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GC-MS Characterization and Antimicrobial Efficacy of Ethanolic Extract of Local Chewing Stick and Mouthwash on *Streptococcus Mutans*.

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ABSTRACT

This study investigates the antimicrobial efficacy of ethanolic extracts from local chewing sticks (*Psidium guajava* and *Dialium guineense*) and a commercial mouthwash (fluoride mouthwash) against *Streptococcus mutans*, a primary causative agent of dental caries. The ethanolic extracts of *Psidium guajava* and *Dialium guineense* were obtained using Soxhlet extraction and subjected to phytochemical analysis, revealing the existence of alkaloids, glycosides, tannins, saponins, and steroids. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified bioactive compounds such as quercetin, gallic acid, lupeol, and β-sitosterol, known for their antimicrobial properties. The antimicrobial activity was evaluated using the disc diffusion method, with the ethanolic extracts showing significant zones of inhibition (18 mm for *P. guajava* and 20 mm for *D. guineense*), compared to the fluoride mouthwash (12 mm). The minimum concentration that inhibits growth of bacteria (MIC) and minimum bactericidal concentration (MBC) values for the extracts were 250 mg/ml and 200-250 mg/ml, respectively, indicating potent antibacterial activity. The results suggest that the ethanolic extracts of *P. guajava* and *D. guineense* possess strong antimicrobial properties against *S. mutans*, making them potential alternatives for oral hygiene products. This study highlights the potential of local chewing sticks as effective, natural, and cost-effective way for maintaining oral health and preventing dental caries.

Keywords: Oral hygiene, chewing sticks, *Streptococcus mutans*, GC-MS characterization, antimicrobial activity.

1.0 Introduction

Good oral hygiene has a major influence on one's general quality of life and well-being. Several chronic and systematic diseases have been attributed to oral health. With the increasing incidence of oral diseases, the global need for alternative prevention, treatment methods, and safe, effective and economical products has expanded. Oral health can primarily be maintained through both mechanical and chemical methods. Utilizing toothbrushes alongside toothpaste is a widely practiced technique for maintaining oral hygiene (WHO, 2018).

In the present era, tooth decay is the most prevalent chronic dental condition that affects 60-90% of the young population. Dental caries has a multifactorial etiology, and hence proper oral hygiene habits are required for its control (Caglar *et al*., 2006). Streptococcus mutans, the microbial species most strongly associated with carious lesions, is normally present in human oral plaque (Culp *et al*.,2011). Mechanical plaque control by use of a toothbrush or by use of chewing stick is the advisable and commonly practiced oral hygiene measure, although numerous antiplaque agents have been in use as auxiliary aids. Employing mouthwash is a reliable and safe way to deliver antimicrobial agents, which are now commonly used. These agents can inhibit bacterial adhesion, colonization, and metabolism, thereby influencing bacterial growth (Sharma *et al*.,2004).

Mouthwashes serve as effective antimicrobial agents and have strong potential in decreasing the level of *S. mutans* in saliva. Mouthwash is a liquid dental product intended to freshen breath. Some types may also eliminate bacteria and/or whiten teeth. The demand for mouthwash arises from a condition known as halitosis, or unpleasant breath. Research indicates that bad breath primarily occurs due to bacterial activity in an unclean mouth. In particular, anaerobic bacteria thrive on the protein-rich food particles trapped between the teeth or on the tongue. As bacteria decompose the proteins, those that contain sulfur release unpleasant-smelling compounds, leading to bad breath. Mouthwashes aim to combat bad breath by eliminating the bacteria that cause the unpleasant smell and also by covering up the odour. Various ancient cultures, such as the Egyptians, Chinese, Greeks, and Romans, had their own formulations for these products (Danaei *et al*., 2011). They utilized a range of components, from palatable items like fruits, honey, or dried flowers to less pleasant substances that can influence dental health.

