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Antibacterial Activities of *Glycyrrhiza Glabra* Roots and *Ocimum Sanctum* Leaves on Cariogenic Bacteria Isolated from Dental Caries Patients in Umuna, Onuimo LGA

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Abstract

This study assessed the antibacterial activity of *Glycyrrhiza glabra* roots and *Ocimum sanctum* leaves against cariogenic bacteria recovered from dental caries patients in Umuna, Onuimo LGA, Imo State, using standard laboratory procedures. Seventy volunteers aged 15 to 70 years were examined for dental health problems according to World Health Organization criteria. Mean Decayed, Missing and Filled Teeth (DMFT) values ranged from 0.16 to 0.75, and the total DMFT of 5.1 pointed to a high prevalence of dental disease. Caries status of each participant was confirmed with the salivary reductase test, which showed active caries in 48% of males and 28% of females, while 22% had no caries. The cariogenic bacteria recovered, with their frequencies, were *Streptococcus mutans* (33%), *Streptococcus sobrinus* (18%), *Lactobacillus fermentum* (15%), *Streptococcus salivarius* (12%), *Streptococcus sanguinis* (12%) and *Streptococcus mitis* (10%). Antibacterial activity was tested by the agar well diffusion method. Aqueous, ethanolic and methanolic extracts of *G. glabra* roots and *O. sanctum* leaves gave inhibition zones of 15.00 to 23.00 mm at 100 to 500 mg/mL, against 19.00 mm for the chlorhexidine control. The combined extracts were more active, giving inhibition zones of 18.00 to 28.00 mm over the same concentration range. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) ranged from 15.63 to 500 mg/mL for *G. glabra* roots and from 62.5 to 1000 mg/mL for *O. sanctum* leaves, while the combination gave lower values of 3.90 to 250 mg/mL. The Fractional Inhibitory Concentration Index (FICI) was used to classify the interaction between the extracts, and a value of 0.49 (≤ 0.5) showed synergy. Gas chromatography and mass spectrometry (GC-MS) of *G. glabra* root revealed 28 bioactive compounds, with mome inositol as the major constituent (28%) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol as the minor one (0.33%). The *O. sanctum* extract contained 23 compounds, led by estragole (19.4%), with trans-4-thujanol present at the lowest level (0.447%). These varied bioactive compounds may explain the synergistic antibacterial effect of the two plants and support their potential use as anticariogenic agents.

I. INTRODUCTION

Oral diseases remain a leading health problem across the world (WHO, 2025). Dental caries and periodontal disease are the most important of these, although oral and pharyngeal cancers and lesions of the oral tissues are also of serious concern. Even with the general gains in health, including oral health, recorded in industrialized countries, caries still affects up to 90% of school children and most adults in those countries (WHO, 2025).

Oral health forms part of general well-being and shapes quality of life well beyond the functions of the mouth and face. A large body of evidence ties poor oral health to chronic conditions; severe periodontal disease, for example, is closely associated with diabetes (Frencken *et al*., 2024). Poor oral health has also been linked with systemic illnesses such as cardiovascular disease, rheumatoid arthritis and osteoporosis (Marsh, 2025), and periodontal disease may raise the risk of pregnancy complications such as preterm, low birth weight delivery (Shouri, 2023).

Loss of teeth from poor periodontal health, which affects up to 20% of adults worldwide, can result in considerable illness and early death (WHO, 2025). The

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cost of oral disease is also a major concern, as curative dental care accounts for up to 10% of public health spending in developed countries. In most developing countries, spending on oral care is low, access to dental services is limited, and care is often restricted to emergencies or pain relief. Although oral health has improved markedly in most developed countries, pockets of dentally disadvantaged people remain within them, often indigenous children and people of low socioeconomic status, among whom oral health is getting worse (Ramchandran *et al*., 2024).

The connection between oral diseases and the activity of microorganisms living in the mouth is well recognized (Clarke, 2023). More than 750 bacterial species occupy the oral cavity, about half of them not yet identified, and several of these are involved in oral disease (Frencken *et al*., 2024).

Dental caries, commonly called tooth decay or a cavity, is a localized and transmissible infection, usually bacterial, that destroys the hard tissues of the tooth. It arises from plaque build-up on tooth surfaces and from the biochemical activity of complex microbial communities. *Streptococcus mutans* is one of the chief opportunistic pathogens involved. In the population of developing countries, the occurrence of caries is about 60 to 65% (Saini *et al*., 2022).

Caries develops through the action of Gram-positive bacteria that both produce acid and tolerate it, mainly the mutans streptococci (*Streptococcus mutans* and *S. sobrinus*), lactobacilli and actinomycetes. These organisms ferment sucrose into organic acids, chiefly lactic acid, which dissolve the calcium phosphate of the tooth and cause decalcification and decay. Caries is therefore a supragingival condition (Marsh, 2009). *Streptococcus mutans* and *Lactobacillus acidophilus* are the most frequent causes of caries. They readily colonize the tooth surface and start producing acid while building extracellular polysaccharides from sucrose-rich foods (Shouri, 2023). For this reason, a caries prevention programme should aim first at reducing cariogenic bacteria.

Periodontal diseases, on the other hand, are subgingival conditions associated with anaerobic Gram-negative bacteria such as *Porphyromonas gingivalis*, *Actinobacillus* sp., *Prevotella* sp. and *Fusobacterium* sp. (Ramchandran *et al*., 2024). In these diseases, the tissues at or below the gingival crevice become infected, and the gums and nearby connective tissue respond with cellular inflammation. The response may appear as gingivitis, which is very common and shows as bleeding gums, or as periodontitis, in which inflammation destroys the collagen attachment of the tooth to bone and leads to bone loss (Frencken *et al*., 2024).

Many mouth rinses on the market can control cariogenic bacteria, but most of them have drawbacks, including altered taste, a burning feeling, diarrhoea and tooth staining. These problems have prompted the search for other rinsing agents, particularly for children (Park *et al*., 2024).

The global demand for safe, effective and affordable options for preventing and treating oral disease stems from the rising incidence of these diseases, especially in developing countries, and from growing resistance of pathogenic bacteria to existing antibiotics and chemotherapeutic agents. Resistance has been recorded, for example, to most if not all antibiotics commonly used for oral infections, including penicillins, cephalosporins, erythromycin, tetracycline and its derivatives, and metronidazole (Velmurgan *et al*., 2024). Other antibacterial agents used against oral diseases, such as cetylpyridinium chloride, chlorhexidine, amine fluorides and products containing them, are reported to be toxic or to stain teeth, and the ethanol found in many mouthwashes has been associated with oral cancer (Van Leeuwen *et al*., 2011). The search for alternatives therefore continues, and natural phytochemicals from plants used in traditional medicine are viewed as good substitutes for synthetic chemicals (Rekha *et al*., 2024).

