: 3121-973x; Volume 2, No. 2, June, 2026
https://fuwijob.com.ng

Evaluation of the antimicrobial activities of dried root and stem extracts of *Senna siamea* against selected clinical isolates

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ABSTRACT
The global burden of infectious diseases caused by bacteria, fungi, and other microorganisms remains one of the most critical challenges to public health, particularly in low- and middle-income countries. This study aimed to investigate the antimicrobial activities of dry stem and root extract of Senna siamea against selected clinical isolates. The Senna siamea stem bark and roost were cut into pieces, then shade-dried, ground into a fine powder and pulverized. Fifty grams (50g) of the steam bark and roots each was mixed with 500ml of ethanol and distilled water. The mixture was left for 72 hours, with occasional stirring. The solution was filtered using Muslin cloth and the filtrates were concentrated. The extracts were dissolved in Dimethyl Sulfoxide (DMSO) which was used to determine the antimicrobial activity against the isolates. Both the extract were subsequently evaluated by agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. The results demonstrated that ethanolic extracts, particularly those derived from the root, exhibited significantly higher antimicrobial efficacy than aqueous extracts, with inhibition zones reaching up to 25 mm at 1 mg/ml concentration and lower MICs, such as 12 mm and 11 mm at 0.5 mg/ml and 0.25 mg/ml values, indicating a dose-dependent response. Conversely, the stem extracts showed moderate activity, especially against Staphylococcus aureus. The study supports the potential of Senna siamea as a source of novel antimicrobial agents and underscores the need for further phytochemical profiling and in vivo evaluations to fully validate its therapeutic applications.

Keywords: Aqueous, Ethanolic, Extract, Root, *Senna siamea,* Stem

INTRODUCTION
The global impact of infectious diseases remains a major public health issue, especially in low- and middle-income countries. Despite medical advances, millions die yearly from infections, many of which are caused by drug-resistant pathogens (Reyes *et al.*, 2021). The World Health Organization warns that antimicrobial resistance (AMR) could undo decades of medical progress, making even minor infections deadly again. Key multidrug-resistant bacteria like *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* are major contributors to AMR-related illness and death (Reyes *et al.*, 2021).
The fast-rising antimicrobial resistance has created an urgent demand for new, effective antimicrobial drugs (Fair and Tor, 2019). However, the development of new antibiotics and antifungal resistance of bacteria and fungi to existing treatments (Fair and Tor,

2019). The widespread misuse and overuse of antibiotics in medicine, farming, and livestock production have sped up the emergence of resistant microbes, making infections that were once easily treatable much harder to cure (Gonzalez-Escalona *et al*., 2020).

Scientists are turning back to natural sources especially plants as promising alternatives for new antimicrobial agents due synthetic drug development is slow. Historically used in traditional medicine to treat infections, medicinal plants are now being scientifically studied to confirm their effectiveness and discover new antimicrobial (Efferth *et al*., 2021). Plants produce several bioactive compounds like alkaloids, flavonoids, tannins, and saponins that have demonstrated strong antimicrobial activity in numerous studies (Silva *et al*., 2020a).

Senna siamea (Fabaceae) is a tropical medicinal tree widely used in Africa and Asia to treat ailments like malaria, digestive problems, and skin infections (Wabo *et al.,* 2021). It contains bioactive compounds such as anthraquinones, flavonoids, and glycosides that likely explain its healing effects (Bongomin *et al*., 2020). However, there is still limited scientific research on the antimicrobial potential of its dry stem and root extracts, especially against multidrug-resistant (MDR) pathogens.

Antimicrobial resistance (AMR) is now a major global health threat, as many bacteria and fungi have developed resistance to common drugs (c*et al*., 2020). The World Health Organization warns that this trend could reverse decades of medical progress, leading to more multidrug-resistant (MDR) infections, higher death rates, longer hospitalizations, and greater healthcare costs (Spellberg *et al*., 2020).

