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Nicotiana Tabacum Essential Oil Extract from Cigarettes Products against Diverse Smokers Mouth Floral Isolates

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ABSTRACT

This study aimed to evaluate the bacterial divesity, and the antimicrobial potential of *Nicotiana tabacum* oil extracts against diverse Smokers Mouth Floral isolates The oral microbiota of smokers is significantly impacted by tobacco use, which may increase the prevalence of pathogenic bacteria, potentially leading to oral health complications.. Five (5) oral samples were collected from five smokers, and microbial analysis was carried out using standard microbiological techniques through the use of NA and MacConkey agar. Isolated were identify via cultural characterstics and through series of biochemical reactions. Antimicrobial activities of *Nicotiana tabacum* oil extracts was performed with varying concertation (100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL) using agar well diffusion assay. The killing dynamics of *Nicotiana tabacum* oil extracts against isolated bacteria was done using UV spectrophotometer. Results showed CFUs ranging from 3.0×10^4 to 9.8×10^4 CFU/mL among the samples. This study identified several bacterial isolates from the oral flora of smokers, including *Enterococcus faecalis*, *Staphylococcus aureus*, *Lactobacillus lactis*, *Pediococcus acidilactici*, *Peptostreptococcus anaerobius*, *Stomatococcus mucilaginosus*, Actinomyces *bovis*, *Streptococcus agalactiae*, *Bifidobacterium bifidum*, and *Corynebacterium diphtheriae*. The oil extracts exhibited significant antibacterial activity, particularly at higher concentrations, with inhibition zones of up to 13.0 mm for *Staphylococcus aureus*. Killing time assays revealed a reduction in bacterial load, with optical density (OD) dropping to 0.000 by 48 hours for most isolates, demonstrating effective bacterial killing.*Nicotiana tabacum* oil extracts showed promising antibacterial properties against oral pathogens from smokers. These findings suggest potential applications of the extracts as adjuncts to oral health treatments for smokers, warranting further research into their molecular mechanisms and long-term efficacy.

Keywords: *Nicotiana Tabacum , essential oil Microbial Diversity*

Introduction

Smoking remains a one of the major public health issue worldwide, with over 300 million people estimated to use and consume tobacco products (1). The harmful effects of smoking on the respiratory and cardiovascular systems are well-known, but its impact on oral microflora is often underemphasized. Smoking is the major cause of oral cancer, periodontitis, colour change on the teeth, halitosis and other health implications. It brings about a drastic decrease in the commensal population of normal flora in the oral cavity leading to an increase of pathogenic microbes. Tobacco smoking could enhance microbial colonization by biofilm formation on oral epithelial cells. This may impair host immune responses against pathogens and also disrupt effective nasal mucociliary clearance (2). These substances can have severe consequences on the oral ecosystem, which consists of a diverse microbial community responsible for maintaining oral health. The mouth flora, or microbiota, is a delicate balance of bacteria species and other microorganisms that coexist to protect the oral cavity from disease (3). In smokers, this balance is often disturbed, leading to a higher prevalence of oral diseases such as periodontitis, dental caries, and fungal infections like oral thrush.

The introduction of harmful chemicals from tobacco smoke fosters an environment conducive to the overgrowth of pathogenic microorganisms, which can outcompete the beneficial bacteria that typically maintain oral health (4).

Nicotiana tabacum, commonly known as the tobacco plant, is a species within the Solanaceae family (5).The Solanaceae family comprises 3000–4000 species that are classified in approximately 90 genera (6). The family is highly diverse, includes perennial trees as well as herbaceous annual species and occupies a wide range of terrestrial habitats from deserts to rainforests Compared with the large size of the family, only few members of the Solanaceae attained importance in human civilizations as ornamentals (petunia, *Datura*, some *Solanum* species, *Schizanthus*) and drugs (tobacco, *Atropa, Hyoscyamus, Mandragora*) (7). This perennial herbaceous plant originates from tropical regions of the Americas and has been cultivated for centuries due to its psychoactive properties. The leaves of *Nicotiana tabacum*are the primary source of tobacco used in a variety of products, including cigarettes, cigars, and chewing tobacco.

Figure 1: Image of Tobacco plant (Source:8,9)

Nicotiana tabacum plant is cultivated globally, with major production occurring in countries such as China, India, Brazil, and the United States (10). Due to its high nicotine content, *Nicotiana tabacum*has played a crucial role in the development of the global tobacco industry, which has significant economic and social implications worldwide.

The oral cavity of non-smokers hosts a diverse and balanced microbiome, predominantly comprising bacterial species that play essential roles in maintaining oral health. One of the most notable bacterial species is Streptococcus, particularly Streptococcus mutans, which is associated with the development of dental caries. S. mutans can metabolize sugars and produce lactic acid, which leads to enamel demineralization and subsequent tooth decay (11). However, the oral cavity also contains beneficial species such as Streptococcus sanguinis and Streptococcus gordonii, which act as antagonists to S. mutans, inhibiting its growth and contributing to overall oral health. These beneficial species help to maintain a delicate microbial balance and prevent the overgrowth of harmful bacteria.

In addition to *Streptococcus species*, non-smokers' oral cavities may harbor *Porphyromonas gingivalis,* a key pathogen linked to periodontal disease. *P. gingivalis* produces virulence factors such as proteolytic enzymes and lipopolysaccharides that contribute to inflammation and tissue destruction, facilitating its evasion of the immune response (12). However, in the oral cavity of non-smokers, the immune system and beneficial bacteria often work synergistically to limit the harmful effects of pathogenic microorganisms, thereby reducing the risk of severe oral diseases.