For thousands of years, and in many parts of the world, medicinal plants have served as traditional remedies for a wide range of human illnesses. In rural areas of developing countries, they may be the main source of medicine, and about 80% of people in these countries rely on traditional medicine for their health care (Ramachandran *et al*., 2024). Natural products from medicinal plants are a rich source of biologically active compounds, and many of them have provided the basis for new lead molecules in drug development (Jain *et al*., 2025). For diseases caused by microorganisms, increasing resistance of common pathogens to current antibiotics and antiviral drugs has revived interest in finding new anti-infective compounds. Of the roughly 500,000 plant species in the world, only about 1% have been studied phytochemically, so the scope for discovering new bioactive compounds is large (Yamani *et al*., 2024).

Several of the mouth rinses now available cause taste disturbance, burning and tooth staining, which has driven the search for alternative rinsing agents, mainly for children. Herbal preparations are regarded as moderately effective and less toxic than the most widely used pharmaceutical rinses (Nayak *et al*., 2024).

Many reports describe the use of traditional plants and natural products in treating oral diseases. Numerous plant medicines from traditional systems appear in pharmacopoeias as anti-infective agents, and several have recently been tested against oral pathogens. The general antimicrobial activity of medicinal plants and plant products such as essential oils has been studied and documented (Van Leeuwen *et al*., 2011).

Herbs such as tulsi patra, neem, clove oil, pudina and ajwain, used singly or in combination, have been shown scientifically to be safe and effective against oral problems such as bleeding gums, bad breath, mouth ulcers and tooth decay (Jazaeri *et al*., 2023).

Ocimum sanctum (OS), widely known as tulsi, is a small shrub of the mint family, Lamiaceae, with small, strongly scented leaves and purple flowers (Pai *et al*., 2023). Tulsi is a long-established and highly valued medicinal herb. It originated in India, where Hindus revere it, and it has been used in Ayurvedic medicine since ancient times. It ranks among the most sacred herbs and is grown widely in Asia and Africa. It has marked antibacterial, antioxidant and anti-inflammatory properties, contains many antimicrobial substances, and is used against illnesses ranging from diabetes mellitus and arthritis to bronchitis and skin disease (Yamani *et al*., 2024). It has also proved active against several oral pathogens and gives relief from toothache (Nair & Shiva, 2023).

Liquorice, or *Glycyrrhiza*, is a herb with several known medicinal uses. In ancient times it served both as a sweetener and as a remedy for many ailments (Ajagannanavar *et al*., 2025). Its roots are rich in saponins and glycyrrhizin; these sweet substances have been found to be excellent cariostatic agents (Awamie & Rees, 2023).

In this study, a combination of *Glycyrrhiza glabra* (liquorice) and *Ocimum sanctum* (tulsi) was used to assess their possible anticariogenic effect on oral pathogens, with a view to developing a simple and inexpensive remedy for preventing and treating cariogenic diseases.



Fig 1 Glycyrrhiza Glabra (Liquorice)



Fig 2 Ocimum Sanctum (Tulsi)

➢ *Justification*

Dental caries is still a major oral health burden worldwide. According to the World Health Organization (WHO, 2022), about 2.3 billion people have caries in their permanent teeth, placing it among the most common chronic diseases. In Africa, an estimated 91% of adults aged 20 to 64 years have had caries in their permanent teeth. This burden adds considerably to health care costs.

Most people experience local episodes of disease at some point in life because of imbalances in their natural oral flora. Much evidence indicates that *S. mutans* is the main organism responsible for caries, although other acid-producing organisms such as *L. acidophilus* also take part in the damage. These pathogens can break down carbohydrates, attach to tooth surfaces and form biofilms.

Several studies on cariogenic organisms suggest that some natural products can reduce the growth and virulence of *S. mutans* and *L. acidophilus* and so help to prevent and treat caries. A safe, effective and affordable treatment approach is needed. Because synthetic drugs have many side effects, this research focuses on assessing natural remedies that are both safe and effective for treating and preventing caries.

➢ *Aim and Objectives*

This research aims to screen the antibacterial efficacy of *Glycyrrhiza glabra* roots and *Ocimum sanctum* leaves against cariogenic organisms.

- *The Objectives Include*

✓ To isolate and identify cariogenic bacteria from patients with dental caries
✓ To prepare different concentrations of the plant extracts and test them against the isolated pathogens
✓ To determine the zones of inhibition of the plant extracts against the pathogens
✓ To evaluate possible synergistic or antagonistic effects of the two plant extracts on the test organisms
✓ To determine the minimum inhibitory concentrations of the extracts against the organisms
✓ To determine the minimum bactericidal concentrations of the extracts
✓ To identify and quantify the bioactive compounds in the two plant extracts

II. MATERIALS AND METHODS

➢ *Study Area*

The study took place in the Department of Microbiology, Bioresources Institute of Nigeria, Umuna, Onuimo LGA, Imo State, in collaboration with the Department of Dental Clinics, Federal Medical Centre, Umuahia, Abia State, Nigeria.

➢ *Sample Collection*

Tooth swabs were taken from 100 in-patients with sterile swab sticks, placed in sterile zip-lock polyethylene bags and transported to the Microbiology Laboratory of

the Bioresources Institute of Nigeria, Umuna, Onuimo LGA, Imo State, for isolation of cariogenic bacteria.

➢ *Salivary Reductase Test*

This test measures the activity of reductase enzymes in saliva by recording how quickly the indicator diazoresorcinol changes from blue to red and then to colourless.

Participants chewed a piece of paraffin to produce saliva, which was spat into a tube containing the reagent and mixed well. The colour change seen between 30 seconds and 15 minutes was taken as a measure of caries activity (Güven *et al*., 1996).

➢ *Microbial Examination*

The 100 swab sticks and one unused swab stick (control) were analysed by the standard spread plate count method (Cheesbrough, 2005). Materials were sterilised and media prepared before samples were inoculated.

- *Sterilization of Materials Used*

All materials except heat-labile ones were sterilised by autoclaving at 121 °C for 15 minutes and then left to cool at room temperature.

- *Media Preparation*

All media for culture, isolation, subculture and biochemical tests were prepared as directed by the manufacturers (Cheesbrough, 2005).

- *Preparation of De Man, Rogosa and Sharpe Agar*

The powdered medium (2.8 g) was weighed accurately on an electronic balance, dissolved in 100 mL of distilled water and poured into sterile Petri dishes.

- *Preparation of Tryptone Yeast Extract Cysteine Sucrose and Bacitracin Agar*

The powdered medium (6.2 g) was dissolved in 100 ml of distilled water, autoclaved at 121 °C for 15 minutes, cooled to 50 to 55 °C and poured into sterile Petri dishes.

➢ *Sample Preparation and Inoculation*

The buccal surfaces of the teeth were swabbed with sterile cotton swabs, and each swab was immersed in 100 mL of sterile buffered peptone water (0.1% w/v) for about two hours at ambient temperature so that the cells were released into suspension.

The swabs were then taken out, and the peptone water suspension was used as the test sample. This suspension was serially diluted from 10^{-1} to 10^{-6}, and 0.1 mL of the 10^{-3} dilution was inoculated onto pre-set MRS and TYECB agar plates by the spread plate method. Plates were incubated at 37 °C for 24 to 72 hours.

