



## **Antibacterial and Biofilm Inhibition Activities of *Nicotiana tabacum* Extracts against *Streptococcus mutans* Isolated from the Oral Cavity**

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### **Author's contribution**

The sole author designed, analysed, interpreted and prepared the manuscript.

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### **ABSTRACT**

**Aim:** To determine the antibacterial activity and biofilm inhibitory activity of *Nicotiana tabacum* leaf extract against *Streptococcus mutans* isolated from oral cavity.

**Place and Duration of Study:** Department of Microbiology, Faculty of Science, Imo State University, Owerri, Nigeria, between April and November 2019.

**Methodology:** The study was carried out using the agar diffusion method for antibacterial activity and microtiter assay method for the biofilm inhibition effect.

**Results:** All four extracts of hot water, ethanol, acetone and methanol tested against *S. mutans* had no antibacterial activity as no zone of inhibition was recorded. Likewise, all the extracts did not inhibit the formation of biofilm by the organism on glass. This is seen in the increase of the optical density (OD) values from 0.572 to 0.804 for water, 0.260 to 1.420 for ethanol, 0.216 to 0.626 for acetone and 0.236 to 0.725 for methanol extracts respectively.

**Conclusion:** This study therefore indicates that *N. tabacum* does not have antibacterial and biofilm inhibitory against *S. mutans*. This also explains why a lot of tobacco users still suffer from dental caries and other oral infections caused by *S. mutans*.

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**Keywords:** Biofilm; optical density; antibacterial; inhibitory; oral cavity; tobacco; Streptococcus.

## 1. INTRODUCTION

Dental caries also known as tooth decay is an infectious disease of the teeth characterized by the dissolution and destruction of the calcified tissues of the teeth often caused by microorganisms particularly *Streptococcus mutans* [1,2]. In Nigeria and other developing countries, dental caries is and remains an epidemic disease especially among the poor with 25% of children under 5 years old having detectable caries lesions [2]. Similarly, the adult population is not left out with the average adult reportedly having at least minor gingivitis, an inflammation of the gums, with a much smaller percentage suffering from moderate to severe periodontitis [3,4]. The high caries rate is most prevalent among the poor rural dwellers and other economically disadvantaged ghetto and slum dwellers most of whom cannot afford to introduce fluoride in their daily life at an early age [5]. Thus, there is need for an effective public health measure to address this problem.

Dental caries is not usually caused by a single bacterial cell but an aggregate of divers' community of bacterial cells referred to as biofilm [6]. These microorganisms bind tightly to one another and to the solid surface of tooth by means of an extracellular matrix consisting of polymers of both host and microbial origin [6]. The biofilm helps the organisms to attach to the teeth surfaces, the epithelia of multicellular organisms, and interfaces [7]. This surface adhesion of bacteria is important if the organisms are to successfully establish themselves and cause disease in the oral cavity [7]. *Streptococcus mutans* is one of such organisms implicated in biofilm formation and causes of dental caries. It plays an important role in cariogenesis processes [8]. The organism ferment carbohydrate food particles while producing acid that brings about the demineralization and cavity formation of the tooth [8]. Biofilm formation by *S. mutans* enhances the virulence of these pathogenic plague causing organisms and helps them resist antibiotics and other antimicrobial agents. This makes efforts to control them relatively difficult [8]. Thus, any effort taken to eliminate *S. mutans* would be vital to the treatment and management of dental caries [7]. However, most of the current antibiotics used in oral infection therapy such as Cephalosporins and Tetracyclines etc distort the balance of oral microflora and causes serious

side effects such as tooth discoloration etc. owing to these and other factors such as high cost of chemically formulated toothpaste and mouthwash which are relatively unaffordable by many rural and urban poor in Africa and Nigeria in particular, there is therefore need to seek for alternative means of prevention and treatment which is relatively cheap and readily available [9]. Plant derived products thus offers such alternatives as it has been previously reported that plant derived antimicrobials inhibit the growth of oral pathogens, reduce the rate of biofilms formation, treat dental plaque, influence the adhesion of bacteria to surfaces and reduce the symptoms of oral diseases [9].

