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Antibacterial Activity of *Khaya senegalensis* Stem Bark Against *Salmonella typhi* Isolated from Meat Handling Environments

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Abstract

Against the backdrop of escalating antibiotic resistance, medicinal plants continue to attract attention as potential sources of alternative antibacterial agents. This study evaluated the antibacterial efficacy of crude ethanol stem bark extract of *Khaya senegalensis* against environmental isolates of *Salmonella typhi* recovered from slaughter slab surfaces and meat handlers' aprons. The stem bark extract was subjected to qualitative phytochemical screening, while environmental swab samples were cultured for the isolation and biochemical identification of *S. typhi*. Antibacterial activity was assessed at concentrations ranging from 12.5 to 100 mg/mL using the agar well diffusion method, whereas the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined through standard broth dilution and subculturing procedures. Data were analyzed using one-way analysis of variance, with statistical significance set at $p < .05$. The phytochemical screening revealed the presence of alkaloids, phenolic compounds, tannins, saponins, and steroids. The extract demonstrated concentration-dependent antibacterial activity against all tested isolates, with

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mean zones of inhibition ranging from 8.00 ± 1.15 mm to 16.00 ± 1.53 mm. The MIC values ranged from 25 to 50 mg/mL, while the MBC was 100 mg/mL for all isolates. Significant differences in antibacterial activity were observed across extract concentrations for most isolates. These findings indicate that *K. senegalensis* stem bark possesses appreciable antibacterial activity against environmental *Salmonella typhi* isolates, thereby supporting its traditional medicinal use. The study contributes to the growing evidence that *Khaya senegalensis* may serve as a promising natural source of antibacterial compounds for addressing antimicrobial resistance and controlling foodborne infections.

Keywords: Ant antibacterial Activity; *Khaya senegalensis*; Medicinal Plants; Phytochemical Screening; *Salmonella typhi*

INTRODUCTION

Medicinal plants have historically been integral to traditional healthcare systems in Africa and other regions, especially in the treatment of infectious disorders (Dar *et al.*, 2023). The growing challenge of microbial infections, exacerbated by the rise of multidrug-resistant bacterial pathogens, has emphasized the urgent need for alternative and more effective treatment options (Davis & Choisy, 2024). In this context, traditional herbal medicine remains a critical alternative to conventional antibiotics in many developing nations. These bioactive compound-rich plants have become well-known for their antiviral, antifungal, and antibacterial qualities (Parham *et al.*, 2020). Consequently, the growing use of medicinal plants has prompted research efforts to separate, identify, and describe their active components, opening up the opportunity for the discovery of new antimicrobial medicines (Najmi *et al.*, 2022).

African mahogany, or *Khaya senegalensis*, is a tropical tree that grows abundantly in West Africa. *K. senegalensis* is well known in traditional African medicine for its ability to heal a wide range of illnesses, including as fever, malaria, gastrointestinal problems, and infections (Hailemariam *et al.*, 2021). This tree's stem bark is very important in ethnomedicine; it is frequently made into infusions or decoctions to cure illnesses, indicating the existence of bioactive substances with anti-inflammatory, antibacterial, and antiparasitic qualities (Abubakar *et al.*, 2024; Mathias *et al.*, 2022). The bark of *K. senegalensis* comprises a variety of secondary metabolites, including tannins, flavonoids, alkaloids, and

saponins, many of which are associated with antibacterial activity, according to phytochemical investigations (Hailemariam *et al.*, 2021; Nethathe *et al.*, 2025).

The Enterobacteriaceae family of bacteria includes the Gram-negative, facultatively anaerobic Salmonella enterica subspecies enterica serovar Typhi (*S. typhi*) (Shah *et al.*, 2021). It is the cause of typhoid fever, a dangerous systemic disease that is often acquired by ingesting contaminated food or water and is mostly transmitted by the fecal-oral route (Fàbrega & Vila, 2013). In many low- and middle-income countries, particularly in sub-Saharan Africa, S. typhi continues to be a serious public health problem. Poor hygiene, inadequate sanitation, and incorrect food handling techniques are associated with frequent outbreaks (Andino & Hanning, 2015; Han *et al.*, 2024).