➢ *Subculturing and Preservation of Isolates*

Colonies were selected on the basis of their cultural features and subcultured aseptically on nutrient agar by streaking. Isolates were kept on nutrient agar slants as stock cultures for later morphological, biochemical and antibacterial tests. Identification to genus level, based on cultural, morphological and biochemical features, followed Cheesbrough (2005).

➢ *Characterization and Identification of the Isolates*

Presumptive isolates were identified from their microscopic and biochemical features. Microscopic examination involved motility testing, spore staining and Gram staining. The biochemical tests performed were catalase, oxidase, coagulase, citrate utilization, urease activity, indole production and Kligler iron agar (Cheesbrough, 2005).

➢ *Identification of Bacteria*

- *Spore Stain*

This stain separates spore-forming from non-spore-forming bacteria. Following Cheesbrough (2005), a heat-fixed smear on a glass slide was covered with 5% malachite green and heated to steaming for 3 to 5 minutes. After cooling, the slide was rinsed with water and counterstained with safranin for 30 seconds, rinsed again, air dried and examined under the oil immersion lens. Vegetative cells stained red and spores stained green.

- *Motility Test*

This test identifies organisms that have organs of locomotion. A drop of the isolate suspension was placed on a slide, covered with a coverslip and sealed with melted petroleum jelly so that no air was trapped. It was examined under the ×10 and ×40 objectives with the condenser iris diaphragm closed enough to give good contrast. Rapid, darting movement in different directions across the field indicated a motile organism.

- *Gram Stain*

Christian Gram introduced this technique in 1884 to identify and classify bacteria as Gram positive or Gram negative according to whether they resist or allow decolourization of the methyl violet stain by acetone (Cheesbrough, 2005).

A thin smear of each isolate was made on a clean, grease-free slide by mixing a loopful of the culture with a drop of distilled water. The smear was air dried and heat fixed by passing it over a Bunsen flame, then covered with crystal violet for 1 minute. The slide was rinsed under slowly running water for 5 seconds, covered with Gram's iodine for 30 seconds and rinsed again. It was decolourized with acetone for 10 to 15 seconds and rinsed once more under slowly running tap water. Carbol fuchsin was then applied as the counterstain for 30 seconds and washed off. The slide was air dried and examined with the ×100 oil immersion objective. Gram-positive organisms kept the purple primary stain, while Gram-negative organisms appeared pink.

➢ *Biochemical Tests*

Biochemical tests are useful for telling apart bacteria of different genera and species on the basis of their biochemical properties.

- *Catalase Test*

This test shows whether an organism produces catalase, an enzyme that releases oxygen from hydrogen peroxide by breaking down this toxic compound into harmless oxygen and water. Catalase is produced by aerobic organisms such as *Staphylococcus aureus*, and the test is used to separate staphylococci from streptococci.

A small amount of each isolate was taken from a nutrient agar slope with a sterile platinum loop and placed in hydrogen peroxide in a small, clean test tube. Gas bubbles indicated a positive result.

- *Oxidase Test*

This test detects the oxidase enzyme, which moves electrons between electron donors in the bacterium and the redox dye tetramethyl-p-phenylenediamine.

A few drops of freshly prepared 1% aqueous tetramethyl-p-phenylenediamine hydrochloride were placed on a small area of Whatman No. 1 filter paper in a Petri dish. The isolate was smeared onto the treated paper with a sterile platinum loop and observed for 5 minutes. A purple colour within 5 to 10 minutes indicated an oxidase-positive culture.

- *Urease Activity Test*

This test identifies bacteria that produce urease, which hydrolyses urea to ammonia and carbon dioxide (Cheesbrough, 2005). Modified Christensen urea agar was prepared as instructed by the manufacturer, dispensed into bijou bottles, sterilised and left to set as slants. The slants were inoculated by streaking the slope and then stabbing the butt, and incubated at 37 °C for 24 hours. A change in the medium from yellow to pink was recorded as positive.

- *Indole Test*

This test identifies organisms that break down tryptophan and release indole, which reacts with Kovac's reagent to give a red compound. Each isolate was grown in peptone water at 37 °C for 24 to 28 hours. About 0.5 mL of Kovac's indole reagent was then added to the tube and shaken gently. A pink layer on the surface of the medium within 10 minutes indicated a positive result.

- *Kligler Iron Agar (Kia)*

This test is based on the fermentation of lactose and glucose and the production of hydrogen sulphide. KIA was prepared according to the manufacturer's directions, dispensed in 6 mL amounts into large tubes, sterilised and left to set as slants with a butt and a slope. The slants were inoculated by stabbing the butt and streaking the slope in a zigzag pattern, incubated at 37 °C for 24 hours, and examined for colour change, cracks in the medium and hydrogen sulphide production.

✓ *Preparation of Plant Extract*

Glycyrrhiza glabra roots and *Ocimum sanctum* leaves were obtained from the organic farm of the Bioresources Institute of Nigeria, Umuna, Onuimo LGA, and identified by a botanist. The materials were dried at 40 °C, ground to powder and kept in airtight containers until use. Extracts were prepared by the method of Jones and Kinghorn (2006). One hundred grams of sliced roots and powdered leaves were soaked in 500 mL of ethanol and of distilled water for 36 hours with shaking at intervals. The extracts were filtered through Whatman No. 1 filter paper and concentrated to dryness under reduced pressure at 37 °C in a rotary evaporator (Strike 202 Steroglass, Italy) to remove the solvents. The crude extracts were stored at 4 °C until needed. Before the bioassay, the reconstituted extract solutions were sterilised by passing them through a 0.45 µm membrane filter.

✓ *Antimicrobial Susceptibility Testing*

The sensitivity of the isolates to *Glycyrrhiza glabra* root and *Ocimum sanctum* leaf extracts was tested by the agar well diffusion method described by the Clinical and Laboratory Standards Institute (CLSI, 2012), with the isolates grown on Mueller-Hinton agar.

Plates were swabbed with cotton wool soaked in the test organism adjusted to the 0.5 McFarland standard. Five wells were bored in each plate with a sterile 6 mm cork borer. Four wells were filled with extract at 250, 200, 100 and 50 mg/mL, and the fifth with the control (chlorhexidine, 1.25 mg/mL). The plates were left to stand for 20 minutes to allow the solutions to diffuse into the medium and were then incubated at 37 °C for 24 hours. Antimicrobial activity was assessed by measuring the zones of inhibition against the test organisms. The test was done twice, and the zones of inhibition are given as mean ± SD.

✓ *Determination of Synergistic or Antagonistic Effects Using the Fractional Inhibitory Concentration Index (FICI)*

The Mueller-Hinton broth method was used to determine whether the extracts showed synergistic, antagonistic, additive or indifferent effects, as described by CLSI (2012). Each extract (*Glycyrrhiza glabra* roots and *Ocimum sanctum* leaves) was serially diluted twofold, and the dilutions were mixed to give a range of concentrations corresponding to 1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 and 1/256 of the MIC values. About 20 µL of each bacterial suspension was added to 180 µL of extract in the wells. The plates were incubated overnight at 37 °C and then checked for colour change.