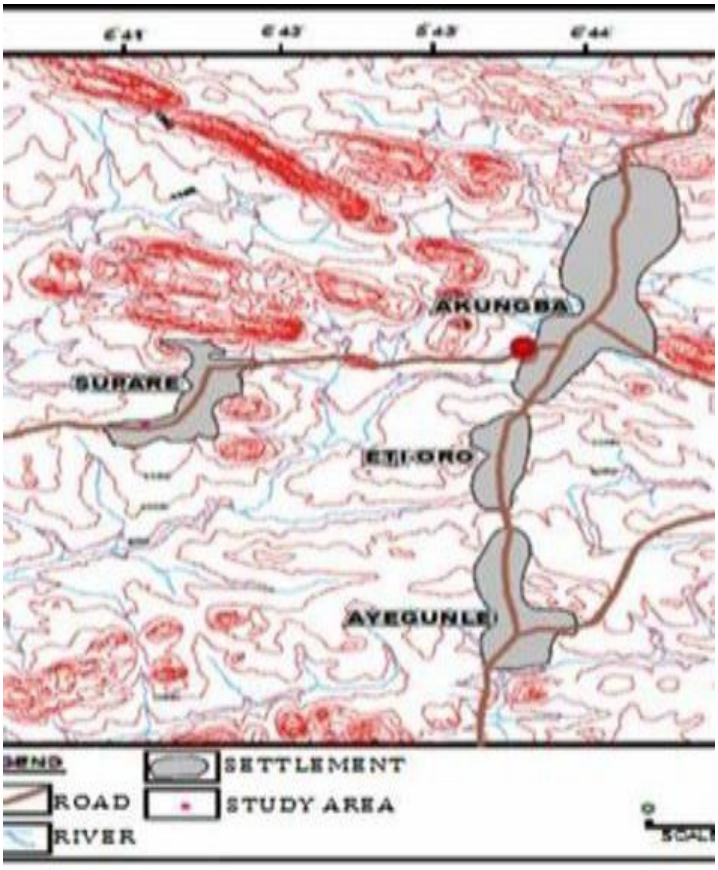
In smokers, the oral microbiome undergoes significant alterations, often leading to an imbalance between beneficial and pathogenic microorganisms. Exposure to tobacco smoke not only affects oral tissues but also alters the microbiome. Studies have demonstrated that tobacco smoke can promote the virulence of certain pathogenic species, particularly fungi. For example, (13) reported that exposure to tobacco smoke extract enhances the adherence and biofilm formation of Candida albicans on epithelial cells. Tobacco compounds appear to alter the expression of adhesion-related genes in C. albicans, facilitating its colonization and contributing to an increased incidence of oral candidiasis in smokers. This shift in microbial dynamics underscores tobacco's role in promoting fungal growth, while potentially inhibiting some bacterial species.

Moreover, tobacco smoke induces pro-inflammatory effects in oral tissues, creating a conducive environment for the growth of pathogenic microbes. (14) showed that tobacco extracts can stimulate the secretion of pro-inflammatory cytokines in oral epithelial cells, leading to inflammation and compromising the integrity of the mucosal barrier. This inflammation exacerbates the risk of oral infections and facilitates the invasion of pathogenic microorganisms, such as *Porphyromonas gingivalis*, worsening periodontal disease. Smokers are thus more susceptible to inflammatory oral conditions due to the pro-inflammatory and toxicological effects of tobacco.

Additionally, the cytotoxic effects of tobacco on oral tissues further compromise oral health. (15) highlighted the detrimental impact of tobacco extracts on oral keratinocytes, which are essential for maintaining the integrity of the oral epithelium. The cytotoxicity observed in smokers impairs the host's immune response, rendering them more vulnerable to infections and the progression of oral diseases. This weakening of both epithelial defense and immune function in smokers contributes to the heightened prevalence of oral health issues, including periodontal disease and fungal infections.

The oral cavity of smokers hosts diverse microbial flora, which can be adversely affected by tobacco use. This alteration is linked to various oral health issues, including periodontal disease and oral cancers. Despite these risks, there is limited research on the specific microbial communities in smokers' mouths and the effects of *Nicotiana tabacum* (tobacco) extracts on these pathogens. Understanding these interactions is essential for addressing health challenges faced by smokers.

Hence, this study aims to investigate the biohazard activity of *Nicotiana tabacum* extract against the microbial flora in smokers' oral cavities, which is critical given the health risks associated with smoking. By isolating and identifying the microbial flora present in smokers which will provide insights into the harmful pathogens linked to smoking-related oral diseases. also by extracting and characterizing *Nicotiana tabacum* using ethanol and n-hexane will help identify potentially antimicrobial compounds that could combat these pathogens.



Map description of Akungba Akoko

MATERIALS AND METHODS

Study Area

The study is in Akungba Akoko, Nigeria, specifically at the Microbiology Department of Adekunle Ajasin University. The geographic coordinates of the study location in Akungba Akoko (Permanent site and Medoline) were approximately 7.3540° N latitude and 5.6132° E longitude. This area is known for its diverse community, which includes a significant number of smokers, making it an ideal location for investigating the microbial flora of the oral cavity.

Collection of cigarette material

Cigarettes made from *Nicotiana tabacum* were collected from various vendors in the Akungba community, Ondo State. The plant material used in this study was identified and authenticated at the herbarium of the Department of Plant Science and Biotechnology (Taxonomic Unit: PSBHT) at Adekunle Ajasin University, Akungba Akoko (AAUA), Ondo State, Nigeria. The rhizomes were designated as PSBHT-273 and deposited in the herbarium for future reference.

Figure 2: Image of selected Cigarettes (Nicotiana tabacum)

Source Saleh et al. (16,17)

Extraction of *Nicotiana tabacum* oil (using N-hexane and ethanol)

A 600 g of the powder sample of *Nicotiana tabacum* was soaked in 900 ml of n-hexane and 1000ml of ethanol in a glass bottle. The bottles was sealed and kept in a dark place for 3 days with continuous agitation. After 3 days, the soaked samples was filtered using a sterile muslin cloth. The filtrate, which contained the solvents and the oil, was transferred to a round-bottom flask and connected to a rotary evaporator. The solvent was evaporated at 40°C and 100 mmHg for 2 h, leaving the oil in the flask. The oil was collected and stored in a glass bottle at 4°C for antimicrobial analysis further analysis (14,15).