The meat processing sector, particularly in informal settings such as local abattoirs and butcher shops, presents a significant risk of bacterial contamination (Zewude *et al.*, 2025). Surfaces like slaughter slabs and items like aprons used by meat handlers can serve as reservoirs and transmission pathways for *S. typhi* and other food borne pathogens (Siluma *et al.*, 2023). This situation is exacerbated by inadequate infrastructure, limited hygiene awareness, and lack of enforcement of public health standards (Adesola *et al.*, 2024). The increasing resistance of *S. typhi* to widely used antibiotics such as ampicillin, chloramphenicol, and third-generation cephalosporins has posed significant challenges to effective treatment and disease control measures (Khadka *et al.*, 2021; Marchello *et al.*, 2020). Antimicrobial resistance (AMR) is one of the top ten worldwide public health problems, according to the World Health Organization (WHO), emphasizing the importance of the problem. As a result, there is a growing urgency to identify and develop novel, effective, and affordable antimicrobial agents, particularly those derived from natural products and medicinal plants (WHO, 2023). A significant but often overlooked source of *S. typhi* contamination is meat processing environments, including slaughter slabs and the aprons worn by meat handlers. These surfaces are frequently contaminated due to exposure to raw meat, blood, and other organic matter in settings with insufficient cleaning and disinfection protocols (Adesola *et al.*, 2024; Zewude *et al.*, 2025)..

Multidrug-resistant *S. typhi* strains have emerged as a result of the widespread and often improper use of antibiotics, making treatment management more difficult and increasing the disease's impact on public health.

Recent studies have documented growing resistance of *S. typhi* to first-line antibiotics such as chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole (Khadka *et al*., 2021; Shaikh *et al*., 2023; Zaffar & Sadiqa, 2025). This trend necessitates the exploration of alternative antimicrobial agents that are both effective and affordable. Medicinal plants offer a promising solution due to their rich bioactive profiles. *Khaya senegalensis*, a widely used plant in traditional medicine across West Africa, has demonstrated antimicrobial properties in several studies (Dougnon *et al*., 2020; Igwe *et al*., 2025; Makosa *et al*., 2021; Malgwi *et al*., 2024; Saadu *et al*., 2023; Usin & Daramola, 2022). However, despite the promising pharmacological profile of *K. senegalensis*, few studies have specifically investigated its efficacy against *S. typhi* strains isolated from environmental sources such as slab surfaces and meat handlers' aprons. The antibacterial activity of *Khaya senegalensis* stem bark crude extract was evaluated in this study against *Salmonella typhi* that was isolated from meat handlers' aprons and slaughter slab surfaces.

MATERIAL AND METHODS

Study Area

This study was conducted in Aleiro town, the administrative center of Aleiro Local Government Area (LGA) in Kebbi State, Northwestern Nigeria. Aleiro was a semi-urban community characterized by commercial and agricultural activities, including livestock rearing, meat processing, and local trading. The town was located within the Sudan savanna ecological zone and experienced a tropical climate with distinct wet and dry seasons, factors that could influence microbial distribution and contamination levels.

Plant Sample Collection and Preparation

Fresh stem bark of *Khaya senegalensis* was obtained from mature trees in Aliero town, Kebbi State, Nigeria. The bark was carefully removed using a sterilized stainless-steel knife to prevent contamination. The collected material was placed in clean, labeled polythene bags and promptly transported to the laboratory for further processing. In the laboratory, the samples were washed thoroughly with clean water to eliminate dust and extraneous particles and then air-dried under shade for two weeks to maintain their phytochemical integrity. Using a clean mortar and pestle, the dried bark was then ground into a fine powder and kept in an airtight container in a dry, cool environment until extraction.

Preparation of Plant Extract

A total of 100 grams (100 g) of powdered *Khaya senegalensis* stem bark was immersed in 500 mL of ethanol in a conical flask and subjected to agitation on a mechanical shaker at room temperature for 72 hours to ensure efficient extraction of bioactive compounds. Whatman No. 1 filter paper was used to filter the mixture after maceration to enable to obtain a clean filtrate. The crude extract was then obtained by concentrating the filtrate using a rotary evaporator which removed the solvent. For further investigation, the resultant extract was kept at 4°C (Hlatshwayo *et al*., 2025; Yannick Stéphane *et al*., 2021).

