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Research Article

Antimicrobial Activity of the Ethanol Extract of Lemongrass (*Cymbopogon citratus*) and Thyme (*Thymus vulgaris*) on Dental Caries-causing Bacteria

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Abstract

This study investigated the antimicrobial activity of the ethanol extract of lemongrass (*Cymbopogon citratus*) and thyme (*Thymus vulgaris*) on dental caries-causing bacteria. Standard microbiological techniques were carried out on samples obtained from patients at St. Luke Hospital, Anua, Uyo. The percentage occurrence of five bacterial isolates obtained included: *Enterococcus faecalis* (20%), *Corynebacterium sp* (10%), *Bacillus sp* (5%), *Lactobacillus acidophilus* (25%) and *Streptococcus mutans* (40%). The ethanol extract of lemongrass and thyme as well as the phytochemical analysis were determined. The results revealed the presence of flavonoids, alkaloids, tannins, glycosides, steroids and phenols in lemongrass. It also showed the presence of flavonoids, alkaloids, saponins, tannins, glycosides, steroids and terpenoids in thyme. Antimicrobial activity and minimum inhibitory concentration (MIC) of the ethanol extracts of lemongrass and thyme were also determined. The results indicated that at 100% concentration, *Bacillus sp* (25.5 mm) exhibited more susceptibility; *Enterococcus faecalis* (19 mm) exhibited the least susceptibility and *Corynebacterium sp* was resistant to the ethanol extract of lemongrass. *Enterococcus faecalis* (32 mm) exhibited the highest susceptibility while *Streptococcus mutans* (14 mm) exhibited the least susceptibility to the ethanol extract of thyme. *Bacillus sp* and *Enterococcus faecalis* exhibited the lowest Minimum Inhibitory Concentration (MIC) value which was 6.25 mg/ml. In this study, lemongrass and thyme proved to be potential antimicrobial agents against dental caries-causing bacteria.

Keywords

Antimicrobial Activity, Bacteria, Dental Caries, Ethanol Extract, Lemongrass, Thyme

1. Introduction

Dental caries, commonly known as tooth decay, is a prevalent oral health issue caused by the demineralization of the tooth surface due to acid production by bacteria, particularly *Streptococcus mutans* and *Lactobacillus species* [1, 2]. These

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bacteria metabolize dietary sugars, producing acids that erode the enamel and dentin, leading to cavities and other dental complications [3]. The rising concern over antibiotic resistance and the side effects associated with synthetic antimicrobial agents has driven the search for natural alternatives. Dental problems date back to as far as 5000 BC when a Sumerian thought the cause of dental caries was a "tooth worm." The term "dental caries" was first reported in the literature around 1634, and it originates from the Latin word "caries," which stands for decay [4]. The term was initially used to describe holes in the teeth. Dental caries is reported to be one of the oldest and most common diseases found in humans. One of the many adverse consequences of dental caries is "pain". Untreated dental caries can lead to dental pain which is known to affect daily activities, with a lasting impact on their quality of life like sleep disturbance, decreased work effectiveness, discomfort, and avoidance of certain types of food. Untreated caries can affect the ability to eat which can impair adequate intake of nutrients and can affect quality of life which may affect the growth of an individual [5].

The prevalence of dental caries is one of the most widespread oral diseases worldwide. It affects about 80% of the human population both adults and children [6]. Oral health is interconnected with overall health and is related to a person's overall quality of life [7]. Dental caries is a biofilm-related oral disease driven by an interaction between the enamel surface and several factors, including microbial biofilm on the tooth's surface; sugars, saliva, immunity, and genetics, in which dental hard tissues undergo phasic demineralization and re-mineralization [8]. Caries can occur at any stage of life, in both primary and permanent dentitions, and result in detrimental effects on the tooth's crown and fatal in later years, on exposed root surfaces.

The demineralization and re-mineralization process on the tooth's surface are related to the capacity of acid production by acidogenic bacteria that accumulate in dental plaque. *Streptococcus mutans* is the main pathogenic acid-producing bacterium associated with dental plaque on tooth surfaces and is responsible for dental caries [9]. The formation of the complex structure of biofilms is a multistep process beginning with bacteria adhering to the implant surfaces using cell wall-associated adhesins. Bacterial exopolysaccharides are employed to promote second colonizer adherence, biofilm formation, and the protection of bacteria from the host immune response and antibiotic therapy [10]. This can limit the population of *Streptococcus mutans* or decrease biofilm formation, a measure to prevent caries development [11].