▪ *The FICI was Calculated as Follows:*

FICI = (MIC of extract A in combination ÷ MIC of extract A alone) + (MIC of extract B in combination ÷ MIC of extract B alone)

The FICI was interpreted as follows: synergistic when FICI ≤ 0.5; additive or indifferent when FICI > 0.5 and < 1; and antagonistic when FICI > 1.

✓ *Determination of Minimum Inhibitory Concentration (Mic90)*

The MIC of the plant extract with the best antimicrobial activity was determined by the microdilution method in 96-well microtitre plates according to CLSI, using twofold serial dilutions. Two to three colonies of

each test organism were grown in Mueller-Hinton broth, and about 20 µL of each bacterial suspension was added to 180 µL of extract in the wells. Control wells received 100 µL of chlorhexidine at 1.25 mg/mL. The plates were incubated overnight at 37 °C.

After incubation, 10 µL of 0.5% aqueous triphenyl tetrazolium chloride (TTC; Sigma-Aldrich) was added to each well, and the plates were incubated for another hour and then checked for colour change. Living microorganisms react with TTC to turn the wells from colourless to red. The MIC90 was taken as the lowest extract concentration that inhibited 90% of bacterial growth.

✓ *Determination of Minimum Bactericidal Concentration (Mbc)*

The MBC was determined by the method of CLSI; About 2 µL from each well of the MIC assay was spread on freshly prepared Mueller-Hinton agar plates, incubated at 37 °C for 24 hours and checked for bacterial growth. The MBC was the lowest concentration that prevented growth on the agar surface.

✓ *Gas Chromatography and Mass Spectrometry (Gc-Ms)*

The chemical constituents of the fractions were analysed on a Hewlett-Packard HP 6890 gas chromatograph coupled to an HP 5973 mass spectrometer and fitted with an HP-5 column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness) and an MS detector. Helium was the carrier gas (1 mL/min), with a split ratio of 1:25 and a scan range of 35 to 425 amu. The oven temperature was raised from 70 °C (after 2 minutes) to 325 °C at 4 °C per minute, and the final temperature was held for 10 minutes at 240 °C. Samples were injected into the GC-MS inlet with a syringe. The ion source was set at 250 °C, with electron ionization at 70 eV. Compounds were identified by matching their mass spectra and retention indices with those in the Wiley 275 library (Wiley, New York).

✓ *Statistical Analysis*

Data were analysed with SPSS version 18.0 (Chicago, IL, USA, 2009). One-way ANOVA was used to test for significant differences in the diameter of inhibition zones between the plant extracts and ciprofloxacin, and in the MIC of the most active extract, fractions and positive control (ciprofloxacin). P values below 0.05 were regarded as significant.

III. RESULTS

Table 1 Distribution of Mean Decayed, Missing and Filled Teeth (DMFT) by Gender and Age

Age (years)	Frequency, n (%)	DMFT ± SD	P value
5–19	5 (7.1)	0.16 ± 0.10	
20–24	6 (8.6)	0.24 ± 0.14	
25–29	7 (10)	0.30 ± 0.23	
30–34	8 (11)	0.20 ± 0.24	0.03
35–39	9 (13)	0.18 ± 0.25	
40–44	8 (11)	0.32 ± 0.30	
45–59	7 (10)	0.60 ± 0.13	
60–64	8 (11)	0.65 ± 0.14	
65–69	7 (10)	0.68 ± 0.16	
70 and above	5 (7.1)	0.75 ± 0.15	
Total	70 (100)	5.1 ± 0.02	

Values were statistically significant at $p < 0.05$.

Seventy volunteers aged 5 to over 70 years took part. The 5 to 19 and 70+ age groups had the lowest participation (7.1% each), and the 35 to 39 age group had the highest (13%).

DMFT ranged from 0.16 to 0.75, with the 5 to 19 age group recording the lowest value and the 70+ group the highest. The total DMFT was 5.1.

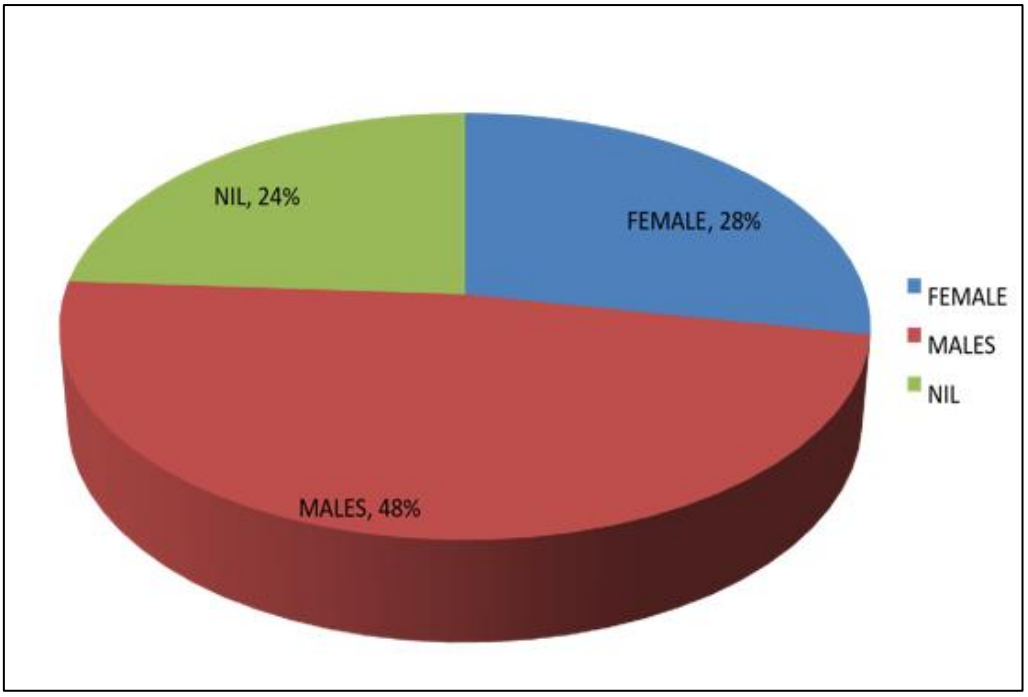


Fig 3 The Salivary Reductase Test Confirming the Caries Status of Participants Showed Females 20(28%), Males 34(48%), Nil 16(24%)