Phytochemical Screening

To determine if the crude extract contained alkaloids, tannins, flavonoids, saponins, phenols, glycosides, terpenoids, and steroids, a preliminary qualitative phytochemical screening was carried out. For this investigation, standard analytical techniques described by Sharma *et al.* (2020) and Dubale *et al.* (2023) were utilized.

Environmental Sample Collection

Environmental swab samples were obtained from slaughter slab surfaces and the front surfaces of meat handlers' aprons at five different sites within Aliero town, Kebbi State, Nigeria. Sampling was conducted aseptically using sterile cotton swabs moistened with sterile normal saline. Each swab was immediately transferred into a sterile bottle containing peptone water, properly labelled according to sampling source and location, and placed in a cooler box with ice packs. For the purpose of timely microbiological analysis, all samples were sent within an hour to the Microbiology Laboratory at Kebbi State University of Science and Technology, Aliero.

Isolation and Identification of *Salmonella typhi*

To enable pre-enrichment, each environmental swab sample was first inoculated into sterile peptone water and incubated for 24 hours at 37°C. A loopful of the enriched culture was spread over Salmonella–Shigella (SS) agar after incubation, and it was then incubated for a further 24 hours at 37°C. Salmonella colonies were identified based on their characteristic morphology, which included colorless or transparent colonies with black centers on SS agar and red colonies with black cores on XLD agar. Suspected colonies were then subcultured onto Nutrient Agar to produce pure isolates. Following established

protocols, the pure cultures were identified using standard biochemical tests, such as Gram staining, the Triple Sugar Iron (TSI) test, motility test, citrate utilization, urease test, indole test, and oxidase test (Safi *et al.*, 2022; Tarabees *et al.*, 2017). Environmental swab samples were collected, pre-enriched, selectively cultured, and biochemically characterized following standard microbiological procedures, as summarized in Figure 1.

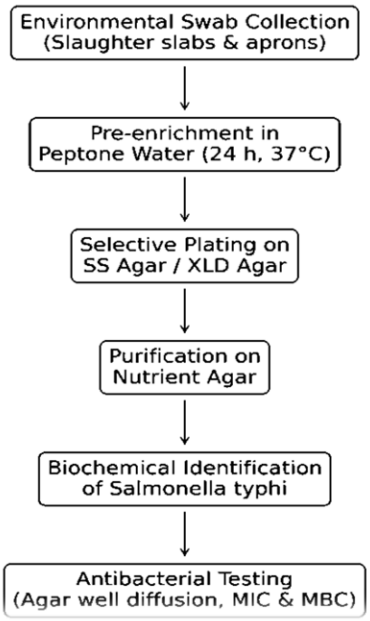


Figure 1: Flowchart illustrating sample collection, the isolation and identification of *Salmonella typhi*.

Preparation of Extract Concentrations

The crude extract was dissolved in 5% dimethyl sulfoxide (DMSO) to yield stock solutions. For the antimicrobial tests, working concentrations of 100.0, 50.0, 25.0, and 12.5 mg/mL were prepared from this stock solution.

Antibacterial Assay using the Agar Well Diffusion Method

The antibacterial activity of *Khaya senegalensis* extract was evaluated using the agar well diffusion technique in compliance with the guidelines set out by the Clinical and Laboratory Standards Institute (CLSI, 2023). Mueller-Hinton agar (MHA) plates were inoculated with bacterial suspensions that were adjusted to the 0.5 McFarland turbidity standard. Wells with a diameter of 6 mm that were aseptically formed on the agar were filled with 100 µL of each extract concentration. The plates were incubated at 37°C for 24

hours. Following incubation, a transparent ruler was used to quantify the zones of inhibition in millimeters. The positive control was 10 μg of ciprofloxacin, whereas the negative control was distilled water. The mean diameters of the inhibitory zones were determined after each test was conducted in triplicate.