Antimicrobial activity kills or inhibits the disease, causing microbes from their performances. Antimicrobial activity may be anti-bacterial, anti-fungal, or antiviral. An antimicrobial can be bacteriostatic or bactericidal. Many essential oils and plant extracts included in herbal pharmacopeias are claimed to possess antimicrobial activities. From study, microorganisms have become resistant to drugs and this has led to the renewed interest in novel antimicrobial agents from plants. Recent studies on medicinal plants and their therapeutic use are quite helpful in solving the antibiotic resistance issue of bacteria. Natural products derived from medicinal plants have proven to be an abundant source of biologically active compounds that have antimicrobial activity. The use of medicinal plants as key drugs to sustain human health is emphasized by the World Health Organization (WHO). Brazil, Latin America and Argentina have gradually increased the use of medicinal plants. About 80% of people in developing countries use medicinal plants as conventional remedies. Many medicinal plants have been investigated phytochemically for their therapeutic use and bioactive compounds including tannins and phenols as growth inhibitors for pathogenic bacteria [12]. Identifying new and effective strategies as an alternative treatment will be the foremost priority.

The genus *Cymbopogon*, commonly known as lemongrass, belongs to the Poaceae family. It originates from southwest Asia (Southern India and Sri Lanka) but currently can grow spontaneously all over the world; especially in the tropical and subtropical regions [13]. It comprises a large number of species, but only two have economic importance as cultivated plants: *C. citratus* and *C. flexuosus* [14]. *C. flexuosus* or East Indian lemongrass is cultivated in Asia, while *C. citratus* or West Indian lemongrass is resistant to drought and low temperatures and because of this can be cultivated over large growth areas; therefore it is commercially more important [15]. It has been cultivated for its medicinal properties, as well as for garden decoration and its repelling effects on insects. It is a tropical plant, grown as an ornamental plant in many temperate areas [16]. The genus *Cymbopogon* is derived from the Greek words kymbe (boat) and pogon (beard), referring to the flower spike arrangement of the plant, while *citratus* is derived from ancient Latin, meaning lemon-scented leaves [17]. However, *Cymbopogon citratus* forms dense clumps up to 3 m tall, with short rhizomes. The whole plant has a lemon-like smell and bitter taste. Its leaves are erect with glabrous plane, more than 1 m long, 5-15 mm wide, whiter upper face and closed edge in the base, with rough margins and membranaceous or arid ligules 4-5 mm long [18]. The upper surface side of the leaves is dark green, while the lower surface is light green. Inflorescences are erect, usually in pairs of terminal spiciform racemes 30-60 cm long. Thyme which belongs to the genus, Thymus, is a perennial evergreen herb of the angiosperm plant family Lamiaceae (mint plant family) that has 350 species and 36 subspecies and is native to Europe, North Africa, and Asia. Because of its distinct aroma, the plant is a popular culinary herb [16, 18]. Wild thyme, *Thymus serphyllum* is the wild relative of all cultivated species. Garden Thyme, *Thymus vulgaris* L., is the most popular and commonly utilized plant for medicinal purposes [17]. Leaf morphology is one of the distinct features unique to the genus Thymus. The leaves are typically tiny (less than 1/8 inch long and 1/16 inch wide), narrow and elliptical, greenish gray, and grouped in whorled phyllotaxy. Thyme flowers are white,

yellow, or purple whorls that terminate branches. *Thymus vulgaris* and *Thymus magnus* have been studied the most and have been shown to exert a range of therapeutic properties that include antimicrobial, antitumor, antifungal, anti-parasitic, anti-oxidative, and anti-inflammatory [19, 20].

Dental caries also known as cavities or tooth decay caused by bacterial species poses a problem for adults and children as oral health is interconnected with overall health and is related to a person's overall quality of life. Dental caries develop when bacteria in the mouth metabolize sugars to produce acid that demineralizes the hard tissues of the teeth. Tooth affected with dental caries are often extracted when they cause discomfort or pain [21].

Microbial communities attach themselves to tooth surfaces and create biofilms. The microorganisms use sucrose and other dietary sugars as food sources; the dietary sugars go through the anaerobic fermentation pathway and produce lactate [22]. The lactate ions demineralize the hydroxyapatite crystals which cause the tooth to degrade. Dental caries occur when the demineralization rate is faster [9]. Dental caries affect poor and disadvantaged countries with less access to prevention and care, therefore its prevalence is due to poverty, poor cleaning of the mouth and receding gums [23]. It is necessary to take care of one's teeth because any harm done to the teeth affects the total well-being of one's health.