Although antimicrobial resistance is widely acknowledged as a global threat, the discovery of new antibiotics has slowed significantly. Many pharmaceutical companies have withdrawn from antibiotic research because of high costs and strict regulations, creating a serious shortage of effective drugs to combat emerging resistant infections. Traditional medicine has long helped manage infections, especially where modern healthcare is limited. *Senna siamea* is one such medicinal plant traditionally used to treat various diseases, but its antimicrobial potential particularly against multidrug-resistant (MDR) pathogens has not been fully validated. Given the rising AMR crisis, exploring and confirming natural plant-based antimicrobials has become increasingly important. This study tackles the lack of scientific evidence on the antimicrobial effects of *Senna siamea*'s dry stem and root extracts against clinical and multidrug-resistant (MDR) pathogens.

As bacteria and fungi evolve into multidrug-resistant (MDR) strains, treatments become less effective leading to higher death rates, longer hospitalizations, and rising healthcare costs. This growing crisis highlights the urgent need for alternative antimicrobial solutions. Pharmaceutical companies have greatly reduced investment in antimicrobial research, because of financial and regulatory challenges. (Fair and Tor, 2019). This has led to a shortage of new antibiotics and antifungal drugs, leaving healthcare systems increasingly exposed to resistant pathogens.

In many developing regions, traditional medicine remains essential, with plants like *Senna siamea* widely used to treat infections. While it's antimicrobial potential is

supported by traditional use, scientific evidence especially against multidrug-resistant (MDR) pathogensis still limited. Given the global AMR crisis and shortage of new drugs, researching and validating *Senna siamea*'s bioactive compounds could help integrate traditional medicine with modern healthcare, providing affordable and accessible treatments. This study aims to evaluate the antimicrobial effects of dry stem and root extracts of *Senna siamea* on selected clinical isolates microbes taken from patients that are often drug-resistant. The goal is to scientifically confirm the plant's traditional use in treating infections and possibly discover new antimicrobial agents effective against resistant pathogens.

MATERIALS AND METHODS

Study design

The study employed a laboratory-based experimental design aimed at evaluating the antimicrobial activities of *Senna siamea* extracts derived from its dry stems and roots. This design was structured to systematically investigate the efficacy of the extracts against selected clinical isolates, which included various bacterial and fungal strains.

Plant material collection and identification for *Senna siamea*

The stems bark and roots of *Senna siamea* was obtained from Marmara area of Wukari metropolis, Taraba State, Nigeria. After careful collecting of mature stems and roots, the materials were cleaned thoroughly to remove contaminants (Efferth *et al*., 2021; Adebayo *et al*., 2023). Identification of the plant was based on morphological characteristics, confirmed and authenticated by a Botanist from the Department of Biological Sciences Federal University Wukari. Comprehensive documentation of the collection process was maintained, including details about the location and identification of the specimens, which aids in ensuring reproducibility and reliability in research (Bongomin *et al*., 2020; Gonzalez-Escalona *et al*., 2020).

Preparation of extraction

The *Senna siamea* stem bark and roost were cut into pieces, then shade-dried (Adebayo *et al*., 2023) at room temperature for one week to ensure that the sample lost most of their moisture content (Brown *et al*., 2024a) to preserve phytochemicals before being ground into a fine powder, enhancing the efficiency of extraction methods (Efferth *et al*., 2021). Therefore, the samples were blended into fine powder using electric blender or pulverized with a sterilized mortar and pestle. The blended pastes were packed into an air tight container and kept in a cool dry place.

Cold maceration

Cold maceration involves soaking the dried plant material in a solvent at room temperature for a specified period. This method allows for the gradual extraction of polar compounds, which include flavonoids, tannins, and phenolic acids, known for their antimicrobial activity (Silva *et al*., 2020b; Adnan *et al*., 2022). Fifty grams (50g) dried powder of *Senna siamea* of the steam bark and roots each was mixed with 500ml of ethanol and distilled water in a clean containers. The mixture was left undisturbed for 72 hours, with occasional stirring, to enhance the extraction process. After this period, the solution was filtered using Muslin cloth to remove solid residues, and the filtrates were concentrated using a rotary evaporator.

Preparation of extract concentrations
The extracts were dissolved in Dimethyl Sulfoxide (DMSO) which was used to determine the antimicrobial activity against the isolates. The stock solution of extracts was prepared by weighing different amounts of extract and dissolving them in 10 ml of DMSO to create a 100% concentration, followed by consecutive dilutions to obtain various concentrations (Brown *et al*., 2024a; 2024b).