Table 2 Identification and Characterization of Cariogenic Bacteria

Isolate	**Morphology**	**Gram reaction**	**Catalase**	**Oxidase**	**Citrate**	**Coagulase**	**Gas / slope / butt**	**Organism**
A	Blue to purple raised colonies on TYEBA	+ cocci in chains	–	+	–	–	+ / Y / R	*Streptococcus mutans*
B	White to grey flat colonies on DMRA	+ cocci	–	+	–	–	+ / Y / R	*Streptococcus sobrinus*
C	Blue, rough, gum-drop colonies on MSA	+ cocci	–	+	–	–	+ / Y / R	*Streptococcus salivarius*
D	Blue, small, flat, hard colonies on MSA	+ cocci	–	+	–	–	+ / Y / R	*Streptococcus mitis*
E	White, small, convex, smooth, glistening colonies on NA	+ rods	–	+	–	–	+ / Y / R	*Lactobacillus fermentum*
F	Light green colonies on Blood agar	+ cocci in chains	–	+	–	–	+ / Y / R	*Streptococcus sanguinis*

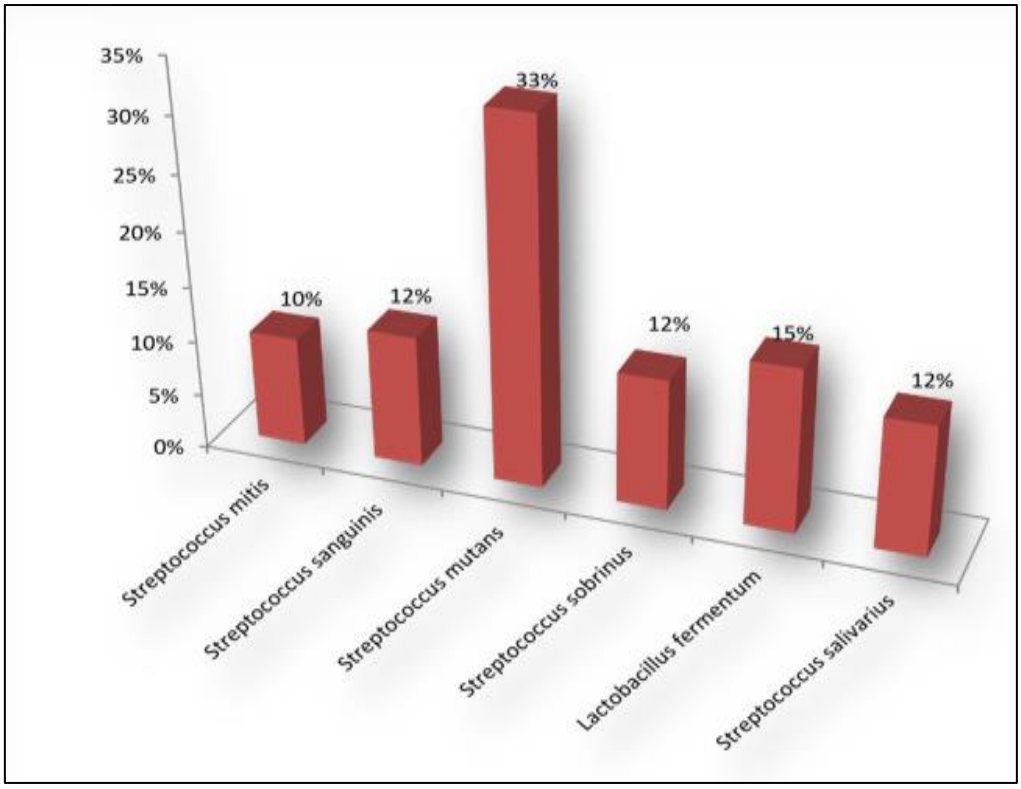


Fig 4 Prevalence of Isolated Cariogenic Bacteria

Table 3 Zones of Inhibition of *G. glabra* Roots on the Isolated Cariogenic Bacteria

Conc. (mg/mL)	Water (mm ± SD)	Ethanol (mm ± SD)	Methanol (mm ± SD)
500	19.0 ± 0.12	21.5 ± 0.45	23.0 ± 0.60
400	18.0 ± 0.45	20.0 ± 0.34	22.0 ± 0.58
300	17.5 ± 0.43	19.0 ± 0.35	21.5 ± 0.48
200	Ni	18.5 ± 0.33	20.0 ± 0.39
100	Ni	Ni	19.0 ± 0.38
Control	19.0	19.0	19.0

Diameter of well used = 5.00 mm. Values were taken in triplicate with standard deviation. Legend: ≤ 17.0 mm = resistant; 17.0 to 19.0 mm = moderate; ≥ 19.00 mm = susceptible; mg/mL = milligram per millilitre; mm = millimetre; SD = standard deviation; Ni = no inhibition.

The aqueous extract showed no inhibition at 100 to 200 mg/mL, while 300 to 500 mg/mL produced zones of 17.5 to 19.00 mm. Compared with the control zone (19.00 mm), the isolated cariogenic bacteria showed moderate susceptibility to this extract. The ethanolic extract, with zones of 18.5 to 21.5 mm, gave higher susceptibility, and the methanolic extract, with zones of 19.0 to 23.0 mm, gave the highest susceptibility. In all cases, zone size increased with concentration.

Table 4 Zones of Inhibition of *O. sanctum* on the Isolated Cariogenic Bacteria

Conc. (mg/mL)	Water (mm ± SD)	Ethanol (mm ± SD)	Methanol (mm ± SD)
500	18.0 ± 0.20	21.5 ± 0.45	23.0 ± 0.60
400	17.0 ± 0.18	21.0 ± 0.35	22.0 ± 0.58
300	16.0 ± 0.16	20.0 ± 0.30	21.0 ± 0.50
200	15.0 ± 0.15	19.5 ± 0.28	20.0 ± 0.48
100	Ni	19.0 ± 0.25	19.5 ± 0.45
Control	19.0	19.0	19.0

Diameter of well used = 5.00 mm. Values were taken in triplicate with standard deviation. Legend: ≤ 17.0 mm = resistant; 17.0 to 19.0 mm = moderate; ≥ 19.00 mm = susceptible; mg/mL = milligram per millilitre; mm = millimetre; SD = standard deviation; Ni = no inhibition.

Relative to the control zone (19.00 mm), the isolated cariogenic bacteria were resistant to the aqueous extract, which gave zones of 15.0 to 18.0 mm. They were susceptible to the ethanolic extract (19 to 21.5 mm) and more susceptible to the methanolic extract (19.5 to 23.0 mm). Again, zone size increased with concentration.

Table 5 Zones of Inhibition of Combined *G. glabra* and *O. sanctum* on the Isolated Cariogenic Bacteria

Conc. (mg/mL)	Water (mm ± SD)	Ethanol (mm ± SD)	Methanol (mm ± SD)
500	23.0 ± 0.45	25.0 ± 0.45	28.0 ± 0.60
400	22.5 ± 0.38	24.5 ± 0.35	27.5 ± 0.58
300	21.0 ± 0.28	23.0 ± 0.30	26.0 ± 0.50
200	20.0 ± 0.25	22.0 ± 0.28	25.0 ± 0.48
100	19.50 ± 0.20	21.0 ± 0.25	25.0 ± 0.45
Control	19.00	19.00	19.00

Diameter of well used = 5.00 mm. Values were taken in triplicate with standard deviation. Legend: ≤ 17.0 mm = resistant; 17.0 to 19.0 mm = moderate; ≥ 19.00 mm = susceptible; mg/mL = milligram per millilitre; mm = millimetre; SD = standard deviation.