Dental caries is a common oral health problem affecting a wide range of people across the globe [24]. This research determines the potential plant extract capable of inhibiting the growth and activity of bacteria associated with dental caries. Dental caries is prevalent among adults and children and untreated caries can lead to functional, aesthetic and psychological problems, as well as poor quality of life. Antimicrobial resistance (AMR) pathogenic bacteria have been an issue of concern across the globe. Some strains of bacteria have become resistant to antibiotics and pose a threat to humanity [25]. Lemongrass and thyme have been used traditionally for health benefits [26, 27]. In this study, ethanol extract of Lemongrass and thyme are evaluated for their antimicrobial effect on dental caries-causing bacteria.

2. Materials and Methods

2.1. Study Area

The study was conducted in the Uyo metropolis. Uyo is the capital city of Akwa Ibom State. It is located between Latitudes $5^{0}02^{'}37^{''}$ North and Longitudes $7^{0}5^{'}6^{''}$ East with a fast-growing population.

2.2. Collection of Samples

The leaves of the lemongrass used for this research work were collected from the University's garden and the thyme samples were obtained from the Akpanandem market in Uyo metropolis, Akwa Ibom State, Nigeria. The samples were collected aseptically, wrapped in a sterile aluminum foil, deposited in sterile containers and transported to the Microbiology laboratory, Department of Microbiology University of Uyo, Akwa Ibom State, Nigeria for analysis using standard methods.

Some of the materials used in this study included: Lemongrass, thyme, swab sticks, Petri dishes, nutrient agar, blood agar, Mueller-Hinton agar, Simmons citrate agar, crystal violet, Lugo's iodine, safranin, hydrogen peroxide, cotton wool, inoculating loop, conical flask, test tubes, cork borer. Dental caries samples were collected from five (5) patients at Anua Hospital dental clinic, Uyo, Akwa Ibom State, Nigeria.

2.3. Microbiological Analysis

Sterile cotton swabs were used for sample collection. Each swab was pressed in the infected area of the affected tooth and inoculated into the nutrient agar medium and incubated at 37 ℃ for 24 hours. Pure cultures were produced; Gram differentiated and biochemically characterized and identified using the standard taxonomic schemes [28]. The isolates were maintained in Nutrient agar slants in McCartney bottles and preserved in a refrigerator at 4 ℃ and for further analysis.

2.4. Preparation of the Ethanol Extract of Lemongrass

The leaves of lemongrass were washed and air dried under shade at room temperature in the laboratory for three (3) weeks after which they were ground into fine powdered form using a laboratory mortar and pestle. 50 g of the dried lemongrass was weighed and macerated by soaking in 500 ml of absolute ethanol in a large container, stirred, covered and left for 72 hours to obtain the ethanol extract. The ethanol extract was filtered and evaporated using a rotatory evaporator.

2.5. Preparation of the Ethanol Extract of Thyme

The leaves of thyme were washed and air dried under shade at room temperature in the laboratory for three (3) weeks after which they were ground into fine powdered form using a laboratory mortar and pestle. 50 g of the dried lemongrass was weighed and macerated by soaking in 500 ml of absolute ethanol in a large container, stirred, covered and left for 72 hours to obtain the ethanol extract. The ethanol extract was filtered and evaporated using a rotatory evaporator.

2.6. Phytochemical Composition of Thyme and Lemongrass Ethanol Extract

The phytochemical screening was carried out on the ethanol extracts of lemongrass and thyme. The procedures were as

follows:

2.6.1. Test for Terpenoids and Steroids

The extract (0.2 g) of the whole plant sample was mixed with chloroform (2 ml) and concentrated tetraoxosulphate (VI) acid (3 ml) was carefully added to form a layer. A reddish-brown coloration of the inner face indicated the presence of terpenoids. 0.2 g of lemongrass and thyme extracts each were added into a test tube and boiled with 2 ml of distilled water in a water bath for two (2) minutes. The solution was filtered. The filtrates were treated with 10 ml of chloroform and concentrated sulfuric acid by the sides of the tubes. The change in colour of the lower layer to yellow and reddish upper layer indicated the presence of steroids.

2.6.2. Test for Flavonoids and Phenolics

The extracts (0.5 g) were mixed with water in a test tube and the tube was shaken. Two (2) drops of tetraoxosulphate (VI) acid were added. The formation of an intense yellow colour indicated the presence of flavonoids. Ferric chloride test was used for the phenolic test; the thyme and lemongrass extracts (5 mg) were added each to distilled water and 2 ml of 1% gelatin solution containing 10% sodium chloride was added. The formation of a white precipitate indicated the presence of phenol.

2.6.3. Test for Glycocides

The Extracts (0.2 g) were boiled each with 20 ml of water in a water bath for 2 minutes. The solution was filtered and 0.5 ml of glacial acetic acid, and a few drops of 5% ferric chloride acid were added to 1 ml of the filtrates. A greenish blue colour which was obtained indicated the presence of glycosides.