The discovery of fluoride's antibacterial properties played a crucial role in the creation of contemporary mouthwash. Generally, there are three categories of mouthwash: the first type includes antibacterial solutions that decrease the number of bacteria in the oral cavity. The second category consists of fluoride mouthwashes that enhance the fluoride coating on dental enamel. Lastly, there are demineralizing mouthwashes designed to assist in the repair of various oral lesions (Almas *et al*., 2005). The practice of using chewing sticks has been recorded since ancient eras. Traditional chewing sticks have been used widely by different civilizations for centuries. It was initially used by Babylonians around 7000 years ago (Almas *et al*., 2005) followed by Greek and Roman empires. Chewing sticks were utilized by Jewish, Egyptian, and ancient Japanese cultures. It was thought that Europeans were unaware of these traditional oral hygiene practices of chewing sticks until about 300 years ago. Today chewing sticks are being widely used in Asia, Africa, South America and throughout Islamic countries (Noumi *et al*.,2010). The cleaning strength of chewing sticks is due to the physical actions of their fibers, the release of helpful compounds, or a combination of these factors (Fayez *et al*., 2016). The continual use of chewing sticks allows fresh sap rich in fluoride to be released, which appears to moisten the tooth enamel and effectively penetrate areas prone to cavities, thus aiding in the prevention of caries. The mild bitter taste also has a bactericidal effect and many other components (Anyiam *et al*., 2016).

Psidium guajava, typically known as guava, is a tropical plant widely recognized for its medicinal properties and nutritional value. It belongs to the Myrtaceae family and has been traditionally used in various cultures for the treatment of gastrointestinal disorders, respiratory infections, and oral health issues (Gutiérrez *et al*., 2008). The leaves, bark, and fruits of *Psidium guajava* are rich in bioactive compounds, including flavonoids, tannins, phenolic acids, and essential oils, which contribute to its antimicrobial, anti-inflammatory, and antioxidant activities (Joseph and Priya, 2011). Studies have shown that the ethanolic extract of *Psidium guajava* leaves exhibits significant antimicrobial activity against a wide range of pathogens, including oral bacteria such as *Streptococcus mutans* (Rahim *et al*., 2017). *Dialium guineense*, commonly known as velvet tamarind or black velvet, is a medicinal plant belonging to the Fabaceae family. It is widely distributed in tropical regions and has been traditionally used for its antimicrobial, antidiarrheal, and anti-inflammatory properties (Akinmoladun *et al*., 2007). The plant's bark, leaves, and fruits contain bioactive compounds such as saponins, tannins, flavonoids, and alkaloids, which contribute to its pharmacological activities (Olekanma *et al*., 2021; Okwu and Uchenna, 2009). Studies have demonstrated that *Dialium guineense* extracts exhibit significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, including oral pathogens like *Streptococcus mutans* (Akinmoladun *et al*., 2007). The ethanolic extract of *Dialium guineense* has been shown to inhibit bacterial growth by disrupting cell wall synthesis and interfering with microbial metabolic processes (Okwu and Uchenna, 2009).

The findings of this work can contribute to the development of natural, plant-based oral care products that are effective in preventing dental caries and promoting overall oral health. Additionally, the study could provide scientific validation for the conventional use of chewing sticks, highlighting their potential as a sustainable and culturally relevant approach to oral hygiene.

2.0 Materials and Methods

Sample collections and preparation of fresh tiwgs of Psidium guajava and Dialium guineense were collected from Federal Polytechnic Nekede, Owerri botanical garden. The twigs were cleaned, dried under shade for four weeks, and ground using an electric grinder to obtain a coarse powder 250ml mouth wash was bought from a supermarket in Owerri.

2.1 Extraction of Plant Material

Ethanolic stock extracts were obtained through soxhlet extraction 10 g of powdered twigs were extracted using 200 ml 95 % ethanol in a soxhlet apparatus for 5 hours. The ethanolic extract of *Psidium guajava* and *Dialium guineense* twings were concentrated using rotary evaporator and stored at 4 ℃ for further experiments.