The aqueous combination gave zones of 19.50 to 23.0 mm and, compared with the control zone (19.00 mm), the isolated cariogenic bacteria showed high susceptibility to it. The ethanolic combination (21 to 25.0 mm) gave higher susceptibility, and the methanolic combination (25.0 to 28.0 mm) gave the highest. Zone size again increased with concentration.

Table 6 Minimum Inhibitory and Minimum Bactericidal Concentrations of *G. glabra*

Organism	MIC (mg/mL)	MBC (mg/mL)
Streptococcus mutans	250	500
Streptococcus sobrinus	125	250
Streptococcus mitis	62.5	125
Streptococcus salivarius	31.2	62.5
Lactobacillus fermentum	15.6	31.2
Streptococcus sanguinis	7.80	3.90

Legend: MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; mg/mL = milligram per milliliter.

Table 7 Minimum Inhibitory and Minimum Bactericidal Concentrations of *O. sanctum*

Organism	MIC (mg/mL)	MBC (mg/mL)
Streptococcus mutans	500	1000
Streptococcus sobrinus	250	500
Streptococcus mitis	125	250
Streptococcus salivarius	62.5	125
Lactobacillus fermentum	62.5	125
Streptococcus sanguinis	31.2	62.5

Legend: MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; mg/mL = milligram per millilitre.

Table 8 Minimum Inhibitory and Minimum Bactericidal Concentrations of Combined *G. glabra* and *O. sanctum*

Organism	MIC (mg/mL)	MBC (mg/mL)
Streptococcus mutans	125	500
Streptococcus sobrinus	62.5	250
Streptococcus mitis	31.2	31.2
Streptococcus salivarius	15.6	62.5
Lactobacillus fermentum	7.80	31.2
Streptococcus sanguinis	3.90	15.6

Legend: MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; mg/mL = milligram per millilitre.

Table 9 Synergistic, Antagonistic and Additive Interaction

Av. MIC of extract A in combination (mg/mL)	Av. MIC of extract A alone (mg/mL)	Av. MIC of extract B in combination (mg/mL)	Av. MIC of extract B alone (mg/mL)
41	140	41	203
ΣFIC	0.49 ≤ 0.5		
Effect	Synergistic		

FICI = (MIC of extract A in combination ÷ MIC of extract A alone) + (MIC of extract B in combination ÷ MIC of extract B alone)

- *Legend:*

MIC = minimum inhibitory concentration

mg/mL = milligram per millilitre.

≤ 0.5 = synergistic

> 0.5 to 1 = additive

≤ 4 = antagonistic.

Table 10 Gas Chromatography Mass Spectometry Analysis Result of *Ocimum sanctum*

GC-MS ANALYSIS OF OCIMUM SANCTUM

No.	Retention time	Essential oil components	Retention index	Content (%)
1	4.002	Ethyl isovalerate	859	0.632
2	5.622	α-Pinene	936	1.236
3	6.554	Sabinene	976	1.045
4	6.654	Neo pinene	-	2.876
5	6.955	β-Pinene	980	0.989
6	8.099	Eucalyptol	1033	13.561
7	8.562	β-Ocimene	1044	7.325
8	8.762	γ-terpinene	1062	0.457
9	8.981	*trans*-4-Thujanol	1065	0.447
10	11.709	α-terpineol	1198	0.506
11	11.996	Terpinen-4-ol	1184	1.210
12	12.816	Estragole	1195	19.410
13	14.148	Chavicol	1203	0.647
14	17.057	Eugenol	1357	16.433
15	17.870	Methyl eugenol	1411	0.655
16	18.202	Caryophyllene	1420	2.964
17	18.446	*Z*, E-Alpha farnesene	1491	2.000
18	18.796	α-Cryophylene	1423	2.138
19	19.303	α-Cubebene	1390	3.153
20	19.766	**R-β-Bisabolene**	1506	8.551
21	20.285	α-Cryophylene	1450	10.834
22	22.143	α-Bisabolol	1687	1.099
23	30.218	Tetratetracontane	-	1.038