2.6.4. Test for Tannins and Saponins

Each portion of plant extracts (0.5 ml) was stirred with distilled water (10 ml), heated and filtered. The filtrates were treated with 0.1% ferric chloride reagent. A brownish-green precipitate was an evidence of the presence of tannins. 0.5 ml of extracts were mixed each with distilled water (5 ml) in a test tube and heated. The formation of frothing and the appearance of creamy mist of small bubbles indicated the presence of saponin.

2.7. Preparation of Lemongrass and Thyme Extract Stock Concentration for Antimicrobial Screening

A 100% w/v stock concentration was prepared by dissolving 1 g of the lemongrass and thyme extracts respectively in 10 of 10% DMSO (Dimethyl sulfoxide) in sterile stock bottles, smaller concentrations were obtained by carrying out a two-fold serial dilution; 50% stock concentration was prepared by using 5 ml of the stock solution and dissolving it in 5 ml of 10% DMSO, 25% stock concentration was prepared by using 5 ml from the 50% concentration and mixed with 5 ml of 10% DMSO, thereafter 5 ml was discarded from the 25% concentration. This mixture was made using well-labeled sterilized stock bottles. DMSO was used as a negative control.

2.8. Preparation of Microorganisms for Antimicrobial Screening

A loopful of each test organism was taken from its respective agar slant and subcultured into test tubes containing nutrient broth. They were incubated at 37 ℃ for 24 hours. The obtained microorganisms in the broth were standardized using normal saline.

2.9. Antimicrobial Sensitivity Assay (Agar Well Diffusion Assay)

The agar well diffusion method was used as the antimicrobial sensitivity test. A loopful of each test organism was taken from its agar slant and sub-cultured in Nutrient broth, and they were incubated at 37 ℃ for 24 hours. About 15 ml of freshly prepared Mueller-Hinton agar was poured into well-labeled sterile Petri dishes. The media plates were allowed to solidify completely and plates were swabbed with 0.1 ml of the sub-cultured test organisms all over the surface of the media. A sterile cork borer of 6 mm diameter was used to bore three (3) wells each into inoculated agar plates according to the number of concentrations used. The cork borer was sterilized at each time, and these wells were inoculated with 0.3 ml of different concentrations (100%, 50% and 25%) of the extracted essential oil. Control plates were also included for each concentration and wells were bored for negative control). 10% DMSO was used as the negative control to confirm that it could not interfere with the normal growth of the test microorganisms. All the plates were incubated at 37 ℃ for 24-48 hours. Antimicrobial activities of lemongrass and thyme extracts were evaluated by measuring the zones of inhibition against the test microorganisms using a transparent meter rule calibrated in millimeters and the result was recorded. The experiments were performed in duplicates to ascertain the results obtained.

2.10. Determination of Minimum Inhibitory Concentration (MIC)

The MIC is defined as the lowest concentration of the compound at which the microorganism does not demonstrate visible growth. The MIC of the essential oil was determined by using the broth dilution method. This test was performed with six (6) concentrations of the essential oil extract (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.25 mg/ml, 6.25 mg/ml, and 3.125 mg/ml) with a positive and negative control employing a 4 ml two-fold serial dilution.

For each bacterial isolate, eight (8) sterile tubes were used; containing 2 ml of nutrient broth, 100 mg/ml stock concentration of extracts was prepared, 2 ml of the stock concentration was taken and added into the first tube containing 2 ml of the broth

media and properly mixed, using a two-fold serial dilution, 2 ml was transferred from the first tube to the second tube and mixed, and subsequent transfer of 2 ml from the second tube to the third tube and mixed, this was done till the sixth (6) tube and 2 ml were discarded from the last tube. This was done to obtain six (6) concentrations (3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml). 0.1 ml was taken from a 24-hour broth sub-culture of each organism in nutrient broth and added to respective test tubes. A test tube containing only broth media and test organism was used as a positive control while a test tube containing only blank culture broth was used as a negative control. All the tubes were incubated at 37 ℃ for 24 hours. After incubation, MIC was considered at the lowest concentration (i.e. highest dilution) in which no bacterial growth occurred in the test tube. The experiments were performed in duplicates to ascertain the results obtained.

3. Results and Discussion

3.1. Biochemical Characteristics of the Bacterial Isolates Obtained from Tooth Samples

The biochemical characteristics of the bacterial species isolated from tooth samples are presented in Table 1. The bacteria species isolated included: *Enterococcus sp, Corynebacterium sp, Bacillus sp, Lactobacillus acidophilus and Streptococcus mutans.*

3.2. Percentage Occurrence of Bacterial Isolates Obtained from Tooth Samples

The bacterial isolates obtained from patients with dental caries in St Luke Hospital Anua, Uyo, are presented in Figure 1. The percentage occurrence was *Enterococcus faecalis* (20%), *Coryebacterium sp* (5%), *Bacillus sp* (5%), *Lactobacillus acidophilus* (20%) and *Streptococcus mutans* (40%).