2.2 Phytochemical Analysis

The ethanolic extracts obtained from the twings of *Psidium guajava* and *Dialium guineense* were subjected to phytochemical screening using standard methods as reported elsewhere (Ojiuko *et al*., 2021).

2.3 Gas Chromatography-mass Spectrometry (GC-ms) Analysis

The quantification of the phytochemicals present in the twings of *Psidium guajava* and *Dialium guineense* ethanol extracts were analyzed using a GC-MS (Bulk M910, USA) equipped with capillary column (30 m x 0.25 mm x 0.25μm). The oven temperature was operated from 70 ℃ to 300 ℃ at a rate of 5 ℃/min. Helium gas was used as the carrier gas at a flow rate of 1ml/mir. The mass spectrometer was operated in flame ionization detector mode.

The compounds were identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) library. The relative percentage of each compound was calculated based on the peak area.

2.4 Isolation and Identification of Streptococcus Mutans

Streptococcus mutans used in this study was obtained from the microbiology laboratory of Federal Medical Centre, Owerri, Nigeria. The culture was grown on Mitis-Salivarius Bacitracin (MSBA) agar at 37 ℃. The bacteria was confirmed using gram staining, catalase and homolysis test (Sarwar *et al*., 2023).

2.5 Antimicrobial Susceptibility Testing

The disc diffusion (agar well) method was used to study the antibacterial activity of the ethanolic extracts and mouthwash. Sterile filter paper discs impregnated with 0.2 ml aliquot of each ethanol extract and mouthwash were placed on agar plates already inoculated with Streptococcus mutans. The inoculated nutrient agar plates were incubated at 37 °C for 24 hours, after which the zones of inhibition were measured.

2.6 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

The MIC was evaluated using a two-fold serial dilution method, and the MBC was assessed by sub-culturing tubes showing no visible growth from the MIC test on nutrient agar plates as reported in our previous study (Ojiuko *et al*., 2021).

3.0 Results and Discussion

3.1 Phytochemical Analysis

The results of qualitative phytochemical analysis of the ethanolic extracts of *Psidium guajava* and *Dialium guineenses* are shown in Table 1. The phytochemical screening of *Psidium guajava* and *Dialium guineenses* extracts revealed the presence of alkaloid glycosides, tannins, saponins and steroids. These phytochemical compounds play a very important role in the antimicrobial effects of plants and these results confirmed the efficacy of plant extracts for medicinal use (Phaiboon *et al*., 2010).

Table 1. Phytochemical Analysis of the ethanol extracts of *Psidium guajava* and *Dialium guineenses.*

Phytochemical Constituents	*P. guajava* Extract	*D. guineenses* Extract
Alkaloid	+	+
Glycosides	+	+
Tannins	+	+
Saponins	+	+
Steroids	+	+
Flavanoids	-	-
Phlobatannins	-	-

3.2 GC- MS Analysis of Psidium guajava and Dialium guineenses Ehtanolic Extrats

The GC- MS analysis results of *P. guajava* and *D. guineenses* ethanol extracts revealed the presence of several bioactive compounds. The identified bioactive compounds and their percentage peak area are presented in Tables 2 and 3, respectively. The result disclosed that the *P. guajava* extract contains various bioactive compounds of flavonoid, phenolic, phytosterol and terpene which are known for their antimicrobial, anti-inflammatory and antioxidant properties that contribute to antibacterial activity against *Streptococcus mutans* (Venugopal *et al*., 2019). *Dialium guineenses* showed bioactive compounds of triterpenuid, phytosterol, and polyphenol. The lupeol and β-sitosterol were the major phytochemical constituents in *D. guineenses* extract. These phytochemical constituents have been reported to exhibit strong antimicrobial activity against oral pathogens (Salahi and Momeni-Danaie, 2006). Similarly, quercetin and gallic acid found in *Psidium guajava* extract have been reported to inhibit biofilm formation and bacterial growth (Cushine and Lamb, 2011).