Collection of oral swab samples

Five (5) oral swab samples were collected from smokers within the Akungba Akoko community. Participants were instructed to refrain from oral hygiene practices for at least one hour prior to sample collection to maximize the yield of microbial flora. Samples were obtained from patient at the the study location exactly 9:00 AM (WAT). Using sterile cotton swabs, each swab was carefully inserted into the oral cavity, focusing on areas such as the buccal mucosa and tongue. The swab was rotated to ensure adequate sample collection. Immediately after collection, the swabs were placed in sterile transport tubes containing 1 mL of sterile saline to maintain the viability of the microorganisms-route to the laboratory (18).

Serial Dilution

6-fold serial dilution was performed to quantify the bacterial load. A sterile saline solution was prepared, and 9 mL of this solution was added to a sterile test tube. Swab from the collected sample were carefully cut into the test tube and mixed thoroughly to create a 10^1 dilution. Following this, 1 mL from the first dilution was transferred to a second sterile test tube containing 9 mL of saline to create a 10^2 dilution. This process was repeated through 10^4 dilutions.

Plating (Pour Plate Method)

The pour plate method was employed for the isolation of bacteria from the oral cavity samples. For each dilution factor (10^3 and 10^6), 100 µL of the sample was carefully pipetted into pre-labeled sterile Petri dishes using a sterile micropipette. A prepared Nutrient agar medium (~ 20 ml),was poured into the Petri dishes containing the 100 µL of sample. The dishes were gently swirled to mix the sample with the agar, ensuring uniform distribution of the bacterial inoculum under an aseptic condition. After the agar had solidified, the plates were inverted to prevent condensation from the lid from dripping onto the agar surface. The plates were then incubated at 37°C for 24 hours for bacterial growth (16).

Colony forming Unit (Cfu/ml)

Following the incubation period, the plates were examined for bacterial colonies, which were counted to estimate the viable cell concentration in the original samples. The results were recorded in colony-forming units per milliliter (CFU/mL).

Sub-culturing

Distinct colonies that appeared on the NA plates were selected based on differences in morphology, color, and size. Each selected colony was carefully picked using a sterile inoculating loop and sub-cultured onto fresh NA plates. The subculturing process involved streaking the loop across the agar surface using the quadrant streaking method to obtain isolated colonies. The plates were labeled appropriately and incubated at 37°C for an additional 24 hours to allow the growth of pure cultures(14).

Identification of Bacterial isolates

Gram Staining

A small amount of each bacterial isolate was placed on a clean microscope slide to create a thin smear. The smear was then heat-fixed by passing it through a flame to adhere the bacteria to the slide. The slide was stained with crystal violet for one (1) minute, followed by rinsing with distilled water. Next, iodine solution was applied for 30 seconds, then rinsed again. The slide was decolorized with ethanol for a 30 seconds until no more dye was washing off, and it was rinsed with water. Finally, the slide was counterstained with safranin for 30 seconds, rinsed, and air-dried. The prepared slides were examined under ax100 oil immersion microscope to identify whether the bacteria were Gram-positive (purple) or Gram-negative (pink).(19)

Biochemical Tests and sugar utilization

The identification of bacterial isolates was further confirmed through a series of biochemical tests. The following tests were conducted: Catalase Test, Oxidase Test, Indole .Citrate**, (19)**

Sugar Fermentation Test

The sugar fermentation is demonstrated by the production of acid or acid and gas (carbondioxide and/or hydrogen).

The ability of an organism to ferment several sugars can be demonstrated by incorporating the sugars into a basal medium (phenol red broth base) and testing for acid and gas production. The sugars used were glucose, fructose, maltose, sucrose, lactose and mannitol. Each of the isolates were inoculated into test tubes containing each of the sugars and incubated at 35°C for 3 days watching daily for color change. Acid production was indicated by the appearance of a yellow color in the medium and gas production by the presence of an air space in the inserted Durham tubes (20).

Standardization of isolated bacteria

Slants of various organisms were reconstituted at aseptic condition, using sterile inoculating loop, approximately one isolated colony of each pure culture was transferred into 5ml of sterile nutrient broth at 37°C for 24hours. After incubation, 0.1ml of the isolated colony was transferred into 9ml of distilled water contained in each test tube using a sterile syringe, and was mixed properly. The liquid now serve as a source of inoculum containing approximately 10^6 cfu/ml of bacterial suspension (21).

Antimicrobial effect of oil extract on test isolate

The bacterial cultures were maintained on nutrient broth. Inoculum size containing 1.5 x10^8 cfu/ml for bacterial(McFarland standard) were used to seed already solidified petri plates of Mueller-hinton agar. The antimicrobial activities of the oil were determined using agar well diffusion method. Ten organisms were used in all three gram positive, five gram negative. A sterile 6mm cork borer was used to make well on already solidified agar where the wells were filled with the the *Nicotiana tabacum*oil ensuring that there was no spill on the agar surface surrounding the well. The plates were allowed to stand for about 2 hours to allow absorption of the oil into the medium after which they were incubated at 37°C for bacterial for 24 hours for bacterial. After 24 hours of incubation, the zones of inhibition was observed and recorded in mm using a calibrated ruler(21).

The killing kinetics and Killing dynamics of bacterial isolates using *Nicotiana tabacum* oil Extracts

The killing kinetics of bacterial isolates were carried out using a UV spectrophotometer. Bacterial cultures were prepared in nutrient broth and incubated overnight at 37°C to ensure active growth. The bacterial suspension was standardized to a specific optical density (OD) at 600 nm using the UV spectrophotometer, ensuring a uniform inoculum. Portions of this suspension were aliquoted into sterile tubes, and the test antimicrobial agent was added at the desired concentrations. A control tube without the antimicrobial agent was set up for comparison. The tubes were incubated at 37°C, and samples were withdrawn at predetermined time intervals (e.g., 0, 30, 60, 120 minutes). These samples were immediately measured for OD at 600 nm using the UV spectrophotometer to monitor bacterial growth reduction over time. A decrease in OD was indicative of bacterial cell death, thus reflecting the killing kinetics.