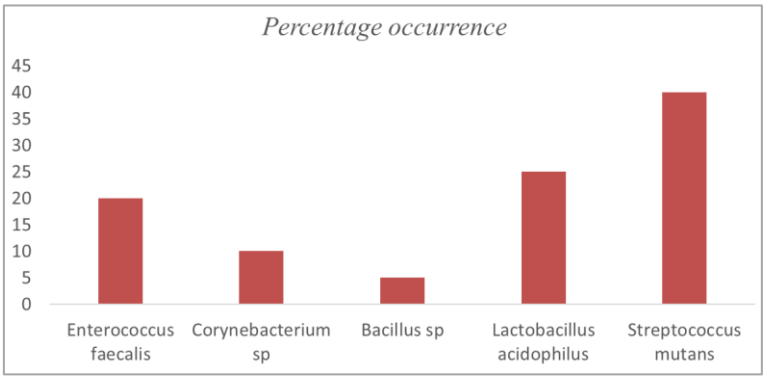

Figure 1. *Percentage Occurrence of Bacterial Isolates.*

Table 1. *Biochemical Characteristics of Bacterial Isolates.*

Isolates	Gram's Reaction	Shape	Motility	Oxidase	Catalase	Indole	MR	VP	Coagulase	Spore	Citrate	Urease	Hemolysis	Probable Microorganism
1.	+	Cocci	-	-	-	-	+	-	-	-	+	+	G	*Enterococcus faecalis*
2.	+	Rod	-	-	+	-	+	-	-	-	-	-	B	*Corynebacterium sp*
3.	+	Rod	-	+	+	-	+	-	-	+	+	+	B	*Bacillus sp*
4.	+	Rod	-	-	-	-	-	+	-	-	-	-	B	*Lactobacillus acidophilus*
5.	+	Cocci	-	-	-	-	-	+	-	-	+	-	A	*Streptococcus mutans*

Key: + = positive; - = negative; G = gamma-hemolytic; B = beta-hemolytic; A = alpha-hemolytic.

3.3. Phytochemical Composition of the Ethanol Extract of Lemongrass and Thyme

The phytochemical composition of the ethanol extract of Lemongrass and Thyme (Table 2). The phytochemical compositions tested in both lemongrass and thyme extract include steroids, flavonoids, tannins, phenols, saponins, glycosides, and terpenoids.

Table 2. *Phytochemical composition of the ethanol extract of Lemongrass and Thyme.*

Phytochemical Composition	Lemongrass Extract	Thyme Extract
Flavonoids	+	+
Alkaloids	+	+
Saponins	-	+
Tannins	+	+
Glycosides	+	+
Steroids	+	+
Phenols	+	-

Key: + Detected - Not Detected.

3.4. Antimicrobial Activity of the Ethanol Extract of Lemon Grass and Thyme on Isolates Obtained from Tooth Samples

The antimicrobial activity of the ethanol extract of lemon grass and thyme on bacterial isolates obtained from tooth samples (Tables 3 and 4). The result showed that some species of bacteria were sensitive to both ethanol extract of lemongrass and thyme at different concentrations at 100%, 50%, and 25% concentration respectively). *Bacillus* sp (25 mm, 22 mm and 19 mm) was more susceptible; *Enterococcus* sp (19 mm, 11 mm and 9 mm) showed the least susceptibility to lemongrass. *Corynebacterium* sp was resistant to lemongrass extract. *Enterococcus sp* (32 mm, 29 mm and 27.5 mm) showed the highest susceptibility to thyme extract while *Streptococcus mutans (*14 mm, 13.50 mm and 12.50 mm) showed the least susceptibility to thyme extract.

Table 3. *Antimicrobial activity of the ethanol extract of Lemongrass at different concentrations against bacterial isolates.*

Lemongrass extract concentrations	100%	50%	25%
Bacterial Isolates	Zone of Inhibition (mm) Mean ±SD		
Enterococcu sp	19.00 ±1.00	11.50 ±0.50	9.00 ±1.00
Corynebacterium sp	-	-	-
Bacillus sp	25.50 ±0.50	22.00 ±1.00	19.00 ±1.00
Lactobacillus acidophilus	20.50 ±1.50	16.00 ±1.00	14.00 ±1.00
Streptococcus mutans	21.00 ±1.00	17.50 ±0.50	14.50 ±0.50

Key: mm = millimeter, SD = Standard Deviation.