Agar well diffusion method
Some clinical isolates of bacteria and fungi were cultured on nutrient agar and incubated at 37°C for 24 hours. After incubation, a standardized inoculum was prepared, ensuring a uniform concentration of approximately 1.0×10^6 CFU/mL. Mueller-Hinton agar plates were inoculated with the standardized microbial inoculum using a sterile swab, ensuring even distribution across the surface. Wells were created in the agar plates using a sterile cork-borer. Different concentrations (100%, 50%, and 25%) of *Senna siamea* extracts were added to the wells, and a control antibiotic (Ciploflaxacin) was placed in the center. The plates were incubated at 37°C for 24 hours for bacterial isolates and at 25°C for 48 hours for fungal isolates. After incubation, the zones of inhibition around the wells were measured in millimeters using a ruler. The wells were visually inspected for bacterial growth.

Determination of minimum inhibitory concentration (MIC)
The Minimum Inhibitory Concentration (MIC) was determined using the method of Brown *et al.* (2025). Two-fold serial dilutions of each of the root and stem extracts were prepared, and 2 ml of each concentration was added to 18 ml of pre-sterilized molten nutrient agar at 40°C to achieve final concentrations of 25, 50, and 100 mg/ml, respectively. The mixtures were poured into sterile petri dishes and allowed to solidify. After the surface dried, 24-hour-old bacterial isolates were streaked onto the medium. Ciprofloxacin was used as a positive control. The plates were incubated at 37°C for 24 hours, after which they were checked for bacterial growth. The MIC was defined as the least concentration of extract that inhibited visible growth.

Determination of minimum bactericidal concentration (MBC)
After the MIC test, wells that showed no visible bacterial growth (clear wells) are selected for MBC determination. This ensured that only concentrations that exhibited complete inhibition (bacteriostatic effect) were tested for their bactericidal properties. A standard antibiotic (ciprofloxacin) was used as a reference. A ten microliter (10 µL) aliquot from each clear well was taken using a micropipette and was streaked onto fresh Mueller-Hinton Agar (MHA) plates, ensuring an even spread of bacteria. The plates were incubated at 37°C for 24 hours under aerobic conditions. After incubation, plates were examined for the presence or absence of bacterial colonies. The MBC is defined as the least concentration of the extract at which no bacterial colonies appeared on the agar surface. If colonies were present, it indicated that some bacteria survived at that concentration, and a higher concentration is required for bactericidal activity.

Data collection
Data were collected from both the agar well diffusion method and the broth microdilution methods. The following metrics were recorded. Zones of Inhibition were

measured in millimeters for each concentration of extract tested in the agar well diffusion assay. Minimum Inhibitory Concentration (MIC) for each isolate was determined using the broth microdilution method.

Statistical Analyses
Descriptive statistics, basic descriptive statistics, including mean, standard deviation, and range, were calculated for the zones of inhibition and MIC values. These provided an overview of the antimicrobial efficacy across different concentrations of extracts (Bongomin *et al*., 2020).

RESULTS
Candida albicans root ethanolic extract (Table 1), produced a zone of inhibition of about 25 mm at 100% and 50% concentrations, with a reduction at 25% (24 mm). *Staphylococcus aureus,* zones were 20 mm at full strength, decreasing to 16 mm and 15 mm at lower concentrations, compared to a control zone of 25 mm. *Escherichia coli,* no inhibition was observed with the extract, while the control showed a 35 mm zone.

Candida albicans stem ethanolic extract (Table 2) showed no inhibition at any concentration, but the control produced a 26 mm zone. *Staphylococcus aureus* zones were slightly larger than the control (22 mm) at higher doses, measuring 24 mm at both 100% and 50% concentrations and 15 mm at 25%. *Escherichia coli*: A modest inhibition of 13 mm was observed across all concentrations, though the control produced a 35 mm zone.

The root aqueous extract (Table 3) of *Candida albicans* showed no inhibition, but the control showed a 20 mm zone of inhibition. *Staphylococcus aureus,* only zones (11 mm at 100% and 10 mm at 50%) were observed; no inhibition at 25% concentration, versus a 15 mm control zone. The extract did not show any inhibition of Escherichia coli, whereas the control showed a 25 mm zone.