Additionally, aliquots from each sample were plated on nutrient agar and incubated to determine colony-forming units (CFU), which were counted to correlate spectrophotometric readings with actual bacterial death. This method was used to quantify the bactericidal effect of the antimicrobial agent over time(21).

RESULTS

Table 1 shows the sample collection data for the study, which includes oral samples from smokers. A total of five samples, labeled M1 to M5, were collected on April 11, 2024, at 11:00 am, indicating a synchronized collection process for the oral microbiota analysis.

Table 2 presents the colony-forming units (CFU) of bacterial isolates from the mouth flora of smokers. The samples, labeled M13, M15, M23, M25, M33, M35, ML13, M45, M53, and M55, exhibit varying bacterial loads. The highest CFU is recorded for sample M23 at 9.8 × 10^4 CFU/mL, while the lowest is found in sample M55 at 3.0 × 10^4 CFU/mL. This variation suggests differences in bacterial presence among the samples.

Table 3 details the cultural characteristics of the bacterial isolates from the mouth flora of smokers. Each isolate exhibits distinct characteristics in terms of color, appearance, elevation, and opacity. For instance, isolate M13 is grayish-white with a smooth, glossy appearance and is flat and translucent, while isolate M23 appears golden-yellow, shiny, and raised with an opaque characteristic. These cultural traits are critical for identifying and classifying the bacterial isolates.

Table 4 summarizes the biochemical reactions and Gram staining characteristics of the bacterial isolates from the mouth flora of smokers. The isolates demonstrate varying reactions to different biochemical tests. For example, isolate M13 is β-hemolytic and identified as *Enterococcus faecalis*, showing positive results for Gram staining and catalase tests but negative for oxidase and indole tests. In contrast, isolate M5, identified as *Lactobacillus lactis*, is non-hemolytic and exhibits different biochemical characteristics. This information is essential for understanding the metabolic capabilities of the isolated bacteria.

Table 1: Oral sample collection from smokers

Samples code	Type	Source	Date	Time
M1	Oral	Smokers	11/April/2024	11:00 am
M2	Oral	Smokers	11/April/2024	11:00 am
M3	Oral	Smokers	11/April/2024	11:00 am
M4	Oral	Smokers	11/April/2024	11:00 am
M5	Oral	Smokers	11/April/2024	11:00 am

Table 2: Colony forming unit of bacterial isolates from mouth flora of smokers

Sample	CFU/mL
M13	8.0 × 10^4
MI5	6.6 × 10^4
M23	9.8 × 10^4
M25	7.1 × 10^4
M33	6.7 × 10^4
M35	5.4 × 10^4
ML13	4.7 × 10^4
M45	3.7 × 10^4
M53	4.1 × 10^4
M55	3.0 × 10^4

Keys: cfu/ml = colony forming unit per milliliter

Table 3: Cultural characteristics of bacterial isolates from mouth flora of smokers

Isolate Name	Color	Appearance	Elevation	Opacity
M13	Grayish-white	Smooth, glossy	Flat	Translucent
Ml5	Cream to white	Milky, creamy	Flat	Opaque
M23	Golden-yellow	Shiny, smooth	Raised	Opaque
M25	White to gray	Circular, smooth	Flat to slightly raised	Opaque
M33	White to off-white	Rough, mucoid	Flat	Opaque
M35	White to pale yellow	Granular, dry	Convex	Opaque
ML13	Creamy white	Smooth, glistening	Flat	Opaque
M45	Cream to light yellow	Smooth, shiny	Flat	Translucent
M53	Grayish-white	Glossy, smooth	Raised	Opaque
M55	Yellow to brown	Irregular, rough	Raised	Opaque

Table 4: Biochemical reaction and Gram staining characteristics of bacterial isolates from mouth flora of smokers

Isolate code	Gram Reaction	Catalase Test	Oxidase Test	Indole Test	Citrate Utilization	Fructose Acid	Fructose Gas	Mannitol Acid	Mannitol Gas	Ribose Acid	Ribose Gas	Naitol Acid	Naitol Gas	Dextrose Acid	Dextrose Gas	Hemolysis Test	Isolate Name
M13	+	+	-	-	-	+	+	+	+	-	-	+	+	+	+	β-hemolytic	*Enterococcus faecalis*
Ml5	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-	Non-hemolytic	*Lactobacillus lactis*
M23	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	β-hemolytic	*Staphylococcus aureus*
M25	+	+	-	+	-	+	+	-	-	-	-	-	+	-	-	β-hemolytic	*Streptococcus agalactiae*
M33	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	Non-hemolytic	*Stomatococcus mucilaginosus*
M35	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	Non-hemolytic	*Corynebacterium spp.*
ML13	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-	Non-hemolytic	*Lactococcus acidophilus*
M45	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Non-hemolytic	*Bifidobacterium bifidum*
M53	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	Non-hemolytic	*Streptococcus anginosus*
M55	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	Non-hemolytic	*Actinomyces bovis*

Key: - = Negative, + = positive

Fig 3 illustrates the zone of inhibition of oil extracts at 100 mg/mL against bacterial isolates from the mouth flora of smokers. The results show varying inhibitory effects of different oil extracts on the bacterial isolates. For instance, *Enterococcus faecalis* shows an inhibition zone of 9.0 mm with YCE and 20.0 mm with control, while *Staphylococcus aureus* exhibits a zone of 13.0 mm with YCE and 18.0 mm with control. This table provides insights into the antimicrobial potential of the oil extracts against oral bacteria.