Table 4. *Antimicrobial activity of the ethanol extract of Thyme at different concentrations against bacterial isolates.*

Thyme extract concentrations	100%	50%	25%
Bacterial Isolates	Zone of Inhibition (mm) Mean ±SD		
Enterococcu sp	32.00 ±1.50	29.00 ±0.50	27.50 ±1.00
Corynebacterium sp	22.50 ±0.50	20.00 ±1.00	18.50 ±0.50
Bacillus sp	31.00 ±1.00	22.00 ±1.00	19.00 ±0.50
Lactobacillus acidophilus	24.00 ±1.00	22.00 ±1.00	20.50 ±0.50

Thyme extract concentrations	100%	50%	25%
Streptococcus mutans	14.00 ±1.00	13.50 ±0.50	12.50 ±1.00

Key: mm = millimeter, SD = Standard Deviation.

3.5. MIC of the Ethanol Extract of Lemongrass and Thyme

The result of the minimum inhibitory concentration of Lemongrass and Thyme ethanol extract (Table 5 and Table 6). Lemongrass showed 50 mg/ml, 100 mg/ml, 6.25 mg/ml, 25 mg/ml and 12.25 mg/ml for *Enterococcus faecalis*, *Corynebacterium* sp, *Bacillus* sp, *Lactobacillus acidophilus* and *Streptococcus* mutans respectively. Then thyme revealed; 6.25 mg/ml, 25.00 mg/ml, 12.50 mg/ml 25.00 mg/ml 100 mg/ml, for *Enterococcus faecalis*, *Corynebacterium* sp, *Bacillus* sp, *Lactobacillus acidophilus* and *Streptococcus* mutans respectively. The minimum inhibition concentration (MIC) showed that *Bacillus* sp exhibited the lowest minimum inhibitory concentration (6.25 mg/ml) for lemongrass extract and *Enterococcus sp* had a minimum inhibitory concentration value of 6.25 mg/ml for thyme extract.

Table 5. *Minimum Inhibitory Concentration of the Ethanol Extract of Lemongrass.*

Bacterial Isolates	Concentration of lemongrass extract						
	100	50	25	12.5	6.25	3.125	MIC (mg/ml)
Enterococcus sp	-	-	+	+	+	+	50.00
Corynebacterium sp	+	+	+	+	+	+	100.00
Bacillus sp	-	-	-	-	-	+	6.25
Lactobacillus sp	-	-	-	+	+	+	25.00
Streptococcus sp	-	-	-	-	+	+	12.50

Key: + = visible growth, - = no growth, mg =Microgram.

Table 6. *Minimum Inhibitory Concentration of the Ethanol Extract of Thyme.*

Bacterial Isolates	Concentration of thyme extract						
	100	50	25	12.5	6.25	3.125	MIC (mg/ml)
Enterococcus sp	-	-	-	-	-	+	6.25
Corynebacterium sp	-	-	-	+	+	+	25.00
Bacillus sp	-	-	-	-	+	+	12.50
Lactobacillus sp	-	-	-	+	+	+	25.00
Streptococcus sp	-	+	+	+	+	+	100.00

Key: + = visible growth, - = no growth, mg = Microgram.

Five (5) bacterial isolates were identified from the tooth samples. They included: Enterococcus sp, Corynebacterium sp, Bacillus sp, *Lactobacillus sp* and *Streptococcus sp*. *Streptococcus sp* (40%) was more abundant than the other bacterial isolates. A similar finding has been explained [29]. The phytochemical constituents of the ethanol extract of Lemongrass (*Cymbopogon citratus*) and Thyme (*Thymus vulgaris*) analyzed in this work revealed the presence of flavonoids, alkaloids, tannins, glycosides, steroids and phenols in the extract of lemongrass. The result also showed the presence of flavonoids, alkaloids, sapo-

nins, tannins, glycosides, steroids and terpenoids in the extract of thyme. The presence of flavonoids, alkaloids, tannins, glycosides, steroids and phenols in the ethanol extract of lemongrass agrees with the work of [30]. Their work proved that flavonoids, alkaloids, tannins, glycosides, steroids, phenols, saponins and terpenoids are present in the extract of lemongrass. The presence of flavonoids, alkaloids, saponins, tannins, glycosides, steroids and terpenoids in the extract of thyme agrees with Shaban et al [31].

