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Antibacterial potential of traditional chewing stick plants *Mezoneuron benthamianum* and S*trychnos afzelii* against cariogenic *Streptococcus spp*. in Côte d'Ivoire

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ABSTRACT

Dental caries is one of the most prevalent oral diseases worldwide and is closely associated with cariogenic microorganisms, particularly bacteria belonging to the genus *Streptococcus*. In western Côte d'Ivoire, several medicinal plants are traditionally used as chewing sticks for oral hygiene, although their antibacterial properties remain poorly documented. This study evaluated the antibacterial activity of aqueous and hydro-ethanolic extracts of *Mezoneuron benthamianum* Baill. and *Strychnos afzelii* Gilg. against *Streptococcus* spp. isolated from dental caries. Plant extracts were prepared by maceration in distilled water and 70% ethanol. Antibacterial activity was evaluated using the agar well diffusion method, while the minimum inhibitory concentration and minimum bactericidal concentration were determined using the broth macrodilution method. Hydro-ethanolic extraction produced the highest yields, reaching 17.60% for *Mezoneuron benthamianum* and 16.90% for *Strychnos afzelii*. Neither the aqueous nor the hydro-ethanolic extracts of *Strychnos afzelii* exhibited antibacterial activity against the tested strains. In contrast, both extracts of *Mezoneuron benthamianum* inhibited the growth of all tested *Streptococcus* spp., with inhibition zones ranging from 13.00 ± 0.50 mm to 18.00 ± 0.58 mm. The minimum inhibitory concentration and minimum bactericidal concentration were both 25 mg/mL, resulting in a minimum bactericidal concentration to minimum inhibitory concentration ratio of 1, indicating a bactericidal effect. These findings provide experimental support for the traditional use of *M. benthamianum* as a chewing stick plant and suggest its potential source of antibacterial compounds for future oral health applications.

Keywords : Dental caries, Oral hygiene, Natural products, chewing sticks, antibacterial activity, Medicinal plants.

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INTRODUCTION

Dental caries is one of the most prevalent chronic infectious diseases worldwide and represents a major public health challenge. It results from complex interactions between dental tissues, dietary factors, host susceptibility, and microbial biofilms developing on tooth surfaces.[1] Among the microorganisms associated with dental caries, bacteria belonging to the genus *Streptococcus*, particularly cariogenic species, play a central role through their ability to adhere to enamel surfaces, produce extracellular polysaccharides, form biofilms, and generate acids responsible for tooth demineralization.[2,3]The prevention and management of dental caries mainly rely on mechanical plaque control, fluoride use, and antimicrobial oral care products. Although chlorhexidine is widely recognized for its effectiveness against oral microorganisms, its prolonged use may cause undesirable effects such as tooth staining, taste alteration, and disturbance of the oral microbiota.[4] Furthermore, the emergence of antimicrobial resistance has increased the interest in identifying alternative natural products with antibacterial properties.[5]

Medicinal plants represent a valuable source of bioactive molecules with potential applications in oral healthcare. Secondary metabolites such as phenolic compounds, flavonoids, tannins, alkaloids, and terpenoids have demonstrated antibacterial effects through different mechanisms, including membrane disruption, inhibition of bacterial adhesion, interference with enzymatic activities, and inhibition of biofilm formation.[6,7] In many African communities, chewing sticks obtained from medicinal plants remain an important traditional method of oral hygiene. These plant materials provide mechanical cleaning of dental surfaces and may release bioactive compounds capable of reducing oral microbial growth.[8] In Côte d'Ivoire, several plant species are traditionally used as chewing sticks, particularly in rural areas where access to dental care remains limited. Among these species, *Mezoneuron benthamianum* Baill. (Fabaceae) and *Strychnos afzelii* Gilg. (Loganiaceae) are commonly used in western Côte d'Ivoire for maintaining oral hygiene. Although species belonging to these genera are known for their richness in secondary metabolites and various biological activities, their antibacterial effects against cariogenic microorganisms remain insufficiently documented.[9,10] Therefore, scientific investigation of these traditional chewing stick plants may contribute to the validation of indigenous oral healthcare practices and the discovery of new natural antibacterial agents. The present study aimed to evaluate the antibacterial activity of aqueous and hydro-ethanolic extracts of *Mezoneuron benthamianum* Baill. and *Strychnos afzelii* Gilg. against *Streptococcus* spp. isolated from dental caries

MATERIALS AND METHOD

Plant material and identification

The plant material consisted of stems of *Strychnos afzelii* Gilg. and leafy stems of *Mezoneuron benthamianum* Baill. The plant samples were collected in Glaogouin, a locality located approximately 5 km from the city of Man, western Côte d'Ivoire.