Fig 4 shows the zone of inhibition of oil extracts at 50 mg/mL against the bacterial isolates. Similar to Table 5, the results indicate varying inhibitory effects. *Enterococcus faecalis* displays a 6.0 mm zone with YCE and 20.0 mm with control, while *Staphylococcus aureus* shows an inhibition of 17.0 mm with YCE and 18.0 mm with control. The consistency in results across different concentrations highlights the potential effectiveness of these extracts.

Fig 5 shows the zone of inhibition of oil extracts at 25 mg/mL against bacterial isolates. The inhibition zones are smaller compared to the higher concentrations. For example, *Enterococcus faecalis* has a 3.0 mm zone with YCE and 20.0 mm with control, while *Staphylococcus aureus* shows a zone of 11.0 mm with YCE and 18.0 mm with control. This decrease in inhibition reflects the impact of extract concentration on antibacterial activity.

Fig 6 shows the zone of inhibition of oil extracts at 12.5 mg/mL against bacterial isolates. The results indicate minimal inhibition, with *Enterococcus faecalis* exhibiting no inhibition with YCE and a 20.0 mm zone with control. Similarly, *Staphylococcus aureus* shows no inhibition with YCE, highlighting the reduced effectiveness of the extracts at lower concentrations.

Fig 3 :Inhibition zone of *Nicotiana Tabacum Essential Oil* Extract at 100 mg/ml against bacterial isolates from mouth flora of smokers

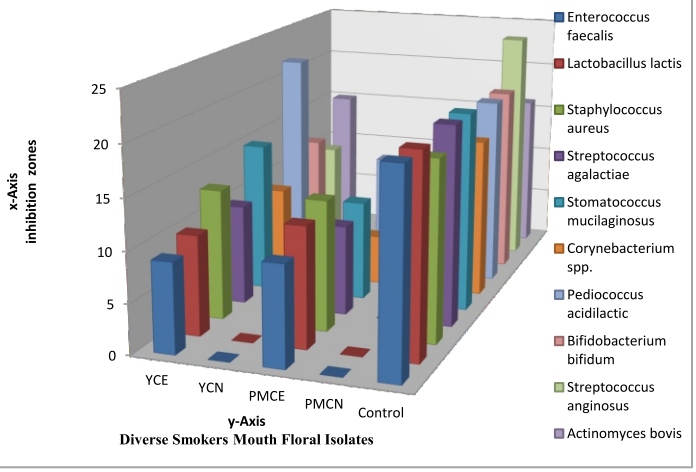


Fig 4 : Inhibition zone of *Nicotiana Tabacum Essential Oil* Extract at 50 mg/ml against bacterial isolates from mouth flora of smokers

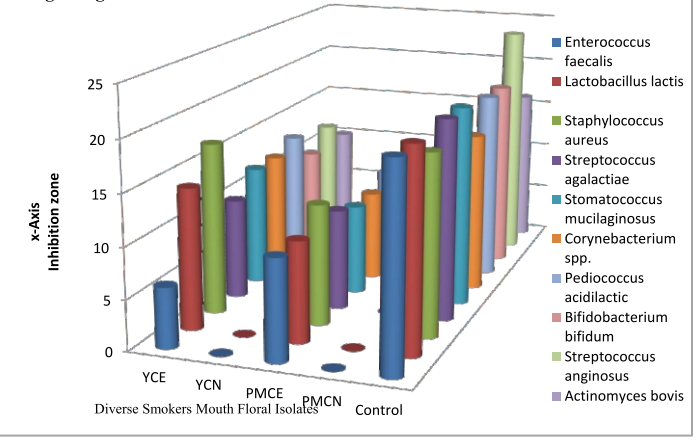


Fig 5 : Inhibition zone of *Nicotiana Tabacum Essential Oil* Extract at 25mg/ml against bacterial isolates from mouth flora of smokers

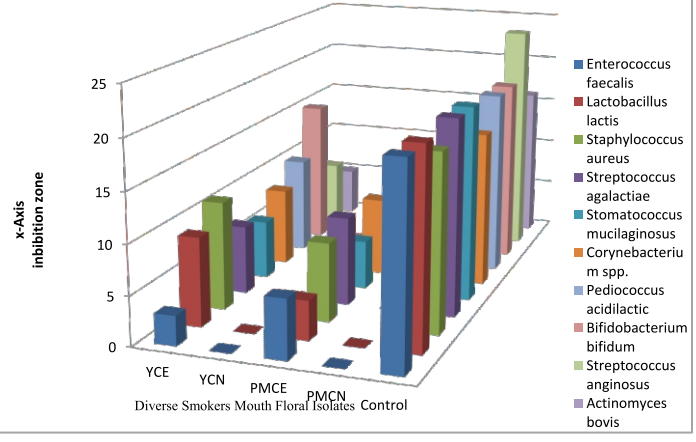
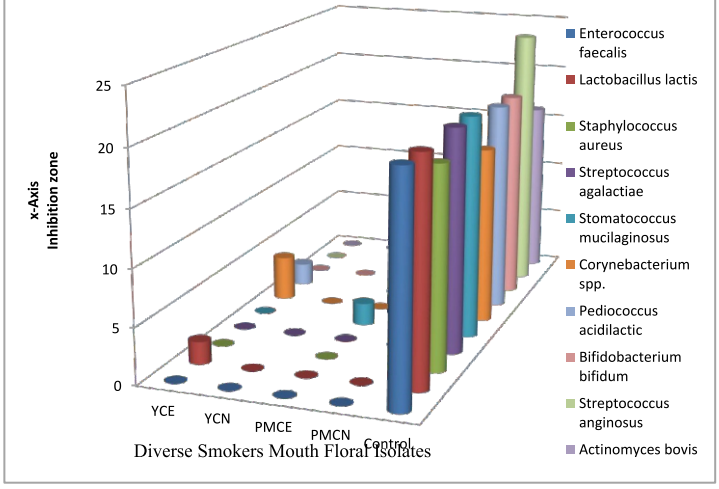