The stem aqueous extract (Table 4) of *Candida albicans*, and *Staphylococcus aureus* showed no inhibition at all of the tested concentrations whereas controls displayed 20 mm and 22 mm, respectively. In comparison to a 32 mm zone of inhibition with the control on *Escherichia coli*, some activity was observed with a 14 mm zone at 100%, 5 mm zone at 50%, and no inhibition at 25%. Compared to aqueous extracts, ethanolic extracts, particularly those derived from the root, typically exhibit stronger and more extensive antibacterial properties. The zones of inhibition typically show a dose-dependent effect as they decrease with dilution (from 100% to 25%).

Table 5 displayed the result of minimum inhibitory concentration of aqueous leaf extract of *Senna siamea* against the test organisms. The MIC results demonstrated that the antimicrobial activity of *Senna siamea* varied depending on the plant part, solvent used for extraction, and extract concentration. The ethanolic root extract exhibited the highest antimicrobial activity, particularly at 1 g concentration, where inhibition was observed against *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*. In contrast, the aqueous extracts showed weak inhibitory effects, suggesting that ethanol was more effective in extracting bioactive compounds from the plant. The root extract

also showed stronger activity than the stem extract, indicating a higher concentration of antimicrobial phytochemicals in the root. Overall, the results suggest that *Senna siamea*, particularly the ethanolic root extract, possesses promising antimicrobial properties that may justify its traditional medicinal use.

The MBC results (Table 6) revealed that *Senna siamea* extracts possess varying bactericidal activities depending on the plant part, extraction solvent, and concentration used. The ethanolic extracts generally showed stronger bactericidal effects than the aqueous extracts, indicating that ethanol was more effective in extracting antimicrobial phytochemicals from the plant material. The stem ethanolic extract demonstrated the highest bactericidal activity, killing *Staphylococcus aureus* at 0.5 g and both *Candida albicans* and *Escherichia coli* at 1 g concentration. In contrast, aqueous extracts showed limited bactericidal activity, with killing observed mainly against *Escherichia coli* at higher concentration. These findings suggest that *Senna siamea* contains bioactive compounds with potential antimicrobial properties and may serve as a valuable source of natural therapeutic agents.

Table 1: Antimicrobial activities of ethanolic extract of *Senna siamea* root showing the zone of inhibition

Organisms	**Concentration (mg/mL)**			**Control**
	100%	**50%**	**25%**	
Candida albicans	25mm	25mm	24mm	-
Staphylococcus aureus	20mm	16mm	15mm	25mm
Escherichia coli	**-**	**-**	**-**	35mm

Key: mm = millimeter

- **-** = No zone of inhibition

Mg/mL = milligram/millilitre

Table 2: Antimicrobial activities of ethanolic extract of *Senna siamea* stem showing the zone of inhibition

Organisms	Concentrations (mg/mL)			Control
	100%	**50%**	**25%**	
Candida albicans	-	-	-	26mm
Staphylococcus aureus	24mm	24mm	15mm	22mm
Escherichia coli	13mm	13mm	13mm	35mm

Key: mm = millimeter

- **-** = No zone of inhibition
- mg/mL = milligram/millilitre

Table 3: Antimicrobial activities of aqueous extract of *Senna siamea* root showing the zone of inhibition

Organisms	Concentrations (mg/mL)			Control
	100%	**50%**	**25%**	
Candida albicans	**-**	**-**	**-**	20mm
Staphylococcus aureus	11mm	10mm	-	15mm
Escherichia coli	-	-	-	25mm

Key: mm = millimeter

- **-** = No zone of inhibition
- mg/mL = milligram/millilitre

Table 4: Antimicrobial activities of aqueous extract of *Senna siamea* stem showing the zone of inhibition

Organisms	Concentration (mg/mL)			Control
	100%	50%	25%	
Candida albicans	-	-	-	20mm
Staphylococcus aureus	-	-	-	22mm
Escherichia coli	14mm	5mm	-	32mm

Key: mm = millimeter

- = No zone of inhibition

Mg/mL = milligram/millilitre

Table 5: Minimium inhibitory concentration (MIC) of *Senna siamea* ethanolic and aqueous root and stem extracts