Botanical identification and authentication were performed at the National Floristic Center (CNF) of Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire. Voucher specimens were deposited under the accession numbers UCJ011524 for *Strychnos afzelii* Gilg. and UCJ009456 for *Mezoneuron benthamianum* Baill.

After collection, the plant organs were washed with distilled water to remove impurities, cut into small pieces, and dried under continuous air-conditioning conditions at 18 °C for one week. The dried materials were subsequently ground into fine powders using an electric grinder (RETSCH SM 100, Germany) at the Central Laboratory of the University of Man. The obtained powders were stored in airtight containers until extraction.

Bacterial strains

Three bacterial strains belonging to the genus *Streptococcus* were used to evaluate the antibacterial activity of the plant extracts (Table 1). The bacterial strains were provided by the Unit of Natural Substances, Antibiotics and Antimicrobial Resistance Surveillance (ASSURMI), Department of Bacteriology and Virology, Institut Pasteur de Côte d'Ivoire (IPCI). The strains were previously characterized according to their antimicrobial susceptibility profiles.

Table 1. Phenotypic characteristics of tested *Streptococcus* spp. strains

Bacterial species	Identification code	Phenotype
Streptococcus sp.	P 01/24	Sensitive
Streptococcus sp.	K 01/24	Sensitive
Streptococcus sp.	R 01/24	Sensitive

Preparation of plant extracts

Two types of crude extracts were prepared from each plant species: aqueous extracts and hydro-ethanolic extracts. Maceration was selected because it is a simple and widely used extraction procedure in ethnopharmacological studies, particularly for medicinal plants traditionally prepared using water or hydro-alcoholic solvents.[11] Hydro-ethanolic mixtures are commonly used because they allow the extraction of compounds with different polarity ranges, including phenolic compounds, flavonoids, tannins, and other secondary metabolites potentially involved in biological activities.[12]

Aqueous extraction

One hundred grams (100 g) of plant powder were macerated in 1 L of distilled water for 24 h under magnetic stirring. The macerate was filtered through sterile cloth followed by cotton wool

filtration. The obtained filtrate was dried in an oven at 50 °C for 24 h. The dried crude extract was weighed, transferred into airtight containers, and stored until antibacterial evaluation.

Hydro-ethanolic extraction

For hydro-ethanolic extraction, 100 g of plant powder were macerated in 1 L of 70% ethanol (v/v) for 24 h under magnetic stirring. After filtration, the solvent was removed by drying the filtrate at 40 °C for 24 h. The resulting crude extracts were stored in airtight containers at 4 °C until use.

Determination of extraction yield

The extraction yield was determined according to the method described by Azwanida.[11] It was calculated as the ratio between the mass of dried extract obtained and the initial mass of plant powder used for extraction using the following equation:

Extraction yield (%) = (Mass of dried extract / Mass of plant powder) × 100

Evaluation of antibacterial activity

Preparation of culture media

Mueller–Hinton agar and Mueller–Hinton broth were prepared according to the manufacturer's instructions. The culture media were sterilized at 121 °C for 15 min. After cooling to approximately 45 °C, sterile blood was added to obtain a final concentration of 5%. The supplemented Mueller–Hinton agar was used for antibacterial susceptibility testing.

Agar well diffusion assay

The antibacterial activity of plant extracts was evaluated using the agar well diffusion method according to Balouiri et al.[13] Bacterial inocula were prepared from 18–24 h cultures. Colonies were suspended in Mueller–Hinton broth and adjusted to approximately 10^6 CFU/mL using the 0.5 McFarland standard. The bacterial suspensions were uniformly inoculated onto supplemented Mueller–Hinton agar using sterile swabs. Extract solutions were prepared at a concentration of 200 mg/mL using sterile distilled water. An aliquot of 80 µL of each extract solution was introduced into wells previously prepared in the agar medium. Sterile distilled water was used as the negative control, while erythromycin was used as the positive control.

After a pre-diffusion period of 45 min at room temperature, plates were incubated at 37 °C under a 5% CO_2 atmosphere for 18–24 h. Antibacterial activity was determined by measuring inhibition zones using a digital caliper.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the broth macrodilution method according to Wiegand et al.[14] Serial two-fold dilutions of active extracts were prepared in Mueller–Hinton broth, with concentrations ranging from 200 mg/mL to 0.78 mg/mL.

For each concentration, 1 mL of bacterial suspension was added to 1 mL of extract solution. Two controls were included:

- sterility control containing culture medium without bacterial inoculum;
- growth control containing bacterial inoculum without extract.

The tubes were incubated at 37 °C under 5% CO_2 atmosphere for 18–24 h.

The MIC was defined as the lowest extract concentration showing no visible bacterial growth.

Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) was determined from tubes showing no visible growth during MIC determination according to Wiegand et al.[14]

Aliquots were subcultured onto Mueller–Hinton agar supplemented with 5% blood and incubated at 37 °C under 5% CO_2 atmosphere for 24 h.

The MBC corresponded to the lowest extract concentration capable of reducing bacterial viability by at least 99.9%.

The bactericidal or bacteriostatic effect was interpreted according to the MBC/MIC ratio:

- MBC/MIC ≤ 4: bactericidal effect;
- MBC/MIC > 4: bacteriostatic effect.[14]

RESULTS AND DISCUSSION

Extraction yields

The extraction yields obtained from aqueous and hydro-ethanolic extracts of *Mezoneuron benthamianum* and *Strychnos afzelii* are presented in Table 2. Extraction yields varied according to both plant species and extraction solvent. Hydro-ethanolic extracts showed the highest yields for both plants, reaching 17.60% for *M. benthamianum* and 16.90% for *S. afzelii*, whereas aqueous extracts yielded 13.70% and 14.00%, respectively. The higher yield obtained with hydro-ethanol may be explained by the ability of ethanol-water mixtures to dissolve compounds with different polarity ranges compared with water alone. Hydro-ethanolic solvents are widely used in phytochemical investigations because they promote the extraction of several classes of secondary metabolites, including phenolic compounds, flavonoids, tannins, and other bioactive constituents.[11,12] Similar variations in extraction efficiency according to solvent polarity have been reported for medicinal plants, highlighting the importance of extraction conditions in the recovery of potentially active molecules.[12]

Table 2: Extraction yields of crude extracts from *Mezoneuron benthamianum* and *Strychnos afzelii*

Plant species	Extract type	Plant powder(g)	Dry extract (g)	Yield (%)
M. benthamianum	Hydro-ethanolic extract (MBHE)	100	17.60	17.60
	Aqueous extract (MBAE)	100	13.70	13.70
S. afzelii	Hydro-ethanolic extract (SAHE)	100	16.90	16.90
	Aqueous extract (SAAE)	100	14.00	14.00

MBAE: aqueous extract of *Mezoneuron benthamianum*; SAHE: hydro-ethanolic extract of *Strychnos afzelii*; SAAE: aqueous extract of *Strychnos afzelii*.

Antibacterial activity of crude extracts

The antibacterial activity of aqueous and hydro-ethanolic extracts was evaluated against three *Streptococcus* spp. strains isolated from dental caries using the agar well diffusion method (Table 3). Sterile distilled water, used as negative control, showed no inhibitory activity, with inhibition diameters corresponding to the well diameter (6 mm), confirming the absence of solvent interference. In contrast, erythromycin exhibited strong antibacterial activity, with inhibition zones ranging from 25 to 30 mm, confirming the susceptibility of the tested strains. No antibacterial activity was detected for aqueous and hydro-ethanolic extracts of *Strychnos afzelii*. The inhibition diameters remained identical to the negative control (6 mm) for all tested strains. This absence of activity suggests that the extracted compounds under the experimental conditions were either insufficiently active against the tested bacteria or present at concentrations below effective levels. Conversely, both extracts of *Mezoneuron benthamianum* inhibited the growth of all tested *Streptococcus* spp. strains. The hydro-ethanolic extract showed the highest activity, with inhibition zones ranging from 16.00 ± 0.50 mm to 18.00 ± 0.58 mm, while the aqueous extract showed inhibition zones between 13.00 ± 0.50 mm and 15.00 ± 1.00 mm. The stronger activity of the hydro-ethanolic extract may be related to its higher capacity to extract antibacterial phytochemicals. Phenolic compounds, flavonoids, tannins, and other plant secondary metabolites have been reported to inhibit oral pathogens through several mechanisms, including alteration of bacterial membranes, inhibition of microbial enzymes, interference with adhesion processes, and reduction of biofilm formation.[5,6] The antibacterial activity observed for *M. benthamianum* supports its traditional use as a chewing stick plant for oral hygiene. Chewing sticks provide mechanical removal of dental plaque while releasing soluble phytochemicals that may contribute to microbial inhibition. Several studies have reported antibacterial effects of medicinal chewing

sticks against oral microorganisms, supporting their traditional applications in dental care.[8,15] The absence of activity observed for *Strychnos afzelii* may be related to differences in chemical composition, extraction conditions, plant organ, or bacterial susceptibility. Previous studies have shown that the antimicrobial activity of plant extracts may vary considerably depending on solvent polarity, extraction procedures, geographical origin, and concentration of active constituents. [16] Although *Strychnos* species are known for their alkaloid diversity and various biological activities, antibacterial effects are not always detected in crude extracts. Recent investigations on other *Strychnos* species have demonstrated that antibacterial activity depends strongly on the nature of the extract and the chemical profile of the fractions obtained, suggesting that fractionation and phytochemical characterization are necessary to reveal potential active compounds. [17] Further investigations are therefore required to clarify the antibacterial potential of *S. afzelii*