Fig 6 : Inhibition zone of *Nicotiana Tabacum Essential Oil* Extract at 12.5 mg/ml against bacterial isolates from mouth flora of smokers



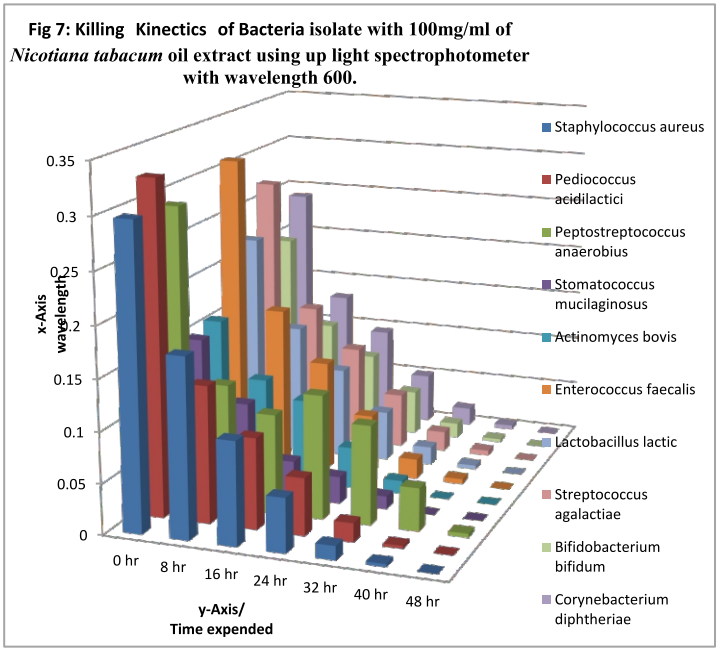
Keys YCE = Yes cigarette socked in solvent (Ethanol), YCN = Yes cigarette socked in solvent (N-hexane), PMCE = Pall mallcigarette socked in solvent (Ethanol) PMCE = Pall mallcigarette socked in solvent (N-hexane), CON = Positive control (Ampicillin)

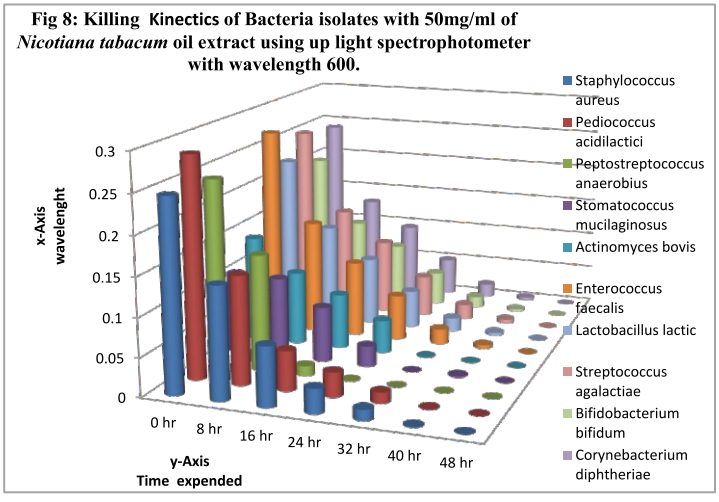
Fig 7 shows the killing time of bacterial isolates with 100 mg/mL of *Nicotiana tabacum* oil extract. The data shows a reduction in optical density over time for different isolates, indicating the effectiveness of the oil extract. For example, *Staphylococcus aureus* shows an initial OD of 0.297 at 0 hours, which decreases to 0.000 by 48 hours, demonstrating a significant reduction in bacterial load. it was observe that between 0 hour to 32 hour the kinetic was bacterotatic and bacterocide between 40 – 48 hours

Fig 8 shows the killing time of bacterial isolates with 50 mg/mL of *Nicotiana tabacum* oil extract. Similar to Table 9, the OD readings decrease over time, indicating the antibacterial activity of the extract. For instance, *Staphylococcus aureus* shows an OD of 0.246 at 0 hours, reducing to 0.000 by 48 hours, again underscoring the efficacy of the extract against this bacterial isolate. it was deduced that between 0 hour to 32 hour the kinetic was bacterotatic and bacterocide between 40 – 48 hours

Fig 9 , shows the killing dynamic of *Nicotiana tabacum* oil extract with the concentration of 25 mg/mL. *Staphylococcus aureus* shows a significant decline in OD, decreasing from 0.234 at 0 hours to 0.000 by 48 hours, indicating effective bacterial killing. Similarly, *Pediococcus acidilactici* demonstrates a drop in OD from 0.244 to 0.000 over the same period, suggesting high susceptibility to the extract. *Peptostreptococcus anaerobius* shows a decline from 0.197 to 0.000, reflecting effective antibacterial action.

Stomatococcus mucilaginosus also exhibits a decrease in OD from 0.130 to 0.000, confirming its susceptibility. *Actinomyces bovis* reduces from 0.136 to 0.000, while *Enterococcus faecalis* drops from 0.270 to 0.000, both indicating effective killing over time. Lactobacillus lactic decreases from 0.220 to 0.000, and *Streptococcus agalactiae* shows a reduction from 0.250 to 0.000. *Bifidobacterium bifidum* decreases from 0.200 to 0.000, and *Corynebacterium diphtheriae* also shows a decline from 0.240 to 0.000, highlighting the antibacterial efficacy of the extract across all isolates. it was observe that between 0 hour to 32 hour the kinetic was bacterotatic and bacterocide between 40 – 48 hours.