Minimum Inhibitory Concentration (MIC) Determination

The extract's Minimum Inhibitory Concentration (MIC) was determined using the broth tube dilution method. The extract was serially diluted twice in nutritional broth, yielding concentrations ranging from 12.5 to 100 mg/mL. Each tube received 0.1 mL of the standardized bacterial suspension, which was then incubated at 37°C for 24 hours. The lowest concentration of the extract that shows no discernible turbidity, indicating inhibition of bacterial growth, is known as the minimum inhibitory concentration (MIC), according to Kowalska-Krochmal and Dudek-Wicher (2021).

Determination of Minimum Bactericidal Concentration (MBC)

Fresh nutrient agar plates were subcultured with aliquots from the tubes that did not exhibit any growth during the MIC determination, and the plates were incubated for 24 hours at 37°C. According to Owuama (2017), the MBC was the extract's lowest concentration at which there was no visible bacterial growth, which indicates bactericidal action.

Data Analysis

Every experiment was carried out in triplicate. The data were analyzed using SPSS version 24 and reported as mean ± standard deviation. The results were presented using both tabular and graphical forms. One-way analysis of variance (ANOVA) was used to compare the mean inhibitory zones across different extract concentrations. The statistical significance was established at $p < 0.05$, and the results were presented as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

The results of *Khaya senegalensis* stem bark crude extract's antibacterial activity against *Salmonella typhi,* which was isolated from slab surfaces and meat handlers' aprons, are presented in this section. The findings include phytochemical screening, identification

of bacterial isolates, antibacterial susceptibility testing using agar well diffusion, and statistical analysis of the zones of inhibition.

Phytochemical Composition of *Khaya senegalensis* Stem Bark Extract

Qualitative phytochemical screening of the crude stem bark extract of *Khaya senegalensis* identified several bioactive secondary metabolites (Table 1). The extract tested positive for alkaloids, phenols, tannins, saponins, and steroids, while flavonoids were absent. The presence of these phytochemical constituents supports the antibacterial potential of *Khaya senegalensis*, as these classes of secondary metabolites have been extensively documented to exhibit antimicrobial activity. Alkaloids are known to interfere with nucleic acid synthesis and protein metabolism in bacteria, while phenolic compounds and tannins exert antibacterial effects by denaturing proteins and disrupting cell membranes (Parham *et al*., 2020). Saponins increase membrane permeability, leading to leakage of cellular contents, and steroids have been associated with membrane destabilization (Davis & Choisy, 2024). Recent studies have consistently reported similar phytochemical profiles for *K. senegalensis* stem bark extracts, supporting the reproducibility of these findings across different geographical locations (Muhammad *et al*., 2024; Sulaiman *et al*., 2025). The absence of flavonoids in the present study may be due to environmental factors, extraction conditions, or plant maturity, as phytochemical composition is known to vary (Chaachouay & Zidane, 2024).

Table 1: Phytochemical Analysis

No.	Phytochemical	Result
1	Alkaloids	+
2	Flavonoids	-
3	Phenols	+
4	Tanning	+
5	Saponins	+
6	Steroids	+

Identification and Characterization of *Salmonella typhi* Isolates

Three bacterial isolates (STA, STB, and STC) obtained from slab surfaces and aprons of meat handlers were identified as *Salmonella typhi* based on cultural, morphological, Gram staining, and biochemical characteristics (Table 2). All bacterial isolates (STA, STB,

and STC) were confirmed as *Salmonella typhi* through Gram staining, cultural characteristics, and biochemical testing. The isolation of *S. typhi* from meat slab surfaces and meat workers' aprons highlights how common poor hygiene procedures are while handling and processing meat. Given that *S. typhi* is the cause of typhoid fever, a disease that is still widespread in many low- and middle-income nations, this result presents serious public health implications (Masuet-Aumatell & Atouguia, 2021). Recent studies have highlighted the presence of *Salmonella* species on meat contact surfaces and food handlers' clothing, underscoring the role of cross-contamination in the spread of foodborne pathogens (Ehuwa *et al.*, 2021; Hammuel & Junan, 2024). The results of this study therefore corroborate earlier findings that meat processing environments can serve as reservoirs for *Salmonella*, emphasizing the need for improved sanitation, regular surveillance, and food safety training among meat handlers (Rufai & Wartu, 2023).