Extract	Concentration in gram (mg/mL)	Organisms *Candida albicans*	*Staphylococcus aureus*	*Escherichia coli*
Root extract				
Ethanol	0.25g	-	-	-
	0.5g	-	+	-
	1g	+	++	+
Aqueous	0.25g	-	-	-
	0.5g	-	-	-
	1g	-	-	+
Stem extract				

	0.25g	-	-	-
Ethanol	0.5g	-	-	-
	1g	+	-	-
	0.25g	-	-	-
Aqueous	0.5	-	-	-
	1g	-	-	+

Key: ++ = sensitive

+ = intermediate inhibition

- = No zone of inhibition

Table 6: Minimum Bacteriocidal Concentration (MBC) of *Senna siamea* ethanolic and aqueous root and stem extracts

Organisms	**Concentration in gram (mg/mL)**	**Solutions Ethanol**	**Aqueous**
Root extract			
Candida albicans	0.25g 0.5g 1g	- - +	— — —
Staphylococcus aureus	0.25g 0.5g 1g	- - -	— — -
Escherichia coli	0.25g 0.5g 1g	- - -	- - +
Stem extract			
Candida albicans	0.25g 0.5 1g	- - +	- - -
Staphylococcus aureus	0.25g 0.5g 1g	- + -	- - -

Escherichia coli	0.25g	-	-
	0.5g	-	-
	1g	+	+

Key: - = Non bacteriocidal,

+ = Bacteriocidal

DISCUSSION

The present study investigated the antimicrobial activity of ethanolic and aqueous extracts of *Senna siamea* (root and stem) against *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*. The results revealed that ethanolic extracts produced larger zones of inhibition compared to the aqueous extracts, indicating a higher extraction efficiency of bioactive compounds when ethanol was used as the solvent. This observation suggests that ethanol was more effective in extracting phytochemicals responsible for antimicrobial activity.

The data indicate a dose-dependent antimicrobial effect. As the concentration of the extract was reduced from 100% to 25%, the zones of inhibition generally decreased. This trend was consistent across both the ethanolic and aqueous extracts and across the different microorganisms tested (Chaudhary *et al.*, 2020).

The MIC and MBC results further substantiated the disc diffusion findings. For the ethanolic root extract, microbial growth was inhibited at 1 g for *Candida albicans* and *E. coli*, while *Staphylococcus aureus* showed weak inhibition at 0.5 g with a more pronounced effect at 1 g. This finding agreed with previous research on leaf extract of fresh *Senna siamea* by Brown *et al.* (2025) which the MBC for both ethanol and aqueous extracts was determined to be 1g/ml for both *Shigella* spp. and *Pseudomonas aeruginosa*, indicating that a concentration of 1g/ml is required to kill these bacteria. In contrast, the aqueous extracts required higher concentrations to achieve similar inhibitory effects, if any. Moreover, bactericidal activity was observed at lower concentrations in some cases most notably with the stem ethanolic extract against *Staphylococcus aureus* (0.5 g), suggesting that not only the extraction solvent but also the specific plant part plays a critical role in antimicrobial potency. The MIC value also aligns with the findings of Brown *et al.* (2024b) and Brown *et al.* (2025), who reported that MIC values for medicinal plant extracts vary based on the plant species, extraction method, and test organism.

The enhanced activity of ethanolic extracts over aqueous extracts observed in this study aligns with findings from previous research. For example, Okon *et al.* (2017) reported that ethanolic extracts of *Senna siamea* exhibited significantly higher antimicrobial activity compared to aqueous extracts, attributing this to the better solubility of phenolic compounds and other bioactive constituents in ethanol. Similarly, Adetunji *et al.* (2018) observed that ethanolic extracts of medicinal plants from Nigeria had notable antimicrobial properties, particularly against Gram-positive bacteria like *Staphylococcus aureus*.

Furthermore, the dose-dependent reduction in the zone of inhibition with decreasing extract concentration is consistent with the work of Chaudhary *et al.* (2020), who demonstrated that lower concentrations of plant extracts produce diminished antimicrobial effects. The observation that *E. coli* was less susceptible to the extracts, especially the aqueous type, is also in agreement with previous studies which have highlighted the inherent resistance of Gram-negative bacteria due to their outer membrane barrier (Nwosu *et al.*, 2018; Olawale *et al.*, 2019).