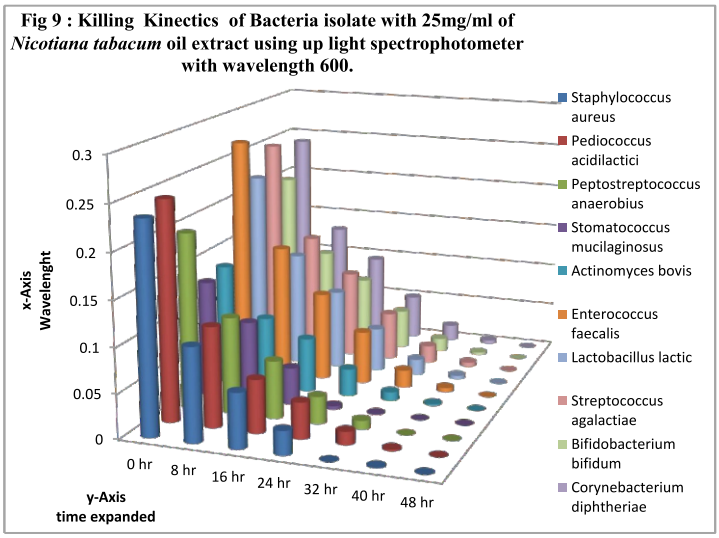
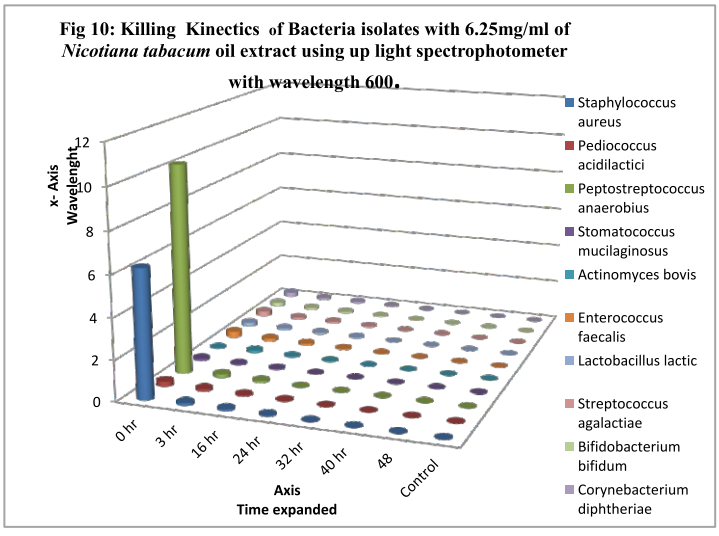
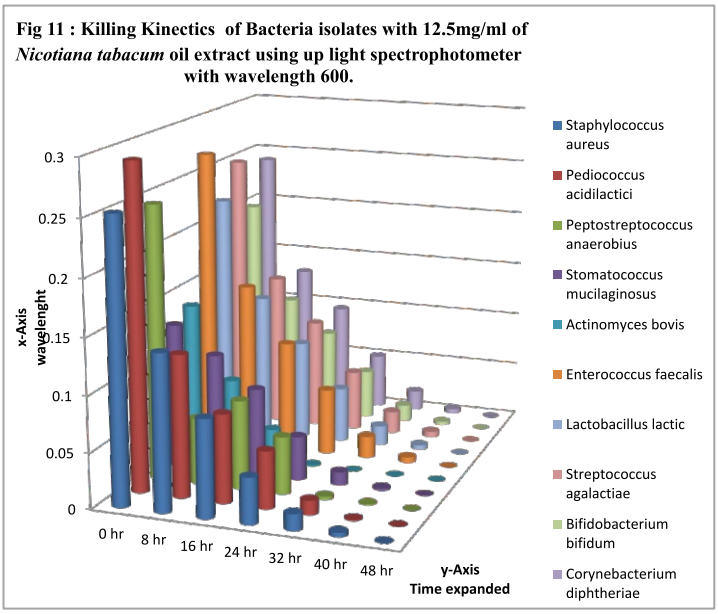


Fig 10 presents the data for a lower concentration of 6.25 mg/mL of *Nicotiana tabacum* oil extract. Here, *Staphylococcus aureus* starts at a high OD of 6.276 at 0 hours, which decreases to 0.000 at 48 hours, indicating that despite the initial high reading, the extract effectively eliminates the bacteria over time. *Pediococcus acidilactici* shows a decrease from 0.245 to 0.00, further demonstrating the extract's efficacy. *Peptostreptococcus anaerobius* has a starting OD of 10.215, which falls to 0.00, indicating substantial antibacterial activity despite the higher initial count. *Stomatococcus mucilaginosus* begins at 0.120 and decreases to 0.00, while *Actinomyces bovis* shows a drop from 0.044 to 0.00, indicating it is also susceptible to the extract. *Enterococcus faecalis* decreases from 0.300 to 0.00, and Lactobacillus lactic goes from 0.200 to 0.00, confirming effectiveness. *Streptococcus agalactiae* shows a decline from 0.250 to 0.00, *Bifidobacterium bifidum* from 0.180 to 0.00, and *Corynebacterium diphtheriae* from 0.220 to 0.00, indicating that even at lower concentrations, the extract remains effective against these isolates..it was observe that between 0 hour, the kinetic was bacterotatic and bacterocide between 3rd– 48 hours.