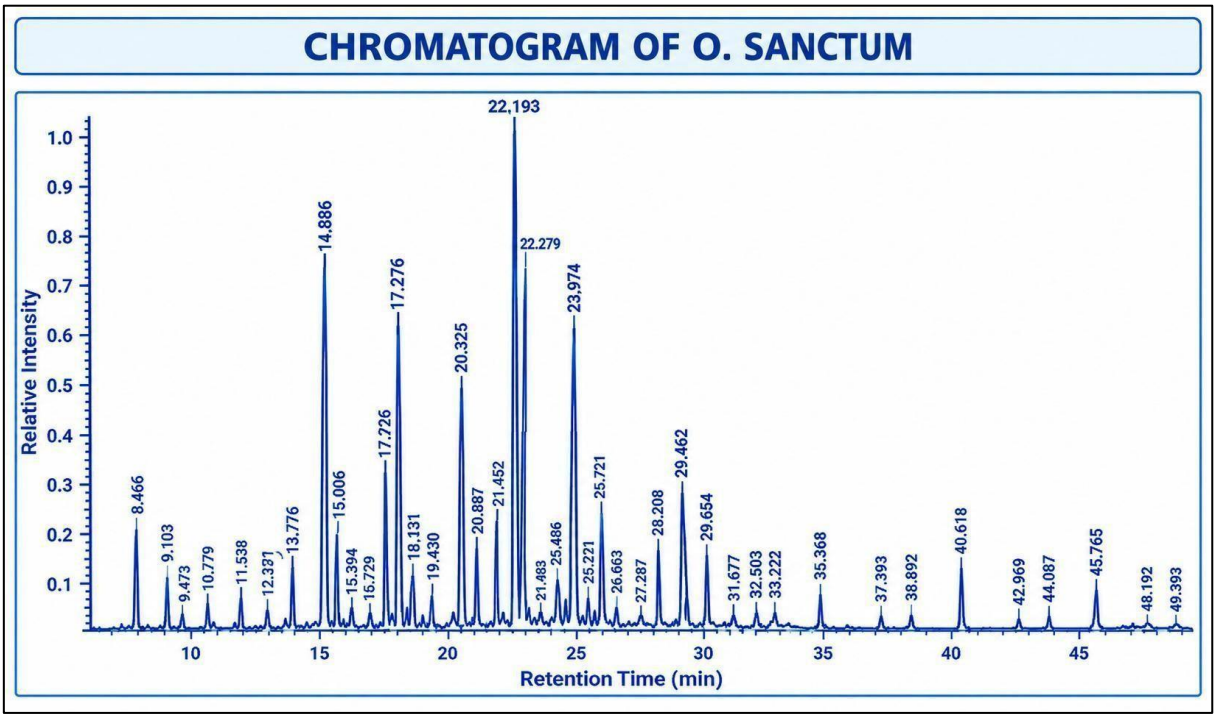


Fig 5 Chromatogram *of O.sanctum*

Table 11 Chromatogram of *G. glabra*

GC-MS ANALYSIS OF GLYCYRRHIZIA GLABRA ROOTS

Peak	R. Time	Area	Area %	Name
1	7.898	4386151	3.91	Ethyl pipecolinate
2	9.627	824567	0.73	2-methoxy-4-vinylphenol
3	10.448	3312915	2.95	5-oxo-pyrrolidine-2-carboxylic acid methyl
4	11.349	3357771	2.99	Cyclopentanol
5	11.962	626586	0.56	Anabassine
6	12.181	1253744	1.12	Phenol, 2,4-bis(1,1-dimethylethyl)-
7	12.291	909809	0.81	2,2'-Isopropylidendifuran
8	12.345	2225274	1.98	Benzoic acid, 4-ethoxy-, ethyl ester
9	13.124	1098304	0.98	Benzenepropanoic acid, 4-hydroxy-, methyl ester
10	13.960	2480795	2.21	Comarin. 3,4,4a.5.6.8a-hexahydro-6,8a-epidioxy-4a,6-dimethyl
11	14.527	31554248	28.12	Mome inositol
12	14.627	3161268	2.82	Mome inositol
13	15.054	488368	0.44	1H-Indole-6-carboxylic acid, 7-(acetyloxy)-2,5-dimethyl-, methyl ester
14	15.453	1117043	1.00	3-heptadecanol
15	15.652	1117980	1.00	Isopropyl myristate
16	15.815	1049728	0.94	2,6,10-trimethyl,14-ethylene-14-pentadecene
17	16.269	375142	0.33	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[R*,R*-(E)]]-
18	16.706	5492512	4.89	Hexadecanoic acid, methyl ester
19	17.111	5863024	5.22	Pentadecanoic acid
20	18.384	3769260	3.36	9,12-octadecadienoic acid (z,z)-, methyl ester
21	18.432	8015058	7.14	9-octadecenoic acid (z)-, methyl ester
22	18.563	327215	0.29	2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[R*,R*-(E)]]- (t-phytol)
23	18.649	1811797	1.61	Methyl stearate
24	18.783	1705852	1.52	9,12-octadecadienoic acid (z,z)
25	18.825	4274753	3.81	Octadec-9-enoic acid
26	19.014	1278112	1.14	Octadecanoic acid
27	19.224	721265	0.64	9,12-octadecadienoic acid (z,z)-, methyl ester
28	20.111	717396	0.64	N-Nonadecanol-1

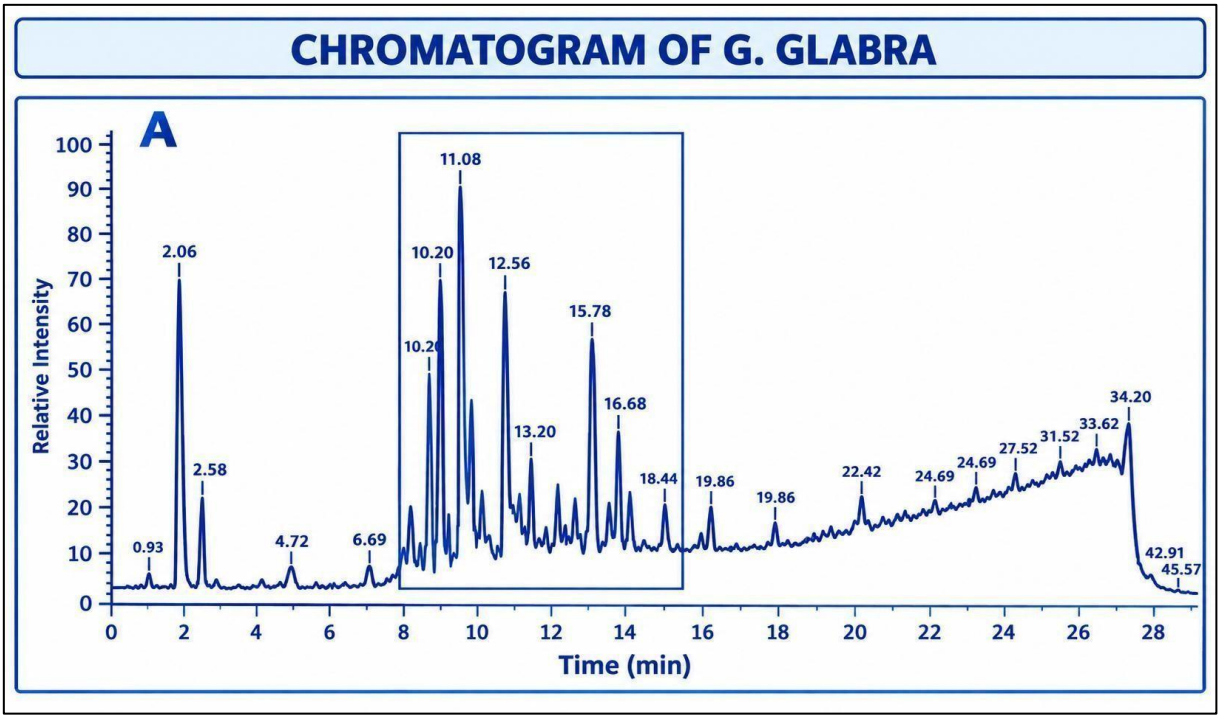


Fig 6 Chromatogram of *G. glabra*

IV. DISCUSSIONS

Dental caries continues to place a heavy burden on oral health across the world. The World Health Organization (WHO, 2025) estimates that about 2.3 billion people have caries in their permanent teeth, which makes it one of the most common chronic diseases. In Africa, about 91% of adults aged 20 to 64 years are thought to have had caries in their permanent teeth, a burden that adds greatly to health care costs.

This study used standard methods to test the antibacterial activity of *Glycyrrhiza glabra* roots and *Ocimum sanctum* leaves against cariogenic bacteria from dental caries patients in Umuna, Onuimo LGA, Imo State.

Seventy volunteers aged 15 to 70 years took part. Participation was lowest in the 15 to 19 and 70+ age groups (7.1% each) and highest in the 35 to 39 age group (13%). DMFT values ranged from 0.16 to 0.75, with the 15 to 19 age group lowest and those aged 70 and above highest at 0.75. The total DMFT of 5.1 was significantly high when compared with the standard DMFT chart.

This finding is in line with the work of Ramachandran *et al*. (2024) on caries prevalence among school children in Namakkal district, Tamil Nadu, India, who also found a high proportion of children with caries. Such a pattern may be related to household income, level of education, access to dental services, knowledge of oral hygiene, dietary habits, employment status and neighbourhood conditions in the study area.