The results highlight the potential of *Senna siamea* as a source of antimicrobial agents, particularly when using ethanol as the extraction solvent. This finding aligns with studies of Brown *et al.* (2025), which reported that ethanol is a better solvent for extracting bioactive compounds. Given the increasing incidence of antibiotic resistance, these findings support further research and investigation into plant-based antimicrobials as complementary or alternative therapeutic agents.

The findings of this study are consistent with the work of Oyebade *et al.* (2021) who reported that ethanolic extracts of *Senna siamea* leaves exhibited stronger antibacterial activity against several pathogenic bacteria compared with aqueous extracts. According to the authors, the higher antimicrobial activity of ethanolic extracts may be attributed to the ability of ethanol to dissolve a wider range of phytochemical compounds such as alkaloids, flavonoids, tannins, and phenolic compounds, which possess antimicrobial properties.

Similarly, Jimoh *et al.* (2019) reported that organic solvent extracts of medicinal plants generally demonstrate higher antimicrobial effectiveness than aqueous extracts. Their study showed that ethanol extracts of *Senna* species inhibited the growth of pathogens such as *Staphylococcus aureus* and *Escherichia coli* more effectively than water extracts. This observation supports the results of the present study, where ethanolic extracts exhibited greater inhibitory activity against the tested organisms.

In another related study, Abera *et al.* (2024) reported that extracts of *Senna siamea* possessed significant antibacterial activity against Gram-positive and Gram-negative bacteria. The authors suggested that the antimicrobial effects of the plant could be linked to the presence of secondary metabolites such as flavonoids, terpenoids, saponins, and tannins, which are known to disrupt microbial cell membranes and interfere with essential metabolic processes.

Furthermore, Kabeer *et al.* (2022) also demonstrated that extracts of *Senna siamea* showed inhibitory activity against pathogenic bacteria, confirming the plant's potential as a source of natural antimicrobial agents. Their findings indicated that the antimicrobial effectiveness increased with higher extract concentrations, which is also similar to the trend observed in the present study.

However, some variations exist between the present findings and those reported by other researchers. For example, Abera *et al.* (2024) have reported greater susceptibility of Gram-negative bacteria such as *Escherichia coli* to *Senna siamea* extracts, whereas in the present study the susceptibility of microorganisms varied depending on the type of extract, plant part, and concentration used. These differences may be attributed to variations in experimental methods, extraction techniques, plant part utilized,

environmental conditions affecting plant phytochemistry, and microbial strain differences.

Generally, the findings of the present study agree with previous research indicating that *Senna siamea* possesses significant antimicrobial properties, particularly when extracted with organic solvents such as ethanol. The higher antimicrobial activity observed in ethanolic extracts further emphasizes the importance of solvent selection in phytochemical extraction and antimicrobial evaluation.

CONCLUSION

The experimental analysis demonstrated that the antimicrobial efficacy of *Senna siamea* extracts is both extract and concentration dependent. The ethanolic extracts, particularly from the root, exhibited stronger antimicrobial activity compared to the aqueous extracts. Specifically, the root ethanolic extract produced a zone of inhibition of approximately 25 mm against *Candida albicans* and showed moderate activity against *Staphylococcus aureus*, although it was ineffective against *Escherichia coli* in the disc diffusion assay. In contrast, the stem ethanolic extract showed moderate inhibition against *Staphylococcus aureus* and slight activity against *Escherichia coli*, while no significant inhibition was observed against *Candida albicans*. The aqueous extracts generally yielded lower inhibitory effects across all tested microorganisms. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) data strengthened these findings. For the root extract, the ethanolic preparation inhibited microbial growth at 1 g for all organisms, with a dose-dependent response evident in the increasing inhibition from 0.25 g to 1 g. The stem extract showed limited inhibitory activity, with bactericidal effects observed at 1 g for *Candida albicans* and *Escherichia coli*, and notably, a bactericidal effect against *Staphylococcus aureus* at 0.5 g using the ethanolic extract. These results indicate that the phytoconstituents responsible for antimicrobial activity are more efficiently extracted with ethanol than with water, and that different parts of the plant may vary in their bioactive compound content.

CONFLICTS OF INTREST

The authors declare no conflicts of interest

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