Tobacco is an agricultural product processed from the leaves of plants in the genus *Nicotiana* [10]. There are more than 70 species of tobacco in the plant genus *Nicotiana* [10]. It is part of the nightshade family (Solanaceae) indigenous to North and South America, Australia, South West Africa and the South Pacific [10]. Tobacco plant (*Nicotianatabacum*) is grown commercially for its leaves and stems, which are rolled into cigars, shredded for use in cigarettes and pipes, processed for chewing, or ground into snuff (a fine powder that is inhaled through the nose). Tobacco nicotine inhibits the growth of pathogens which is dose dependent [11,12]. It is equally effective against gram-positive and gram-negative bacteria, along with the acid-fast *Mycobacterium phlei* and the opportunist fungi *Candida albicans* and *Cryptococcus neoformans*. Levels of inhibition  $\geq 50\%$  occurred when most of the affected organisms were cultured with nicotine at 100-250  $\mu\text{g/mL}$  [13]. The above mentioned concentrations of nicotine can be found *in vivo* [13] especially in the oral cavity of smokeless tobacco users, making these findings physiologically relevant. Yildirim et al. [14] reported that the ether extracts of both the leaves and seeds and ethanol extract of leaves had shown antimicrobial activities on *Staphylococcus*. Wang et al. [11] reported inhibition of the activities of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* by Crude polyphenols extracted from tobacco leaf by 80% ethanol solution. Strong antimicrobial activities against *Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus faecalis*, *Mycobacterium phlei*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and the human pathogenic yeast, *Candida albicans* were detected in methanolic

extracts of 24 plants used medicinally in the treatment of skin infections in four different regions of Colombia. Twenty-two extracts displayed activity against Gram positive bacteria whereas none was active against the Gram-negative species [15].

Present study is therefore focusing on identifying plants that can be used as an herbal alternative to chemical drugs to treat dental infections. We are studying the efficacy of tobacco leaves plant parts extracts against dental plaque forming *Streptococcus mutans*.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

The test organism *Streptococcus mutans* used for this research was obtained from the Federal Medical Centre (FMC) Owerri, Imo State, Nigeria. While the tobacco leaves were purchased from different commercial traders from Eke Ukwu Owerri Market in owerri, Imo State Nigeria and was authenticated by the Taxonomist in Botany department of Imo State University Owerri, Nigeria.

### 2.2 Preparation of Plant Extracts

Already dried tobacco leaves procured from traders were grounded using a sterile mortar and pestle and used for extraction with different solvents (hot water, acetone, ethanol and methanol). For hot water extraction, 50 grams of the grounded leaves were into a flask containing 500 ml of water and heated in a water bath until boiled. The resulting solution was filtered using a filter paper and the residue separated. The water content of the filtrate was evaporated completely by heating leaving a dried extract. While for the acetone, ethanol and methanol extraction, 100 grams of the grounded leaves were soaked in 500 ml of each of the solvent in a conical flask for 24 hours. After which the resulting solution was filtered using a filter paper and the residue separated. The solvent content of the filtrate was evaporated completely by heating leaving a dried extract.

### 2.3 Antibacterial Assay of Plant Extracts by Agar Well Diffusion Method

Well Diffusion method was used to test the antibacterial activities of the different extracts [16]. The extracts (hot water, acetone, methanol, ethanol) were tested for antibacterial activity using agar diffusion on Mueller Hinton Agar. The

Media was sterilized by autoclaving at 121°C at 15 lbs pressure for 15 minutes. The molten agar was allowed to cool to 45°C and then 20 ml of Mueller Hinton agar was poured aseptically into Petri plates. The agar was allowed to set and harden. Agar test plates of the test organism prepared. Using a sterile swab, lawn of the test organism was spread onto the Mueller Hinton agar plates. The wells were punctured in the centre by using a sterile cork borer. Then the wells were filled with various leave extracts. The plates were incubated at 37°C for 24 hrs. After incubation the plates were observed for the zone of inhibition. The zones were measured using zone measuring scale.

### 2.4 Biofilm Inhibition Activity

Biofilm formation assay was carried out by the modified method of crystal violet staining assay in a glass test tube [17]. Tryptic soy broth was prepared and 30 µl of the broth was transferred sterile test tube. The broth was then sterilized by autoclaving at 121°C at 15 lbs pressure for 15 minutes. The test tube was then inoculated with 3 µl of overnight grown culture of *S. mutans* strain. The hot water, acetone, methanol and ethanol leave extracts were added into each tube. The tubes were then incubated at 37°C for 18 hrs in a slanted manner. After incubation the supernatant (non adherent cells) was carefully decanted without disturbing the adhering cells. The tube containing biofilm were washed with saline (0.85% NaCl). Then 30 µl saline was added and mixed well to separate the cells adhered to surface and to remove loosely attached cells. 1% Crystal violet solution was added to observe for the adhered cells. The optical density was measured at 550 nm using a spectrophotometer.

### 2.5 Data Analysis

The mean and standard deviation of the measurements were used to present the findings of the study. The difference in mean values was analyzed using ANOVA with SPSS.