The caries status of each participant was confirmed with the salivary reductase test. As shown in Fig 1.0, 48% of males and 28% of females had active caries, while 22% were free of caries. This is consistent with the report of Elamin *et al*. (2021) on factors associated with dental caries in children in the Middle East and North Africa. The higher level of caries among males may be due to less frequent tooth brushing, poor access to health facilities, poor oral habits, poor feeding practices and high sugar intake.

The cariogenic bacteria recovered, with their frequencies, were *Streptococcus mutans* (33%), *Streptococcus sobrinus* (18%), *Lactobacillus fermentum* (15%), *Streptococcus salivarius* (12%), *Streptococcus sanguinis* (12%) and *Streptococcus mitis* (10%).

Streptococcus mutans was recovered from more than 80% of the caries patients in this study, which supports earlier reports that it is the main cause of human dental caries (Yamani *et al*., 2024). *S. mutans* serves as a microbial tool for assessing dental caries (Chotvorrarak *et al*., 2022) and has been used in a semi-quantitative way to estimate oral infection risk in preschool children (Amadi *et al*., 2007). The isolation of *Lactobacillus fermentum* agrees with an earlier finding that oral lactobacilli may also play a part in causing caries (Saha *et al*., 2024).

The aqueous extract of *G. glabra* roots showed no inhibition at 100 to 200 mg/mL, while 300 to 500 mg/mL gave zones of 17.5 to 19.00 mm. Compared with the control zone (19.00 mm), the isolated cariogenic bacteria showed moderate susceptibility to this extract. The ethanolic extract (18.5 to 21.5 mm) produced higher susceptibility, and the methanolic extract (19.0 to 23.0 mm) the highest, with zone size increasing with concentration.

This agrees with Mahboubeh *et al*. (2010), who found that the ethanolic extract had stronger antibacterial activity than the aqueous extract. Ethanol is often a better extraction solvent than water because its polarity is more balanced, allowing it to dissolve a wider range of compounds, both polar and non-polar.

The aqueous extract of *O. sanctum* leaves gave zones of 15.0 to 18.0 mm, to which the isolated cariogenic bacteria were resistant. The ethanolic extract (19 to 21.5 mm) produced susceptibility, and the methanolic extract (19.5 to 23.0 mm) produced higher susceptibility, again following the increase in concentration.

This supports the account of Trinette *et al*. (2020) that *Ocimum sanctum* L. extracts have been used to treat various ailments since ancient times and are mentioned in old texts such as the Atharvaveda. References to *Ocimum sanctum* L. date back to 1200 to 1000 BC, where it is described as the "elixir of life". The plant has been used for oral care and against bad breath because of its anticariogenic properties. Its anti-inflammatory compounds also help to reduce gum inflammation, a feature of periodontal diseases such as periodontitis and gingivitis. In vitro tests on oral pathogenic bacteria have shown that toothpastes, mouthwashes and gels containing *Ocimum sanctum* L. extract are active against *Streptococcus mutans* and *Enterococcus faecalis*.

Used singly, the aqueous, ethanolic and methanolic extracts of *G. glabra* roots and *O. sanctum* leaves gave zones of inhibition of 15.00 to 23.00 mm at 100 to 500 mg/mL, increasing with concentration, which represented fair susceptibility relative to the chlorhexidine control (19.00 mm). When combined, however, the extracts produced larger zones of 18.00 to 28.00 mm over the same concentration range, indicating high susceptibility compared with the control (19.00 mm).

Other studies have reported the in vitro antibacterial and anticaries activity of local medicinal plants. Ghosh *et al*. (2023) described the effect of *Ocimum sanctum*, and Amadi *et al*. (2007) reported the anticaries activity of *Breynia nivosus* and *Ageratum conyzoides*. The stronger inhibition seen here may result from synergistic interaction between the two herbs, and increased activity of herbal combinations has been reported before (Park *et al*., 2024; Amadi *et al*., 2000).

The MIC and MBC of *G. glabra* roots and *O. sanctum* leaves were 15.63 to 500 mg/mL for *G. glabra* roots and 62.5 to 1000 mg/mL for *O. sanctum* leaves, while the combination gave lower MIC and MBC values of 3.90 to 250 mg/mL.

The interaction between the combined extracts was assessed with the Fractional Inhibitory Concentration Index (FICI) and found to be synergistic at FICI 0.49 ($\leq$ 0.5). A synergistic effect of *Spondias mombin* and *Baphia nitida* on dental caries organisms has been reported previously (Amadi *et al*., 2007).

GC-MS analysis of *G. glabra* root revealed 28 bioactive compounds, with mome inositol the most

abundant (28%) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol the least (0.33%). The anticariogenic properties of liquorice have been the subject of several studies, many focusing on its primary and secondary metabolites, glycosides and triterpenoid saponins. Glycyrrhizin is reported to block the glucosyltransferase activity of *S. mutans*, the enzyme behind the insoluble glucans that biofilms need. In acidulated phosphate fluoride, glycyrrhizin coats the enamel surface and settles in the pores of demineralized enamel, lowering solubility and increasing fluoride uptake. An in vivo study found that glycyrrhizin had no significant effect on mineral loss in artificial caries lesions, perhaps because the concentration or exposure time was too low. Glycyrrhizin also lessens enamel breakdown by suppressing acid production in plaque. Together, these studies support randomized clinical trials of liquorice in forms such as herbal lollipops (Awamie & Rees, 2023).

In the same way, GC-MS analysis of *O. sanctum* revealed 23 bioactive compounds, with estragole the most abundant (19.4%) and trans-4-thujanol the least (0.447%). Nair and Shiva (2023) reported that eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituent of *O. sanctum*, is mainly responsible for the therapeutic value of tulsi, with ursolic acid and carvacrol as other important constituents to which its antimicrobial activity can be attributed.

The range of bioactive compounds present in both plants explains their synergistic antibacterial effect and could be exploited for anticariogenic therapy.

➢ *Contributions to Knowledge*

- Established a high prevalence of dental caries in the sampled population
- Identified the age group at greatest risk of dental caries
- Isolated several causative agents of dental caries in addition to *S. mutans* and *S. sobrinus*
- Identified two potent, synergistic anticariogenic plants

V. CONCLUSION

Dental caries remains a major oral health problem worldwide. The World Health Organization (WHO, 2022) estimates that about 2.3 billion people have caries in their permanent teeth, placing it among the most common chronic diseases. In the present study, 91% of adults aged 15 to 70 years were found to have had caries in their permanent teeth, a burden that adds substantially to health care costs.

Previous work on cariogenic microorganisms indicates that some natural products can affect the growth and virulence of cariogenic bacteria and so help to prevent and treat dental caries. A safe, effective and affordable treatment approach is needed. Because synthetic drugs carry many adverse effects, this research assessed the efficacy of *G. glabra* roots and *O. sanctum* leaves and found that they have significant, synergistic antibacterial activity against the isolated pathogens. After toxicity and pharmacokinetic studies, these natural remedies could be developed into mouthwashes for the treatment and prevention of dental caries.

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