The presence of these bioactive compounds contributes to the inhibitory properties of the ethanolic extract of thyme and lemongrass. Flavonoids are a diverse group of natural compounds that are widely distributed in the plant kingdom. They have antioxidant and anti-inflammatory properties [32]. Saponins are a class of compounds that are found in a wide variety of plants. They also possess the ability to form stable complexes with cholesterol and other lipids, leading to an increase in permeability and consequent leakage of cytoplasmic contents [33]. This mechanism of action makes saponins effective against a wide range of bacteria. Tannins have been shown to have antibacterial properties [34], and may act by disrupting the bacterial cell membrane. Glycosides are a class of compounds that exhibit antibacterial properties. For example, arbutin, a glycoside found in the leaves of many plants, exhibits antibacterial activity against several strains of bacteria [35]. Phenols are a class of organic compounds that have antibacterial properties. For example, thymol, a phenol found in thyme oil, displays antibacterial activity against several strains of bacteria [36].

The antimicrobial properties of various natural products have been extensively studied recently for their potential applications in preventing and treating dental caries. Among these, lemongrass (*Cymbopogon citratus*) and thyme (*Thymus vulgaris*) have gained attention in this study due to their antimicrobial activities against a wide range of bacteria. The antimicrobial activity of the ethanolic extract of lemongrass and thyme against five(5) bacterial isolates namely; *Enterococcus* sp, *Corynebacterium* sp, *Bacillus* sp, *Lactobacillus* sp *and Streptococcus* sp explained that *Bacillus* sp (25 mm) at 100 ml/ml concentration was more susceptible; *Enterococcus* sp (19 mm) at 100 ml/ml concentration showed the least susceptibility to lemongrass. *Enterococcus* sp (32 mm) at 100 ml/ml concentration showed the highest susceptibility to thyme extract while *Streptococcus* sp (14 mm) at 100 ml/ml showed the least susceptibility to thyme extract. It was observed that *Corynebacterium* sp was resistant to the lemongrass extract expressed by no zone of inhibition.

This study revealed that both the ethanol extract of lemongrass and thyme have significant antimicrobial effects on bacterial isolates. It was generally observed that the ethanol extract of thyme exhibited a higher antimicrobial effect against the bacterial isolates which agrees with the study conducted by Fani et al and Ajijolakewu et al [37, 38]. It was also observed that the susceptibility of the *Streptococcus* sp to both extracts was minimal when compared with other bacterial isolates.

The minimum inhibition concentration observed showed *Bacillus* sp with the lowest minimum inhibitory concentration value of 6.25 mg/ml for lemongrass extract and *Enterococcus* sp with the minimum inhibitory concentration value of 6.25 µg/ml for thyme extract. *Lactobacillus* sp had the same minimum inhibitory concentration (25 mg/ml) for both lemongrass and thyme extract.

The susceptibility of microorganisms to antimicrobial agents depends on many factors such as the species of organism involved, its antimicrobial resistance versatility, time of exposure, and concentration of antimicrobial agents. According to the result obtained in this study, the variations in the zones of inhibition of each extract against the *Corynebacterium* sp and *Streptococcus mutans* were probably due to the mechanism in place by the bactericidal and bacteriostatic action of the extracts. *Streptococci* species which is considered one of the most cariogenic amongst oral microflora have many resistant mechanisms. One of the resistant mechanisms is the production of extracellular polysaccharide which offers protection against antimicrobials by preventing it from getting to them.

4. Conclusion

The ethanol extracts of Lemongrass (*Cymbopogon citratus*) and Thyme (*Thymus vulgaris*) were revealed in this study to exhibit antimicrobial activity against dental caries-causing bacteria. The ethanol extract of thyme was more effective against the bacterial species than the ethanol extract of lemongrass except for the *Streptococcus* sp which was more sensitive to lemongrass than the thyme extract. Indeed, both lemongrass and thyme extracts should be included in oral pharmaceutical formulations as preventive therapy for dental caries. Interventions are necessary for the enlightenment of the public on the oral health benefits of both lemongrass and thyme.

Abbreviations

AMR	Antimicrobial Resistance
DMSO	Dimethyl Sulfoxide
MIC	Minimum Inhibitory Concentration
SD	Standard Deviation
WHO	World Health Organization

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Spatafora, G., Li, Y., He, X., Cowan, A., & Tanner, A. C. (2024). The evolving microbiome of dental caries. *Microorganisms*, 12(1), 121. https://doi.org/10.3390/microorganisms12010121

[2] Zhu, Y., Wang, Y., Zhang, S., Li, J., Li, X., Ying, Y., & Wang, Q. (2023). Association of polymicrobial interactions with dental caries development and prevention. Frontiers in Microbiology, 14, 1162380. https://doi.org/10.3389/fmicb.2023.1162380

[3] Abbas, F. N. (2024). Oral Microbiota and it's Role in Dental Caries. University of Thi-Qar Journal Of Medicine, 27(1), 54-66. Available at http://jmed.utq.edu.iq/index.php/main/article/download/486/556 (Accessed April 14, 2024).