## 3. RESULTS AND DISCUSSION

The antibacterial and biofilm inhibition activity of different solvent extracts of tobacco leaves against *S. mutans* were investigated and the results presented in Table 1. From the results obtained, the hotwater, ethanol, acetone and methanol extracts of the tobacco leaves recorded zero diameter zone of inhibition which is

indicating that the extracts had no antibacterial activity against the test organism.

From the findings, the tobacco leave extracts did not exhibit any form of antibacterial activity against *S. mutans* which thus indicates that it is not an effective agent or means of treating and managing dental caries. This explains why there are still increased cases of dental caries among tobacco users who do not observe good personal oral hygiene. This corroborates previous reports that there is a positive relationship between smoking or chewing of tobacco and caries [18,19]. Others have reported that smoking increases the growth of *Streptococcus mutans*, while the condensates enhances the ability of the organism to adhere to surfaces of the teeth [20]. However, there are other contradicting and conflicting reports which are at variance with our findings stating a significant antibacterial activity of tobacco leave extracts against *streptococcus* sp and other Gram positive bacteria [21,22, 23,24].

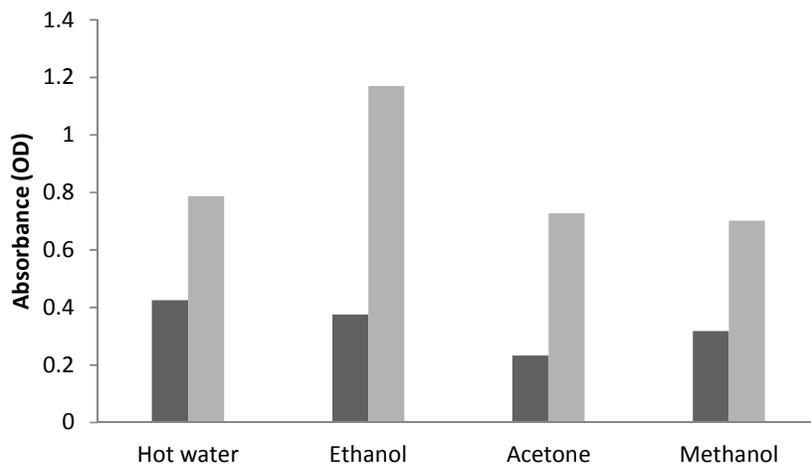
For the biofilm inhibition activity, the results showed that the hot water extract produced an

increase in the turbidity from 0.426 to 0.787, while ethanol extract produced an increase in turbidity from 0.376 to 1.170, acetone recorded an increase from 0.233 to 0.727 and finally methanol extract also recorded an increase in turbidity with OD of 0.318 to 0.702 at 550nm (Table 1).

The tobacco leave extracts was observed not to inhibit the formation of biofilm of the test organism *Streptococcus*. This was shown by the increase in the optical density (OD) recorded for all for extracts at 550 nm. This is very instructive and worthy of note because of the critical role biofilm plays in the cause and development of dental caries. This is in line with previous reports stating that nicotine extracted from tobacco significantly enhanced the growth of *Streptococcus mutans* biofilms [25]. Therefore, the inhibition of *Streptococcus mutans* biofilm formation is key to the prevention, control and management of dental caries. No report has been previously put forward to support the effectiveness or otherwise of tobacco leave extracts in inhibiting the formation of oral *Streptococcus* biofilm.

**Table 1. Antibacterial and biofilm inhibition activities (optical density) of tobacco leave extracts against *S. mutans***

Well No.	Extract used	Diameter zone of inhibition	OD before addition of extract	OD after addition of extract
1	Hot water	Nil	0.426	0.787
2	Ethanol	Nil	0.376	1.170
3	Acetone	Nil	0.233	0.727
4	Methanol	Nil	0.318	0.702



**Fig. 1. Biofilm Inhibition of *Streptococcus mutans* after 24 hrs at 490 nm before and after addition of Tobacco leave extracts**

#### 4. CONCLUSION

Tobacco usage has remained a prominent and daily part of the lives of millions of people across the world. The leaves are processed and consumed in the forms such as cigarettes which is smoked through the mouth, chewed or sniffed as a ground powder which is also applied on the teeth and applied in the mouth for different reasons. It is for this reason that the antibacterial and biofilm inhibition activity of the leaves against *S. mutans* which has been implicated as the primary and most important causative organism of dental caries was investigated.

It is therefore logical to state that from this study, that tobacco leave extracts although may be having other medicinal values, is not effective as a dental caries treatment or management agent which should be recommended for any oral therapeutic use. As earlier stated, this may account for why there are still increased cases of dental plaque among tobacco users. In fact, it may even be a predisposing factor in the increasing the occurrence of dental caries.

#### COMPETING INTERESTS

Author has declared that no competing interests exist.

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