Table 2: Identification and Characterization of Bacterial Isolates

Isolate ID	Gram Stain	Size	Colour on Agar	Shape	Urease	Citrate	Catalase	Oxidase	Indole	Species Identified
STA	-	Medium	Black on SS Agar	Rod	+	+	+	-	-	*Salmonella typhi*
STB	-	Medium	Black on SS Agar	Rod	+	+	+	-	-	*Salmonella typhi*
STC	-	Medium	Black on SS Agar	Rod	+	+	+	-	-	*Salmonella typhi*

Key: + = Positive, - = Negative

Antibacterial Activity of *Khaya senegalensis* Stem Bark Extract

Table 3 displays the antibacterial activity of stem bark extract from *Khaya senegalensis* against *Salmonella typhi*. The data is presented as mean ± standard error of the mean (SEM) using three independent replicates. Within the same isolate, mean values with different superscript letters indicate statistically significant differences ($p < 0.05$). A significant concentration-dependent increase in the zones of inhibition was observed for isolates STA and STB, whereas no statistically significant variation was detected across the different concentrations for isolate STC. The observed antibacterial activity aligns with previous

studies that reported inhibitory effects of *K. senegalensis* extracts against Gram-negative bacteria, including *Salmonella* species (Dlama *et al*., 2016; Dougnon *et al*., 2021). The lack of inhibition observed in the negative control confirms that the antibacterial activity was solely due to the plant extract and not the solvent used for extraction. The stronger response observed in STB is consistent with its lower MIC value (25 mg/mL), indicating greater susceptibility of this isolate to the extract. In contrast, STA and STC required higher concentrations to achieve growth inhibition, reflecting isolate-specific variability. Such variability has been widely reported in antimicrobial studies and may be attributed to differences in membrane permeability, efflux pump activity, or intrinsic resistance mechanisms (Oscar *et al*., 2020).

Table 3: Antibacterial activity of *Khaya senegalensis* stem bark extract against *Salmonella typhi* expressed as mean ± SEM

Isolate	Concentration (mg/mL)	Replicate1(mm)	Replicate2(mm)	Replicate3(mm)	Zone of inhibition (mm) Mean ± SEM	F-value	F-value
STA	**100.0**	**14**	**16**	**18**	**16.00 ± 1.15[a]**	**9.83**	**0.0047**
	50.0	12	12	14	12.67 ± 0.67[b]		
	25.0	11	15	13	13.00 ± 1.15[b]		
	12.5	8	10	6	8.00 ± 1.15[c]		
	Ciprofloxacin	26	24	27	25.67 ± 0.88[a]		
	Negative control	0	0	0	0.00 ± 0.00[c]		
STB	100.0	13	18	17	16.00 ± 1.53[a]	7.45	0.0105
	50.0	12	12	10	11.33 ± 0.67[b]		
	25.0	10	13	8	10.33 ± 1.45[b]		
	12.5	8	10	7	8.33 ± 0.88[c]		
	Ciprofloxacin	28	25	20	24.33 ± 2.40[a]		
	Negative control	0	0	0	0.00 ± 0.00[c]		

Isolate	Concentration (mg/mL)	Replicate1(mm)	Replicate2(mm)	Replicate3(mm)	Zone of inhibition (mm) Mean ± SEM	F-value	F-value
STA	**100.0**	**14**	**16**	**18**	**16.00 ± 1.15[a]**	**9.83**	**0.0047**
STC	100.0	14	12	10	12.00 ± 1.15[a]	3.49	0.0701
	50.0	12	13	15	13.33 ± 0.88[a]		
	25.0	11	12	11	11.33 ± 0.33[a]		
	12.5	9	9	11	9.67 ± 0.67[a]		
	Ciprofloxacin	18	22	20	20.00 ± 1.15[a]		
	Negative control	0	0	0	0.00 ± 0.00[b]		