Table 2: Phytochemical Constituents Identified in the Ethanolic Extract of *P. guajava*

Phytochemicals	Class of phytochemicals	Percentage Peak area
Quercetin	Flavonoid	22.8
Gallic acid	Phenol	18.5
Catechin	Flavonoid	13.1
β-sitosterol	Phytosterol	10.8
Limonene	Terpene	6.0

Table 3: Phytochemical Constituents Identified in the Ethanolic Extract of *D. guineenses*.

Phytochemicals	Class of phytochemicals	Percentage Peak Area
Lupeol	23.5	Triterpenoid
β-sitosterol	20.8	Phytosterol
Stigmasterol	17.3	Phytosterol
Tannic acid	15.6	Phytosterol
Oleanolic acid	11.4	Triterpenoid

3.3 Antibacterial Susceptibility Test

The ethanolic extracts of *P. guajava* and *D. guineenses*, and an antiseptic mouthwash on antibacterial activity against *Streptococcus mutans* were evaluated. The results of the antibacterial susceptibility test of the extracts and antiseptic mouthwash against S. mutans are shown in Table 4. The zones of inhibition observed on ethanolic extracts of *P. guajava*, *D. guineenses*, fluoride mouthwash and control (Chloramphenicol) were 18, 20, 12, and 52 mm, respectively. The fluoride mouthwash showed the least activity (12 mm) while the control (chloramphenicol) showed the highest activity (52 mm).

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

The MIC and MBC of *Psidium guajava*, *Dialium guineenses*, fluoride mouthwash and chloramphenicol (control) were assessed on streptococcus mutans. The ethanolic extracts of *P. guajava* and *D. guineenses* possessed antibacterial activity against S. mutans with MICs of 250mg/ml each and MBCs of 200mg/ml and 250mg/ml respectively. The fluoride mouthwash showed MIC of 100mg/ml and MBC of 250mg/ml, respectively. The MIC and MBC results are tabulated in Table 5. The results obtained in the present study were in agreement with the study of Salehi and Momeni-Danaie, 2006. The minimum bacterial concentration values were consistent with the minimum inhibitory concentration results, indicating that both extracts possess bactericidal properties.

Table 4: Antimicrobial susceptibility testing of plants extracts and fluortide mouthwash

Test Agents	Zone diameter of inhibition (mm)
P. guajava	18
D. guineenses	20
Fluoride mouthwash	12
Chloramphenicol	50

Table 5: MIC and MBC of plants extracts and mothwash against streptococcus mutans.

Test Agents	MIC (mg/ml)	MBC (mg/ml)
P. guajava	250	200
D. guineenses	250	250
Fluoride mouthwash	100	250

4.0 Conclusion

The study revealed that ethanolic extracts of *Psidium guajava* and *Dialium guineense*, traditional chewing sticks, exhibit significant antimicrobial activity against *Streptococcus mutans*, a key bacterium associated with dental caries. Phytochemical analysis displayed the existence of bioactive compounds such as alkaloids, glycosides, tannins, saponins, and steroids, which contribute to their antimicrobial properties. GC-MS analysis further identified specific compounds like quercetin, gallic acid, lupeol, and β-sitosterol, known for their antibacterial, anti-inflammatory, and antioxidant effects. The ethanolic extracts showed greater zones of inhibition (18 mm for *P. guajava* and 20 mm for *D. guineense*) compared to a commercial fluoride mouthwash (12 mm), indicating their superior efficacy in inhibiting *S. mutans* growth. Additionally, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values further confirmed the potent antibacterial activity of the extracts. These findings suggest that *P. guajava* and *D. guineense* extracts have the potential to serve as natural, safe, and cost-effective alternatives for oral hygiene products, particularly in regions where access to commercial dental care products is limited.

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