Table 3: Inhibition zones (mm) of plant extracts and erythromycin against *Streptococcus* spp. strains

Bacterial strain	SAAE	SAHE	MBAE	MBHE	Erythro mycin	Sterile distilled water
Streptococcus sp. P 01/24	6	6	15 ± 1	18 ± 0.58	25	6
Streptococcus sp. K 01/24	6	6	13 ± 0.5	17 ± 1	30	6
Streptococcus sp. R 01/24	6	6	14 ± 1	16 ± 0.5	30	6

SAAE: aqueous extract of *Strychnos afzelii*;SAHE: hydro-ethanolic extract of *Strychnos afzelii* ; MBAE: aqueous extract of *Mezoneuron benthamianum*; MBHE: hydro-ethanolic extract of *Mezoneuron benthamianum*.

Values represent mean ± standard deviation of three independent experiments.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Only *M. benthamianum* extracts showing antibacterial activity in the agar diffusion assay were selected for MIC and MBC determination (Table 4). Both aqueous and hydro-ethanolic extracts exhibited identical antibacterial parameters against all tested *Streptococcus* spp. strains. The MIC values were 25 mg/mL, while the MBC values were also 25 mg/mL for all tested combinations. The MBC/MIC ratio was equal to 1, indicating a bactericidal effect according to commonly accepted antimicrobial interpretation criteria.[13,14] The ability of *M. benthamianum* extracts to eliminate bacterial cells at the same concentration required for growth inhibition suggests a strong antibacterial potential against cariogenic bacteria. This bactericidal effect is particularly relevant because cariogenic *Streptococcus* species contribute significantly to dental biofilm formation and

caries progression.[2,3] Reduction of these bacterial populations may therefore contribute to limiting biofilm development and supporting oral health

Table 4. Minimum inhibitory concentration and minimum bactericidal concentration of *Mezoneuron benthamianum* extracts against *Streptococcus* spp.

Bacterial strain	Extract	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Interpretation
Streptococcus sp. P 01/24	MBAE	25	25	1	Bactericidal
	MBHE	25	25	1	Bactericidal
Streptococcus sp. K 01/24	MBAE	25	25	1	Bactericidal
	MBHE	25	25	1	Bactericidal
Streptococcus sp. R 01/24	MBAE	25	25	1	Bactericidal
	MBHE	25	25	1	Bactericidal

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; MBAE: Aqueous extract of *Mezoneuron benthamianum*; MBHE: Hydro-ethanolic extract of *Mezoneuron benthamianum*.

MIC and MBC were determined only for the active extracts of *M. benthamianum*. An MBC/MIC ratio ≤4 indicates bactericidal activity, whereas a ratio >4 indicates bacteriostatic activity.

The present study demonstrates that *Mezoneuron benthamianum*, traditionally used as a chewing stick in western Côte d'Ivoire, possesses significant antibacterial activity against cariogenic *Streptococcus* spp. In contrast, *Strychnos afzelii* did not show inhibitory activity under the experimental conditions. The antibacterial effect of *M. benthamianum* may be associated with its phytochemical composition, particularly secondary metabolites commonly reported in Fabaceae species. These compounds may contribute to antimicrobial activity through multiple mechanisms.[14] However, chemical characterization of active constituents, evaluation of antibiofilm activity, toxicity assessment, and investigation of effects on oral microbiota are necessary before considering potential applications in oral care formulations.

Overall, these findings provide scientific support for the traditional use of *M. benthamianum* as a natural oral hygiene resource and highlight its potential as a source of antibacterial compounds for dental health applications

CONCLUSION

This study demonstrated that extracts of *Mezoneuron benthamianum* Baill., a traditional chewing stick plant used in western Côte d'Ivoire, exhibited antibacterial activity against *Streptococcus* spp. isolated from dental caries. Both aqueous and hydro-ethanolic extracts inhibited bacterial growth, with the hydro-ethanolic extract showing the strongest activity. The bactericidal effect confirmed by the MIC and MBC values highlights the potential of this plant as a source of natural

antibacterial compounds for oral health applications. In contrast, *Strychnos afzelii* extracts showed no activity under the experimental conditions. These results provide experimental support for the traditional use of *M. benthamianum* in oral hygiene and encourage further investigations aimed at identifying active compounds and evaluating its potential application in plant-based oral care products.

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