Values bearing different superscript letters within the same isolate differ significantly at $p < 0.05$. Data are presented as mean ± standard error of the mean (SEM) from three independent replicates.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the crude *Khaya senegalensis* stem bark extract against *Salmonella typhi* isolates are summarized in Table 4. The MIC values varied among the isolates, with isolate STB showing the lowest MIC (25 mg/mL), indicating higher susceptibility to the extract. In contrast, isolates STA and STC exhibited MIC values of 50 mg/mL. The MBC for all isolates (STA, STB, and STC) was determined to be 100 mg/mL, suggesting that higher concentrations of the extract were necessary to achieve bactericidal effects. These results demonstrate that the particular *S. typhi* isolate affects the antibacterial effectiveness of *Khaya senegalensis* stem bark extract. The comparatively high MIC and MBC values found are consistent with other research on crude plant extracts, especially when it comes to Gram-negative bacteria (Dougnon *et al.*, 2021; Sulaiman *et al.*, 2025). Higher quantities of

antimicrobial drugs are necessary to successfully hinder growth and kill Gram-negative bacteria because their outer membrane acts as a barrier preventing their entrance (Helmy *et al.*, 2023; Lamichhane *et al.*, 2024). The isolate-dependent sensitivity to the extract is further supported by the variance in MIC and MBC values across *S. typhi* isolates, as seen in Figure 2.

Table 4: MIC and MBC Determination of *Khaya senegalensis* Extract against *Salmonella typhi*

Isolate	12.5 mg/mL	25 mg/mL	50 mg/mL	100 mg/mL	MIC (mg/mL)	MBC (mg/mL)
STA	+	+	−	+	50	100
STB	+	-	+	+	25	100
STC	+	+	−	+	50	100

Key: + = Visible bacterial growth **−** = No visible growth

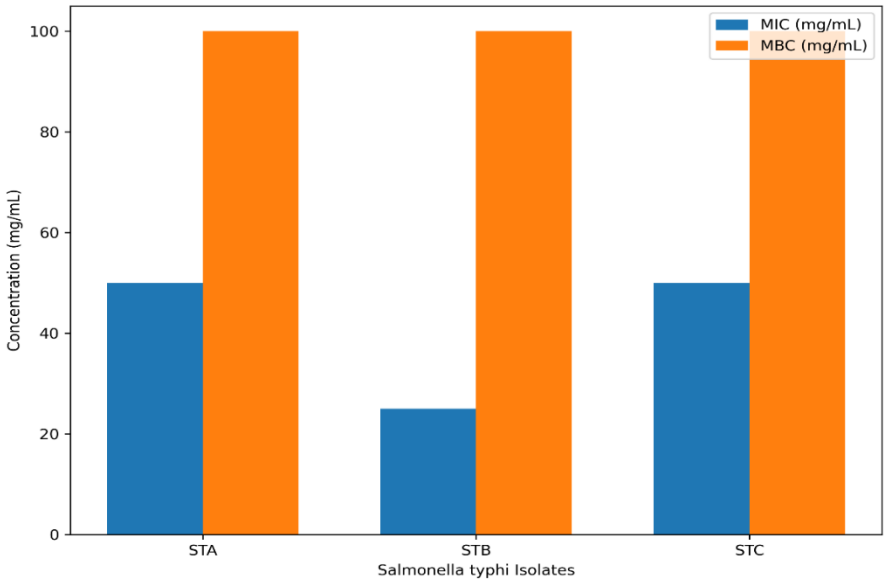


Figure 2: Minimum inhibitory and bactericidal concentrations (MIC and MBC) *of Khaya senegalensis* stem bark extract against Salmonella typhi isolates

Despite the promising results, this study has some limitations. The use of crude extract may mask the activity of individual bioactive compounds, and the agar well diffusion method may underestimate activity due to limited diffusion of some compounds. Additionally, in vivo efficacy and toxicity were not evaluated. Future studies should focus

on extract fractionation, molecular characterization of active compounds, cytotoxicity assessment, and in vivo anti-bacterial evaluation.

CONCLUSION

The outcomes of this investigation show that *Salmonella typhi* isolated from meat processing settings is significantly inhibited by the crude stem bark extract of *Khaya senegalensis*.The observed antibacterial activity was concentration-dependent and statistically significant for most of the isolates, with bactericidal effects evident at higher extract concentrations. These findings demonstrate K. senegalensis's potential as a useful source of natural antibacterial chemicals for the management of foodborne pathogens and the reduction of antimicrobial resistance, and they justify the ethnomedical use of the plant in the treatment of infectious disorders.

Conflicts of Interest

The authors affirm that they have no competing interests with respect to the publishing of this work.

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