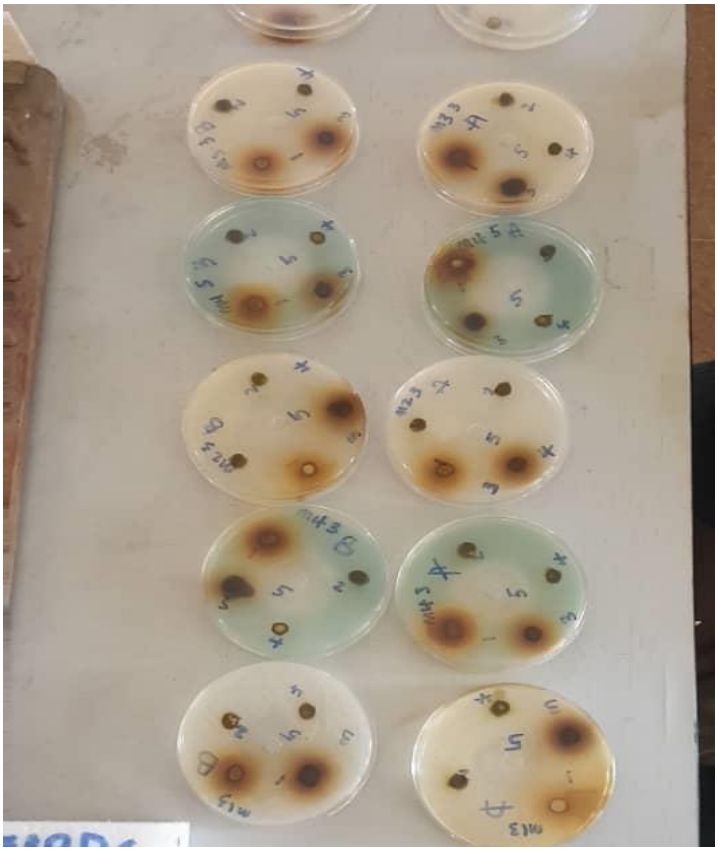


Plate 1: Antimicrobial susceptibility test analysis of plant extracts (Personal photograph)

Discussion

This study aimed to evaluate the bacterial divesity, and the antimicrobial potential of *Nicotiana tabacum* oil extracts against diverse Smokers Mouth Floral isolates The saying that' Smokers a liable to die young" it is not only the smokers that a liable, but also the microbiota i.e Microbioal diversity, of the mouth flora are also liable to die young .The *Nicotiana tabacum* oil extract from the cigarette has a varing degree of effects on the microbial community in the smokers mouth which are ordinarily the normal mouth flora.

The results of the study investigating the oral microbiota of smokers reveal significant insights into bacterial load, cultural characteristics, biochemical traits, and the antimicrobial potential of *Nicotiana tabacum* oil extracts.

The results in this study highlight variations in colony-forming units (CFUs) among different samples, as well as the differential responses of bacterial isolates to varying concentrations of oil extracts. This study found that sample the cfu/ml ranged from 3.0 x 104, CFU/mL to9.8 x 104, CFU/mL. This variability is consistent with findings by (2), who noted that tobacco smoking alters the oral microbiome, leading to shifts in bacterial populations. The presence of pathogenic bacteria is often heightened in smokers, potentially contributing to increased risks of oral diseases. Such differences underscore the impact of environmental factors, like smoking, on microbial load, affirming the need for continuous monitoring of oral health in smokers.

The distinct cultural traits and biochemical properties of isolates such as Enterococcus faecalis and Lactobacillus lactis are crucial for identification and classification, as supported by (22). These characteristics can provide insights into the metabolic capabilities and pathogenic potential of these bacteria, with some isolates showing hemolytic activity indicative of virulence. This finding reinforces the narrative that smoking can foster a pathogenic oral environment, potentially leading to conditions such as periodontal disease and oral cancers, as discussed by (23).

The results of this study also highlight the varying zones of inhibition exhibited by *Nicotiana tabacum* oil extracts against isolated bacterial from smokers. Notably, the extract demonstrated significant antibacterial activity at higher concentrations (100 mg/mL), with *Enterococcus faecalis* showing an inhibition zone of 9.0 mm and *Staphylococcus aureus* of 13.0 mm. The effectiveness diminished with lower concentrations, as seen in Tables 7 and 8, where minimal inhibition was noted at 12.5 mg/mL. These results are consistent with studies indicating that higher concentrations of plant extracts often yield greater antimicrobial effects (24). However, the reduction in efficacy at lower concentrations aligns with the results of (25), which emphasizes the importance of optimizing extract concentrations for effective antibacterial action.

The killing time data presented in this study demonstrate a clear trend of bacterial load reduction over time with varying concentrations of *Nicotiana tabacum* oil extract. For instance, *Staphylococcus aureus* exhibited a complete reduction in optical density (OD) from 0.297 to 0.000 over 48 hours at 100 mg/mL, reflecting the extract's potent antibacterial properties. This finding corroborates previous research by (19,26), which illustrated the detrimental impact of tobacco smoke on salivary bacterial populations, potentially exacerbated by the pathogenic bacteria present in the oral cavity of smokers.

The results of this study not only align with existing literature but also expand upon it by illustrating the potential of *Nicotiana tabacum* extracts as a therapeutic agent against oral pathogens commonly found in smokers. The ability of these extracts to inhibit bacterial growth could offer a valuable adjunct to conventional oral health strategies, especially for populations at increased risk due to tobacco use. Future research should aim to explore the molecular mechanisms behind the antibacterial properties of *Nicotiana tabacum* and investigate the long-term effects of its application in oral health care.

The study on the oral microbiota of smokers reveals critical insights into the interplay between tobacco use and microbial dynamics in the oral cavity. The significant variations in colony-forming units (CFUs) and the presence of pathogenic bacteria underscore the detrimental effects of smoking on oral health.

The cultural characteristics and biochemical traits of bacterial isolates, particularly *Enterococcus faecalis* and *Lactobacillus lactis*, highlight the need for precise identification methods and underscore the potential virulence of these bacteria in smokers. Moreover, the antibacterial efficacy of *Nicotiana tabacum* oil extracts demonstrates its potential as a therapeutic agent against oral pathogens, particularly at higher concentrations. Overall, these findings contribute to a growing body of evidence linking smoking to altered oral microbiota and increased susceptibility to oral diseases.

Recommendations

I. Establish regular evaluations to monitor bacterial load and detect early signs of oral diseases, along with educational programs on tailored oral hygiene practices.

II. Investigate the molecular mechanisms of its antibacterial properties and conduct clinical trials to assess the long-term efficacy and safety of its extracts as adjuncts to standard oral health treatments for smokers.

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COMPETING INTERESTS

The authors have declared that no competing interests exist.

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