[4] Bowen, D. M. (2016). Effectiveness of professionally-applied silver diamine fluoride in arresting dental caries. American Dental Hygienists' Association, 90(2), 75-78.

[5] Dworkin S. F., Chen A. C., Schubert M. M. and Clark D. W. (1984). Cognitive modification of pain: information in combination with N_2O. *Pain, 19*(4),: 339–351. https://doi.org/10.1016/0304-3959(84)90080-0

[6] Al‐Nasser, L. and Lamster, I. B. (2020). Prevention and management of periodontal diseases dental caries in the older adults. *Periodontology 2000*, *84*(1): 69–83. https://doi.org/10.1111/prd.12338

[7] John, J. R., Daniel, B. R., Paneerselvam, D. and Ganesh, R. (2017). Prevalence of dental caries, oral hygiene knowledge, status, and practices among visually impaired individuals in Chennai, Tamil Nadu. *International Journal of Dentistry*, 1–6. https://doi.org/10.1155/2017/9419648

[8] André, C. B., Rosalen, P. L., De Carvalho Galvão, L. C., Fronza, B. M., Ambrosano, G. M. B., Ferracane, J. L. and Giannini, M. (2017). Modulation of *Streptococcus mutans* virulence by dental adhesives containing anti-caries agents. *Dental Materials*, *33*(10): 1084-1092. https://doi.org/10.1016/j.dental.2017.07.006

[9] Rainey, K., Michalek, S. M., Wen, Z. T. and Wu, H. (2019). Glycosyltransferase-mediated biofilm matrix dynamics and virulence of *Streptococcus mutans*. *Applied and Environmental Microbiology*, *85(*5). https://doi.org/10.1128/AEM.02247-18

[10] Pedraza, M. C. C., Novais, T. F., Faustoferri, R. C., Quivey, R. G., Terekhov, A. I., Hamaker, B. R. and Klein, M. I. (2017). Extracellular DNA and lipoteichoic acids interact with exopolysaccharides in the extracellular matrix of *Streptococcus mutans* biofilms. *Biofouling*, *33*(9): 722–740. https://doi.org/10.1080/08927014.2017.1361412

[11] Jiao, Y., Tay, F. R., Niu, L. N. and Chen, J. H. (2019). Advancing antimicrobial strategies for managing oral biofilm infections. *International Journal of Oral Science*, *11*(3). https://doi.org/10.1038/s41368-019-0062-1

[12] Cui, P., Niu, H., Shi, W., Zhang, S., Zhang, H., Margolick, J. B., Zhang, W. and Zhang, Y. (2016). Disruption of membrane by colistin kills uropathogenic *Escherichia coli persisters* and enhances killing of other antibiotics. *Antimicrobial Agents and Chemotherapy*, *60*(11): 6867–6871. https://doi.org/10.1128/aac.01481-16

[13] Machraoui M., Kthiri Z., Ben-Jabeur M. and Hamada W. (2018). Ethnobotanical and phytopharmacological notes on *Cymbopogon citratus* (DC.) Staph. *Journal of New Sciences*, *55*(05): 3642-3652.

[14] Avoseh O., Oyedeji O., Rungqu P., Nkeh-Chungag B. and Oyedeji A. (2015). *Cymbopogon* species; Ethnopharmacology, phytochemistry and the pharmacological importance. *Molecules*, *20*(5): 7438-7453. https://doi.org/10.3390/molecules20057438

[15] Prins, C. L., Freitas, S. P., De Menezes De Assis Gomes, M., Vieira, I. J. C. and De Amaral Gravina, G. (2013). Citral accumulation in *Cymbopogon citratus* plant is influenced by N 6-benzylaminopurine and light intensity. *Theoretical and Experimental Plant Physiology*, *25*(2): 159–165.

[16] Vassiliou, E., Awoleye, O., Davis, A. and Mishra, S. (2023). Anti-inflammatory and antimicrobial properties of thyme oil and its main constituents. *International Journal of Molecular Sciences*, *24*(8): 6936. https://doi.org/10.3390/ijms24086936

[17] Negrelle, R. R. B., & Gomes, E. C. (2007). Cymbopogon citratus (DC.) Stapf: chemical composition and biological activities. Revista Brasileira de Plantas Medicinais, 9(1), 80-92.

[18] Aćimović, M., Čabarkapa, I., Cvetković, M., Stanković Jeremić, J., Kiprovski, B., Gvozdenac, S., & Puvača, N. (2019). Cymbopogon citratus (DC.) Staph: Chemical composition, antimicrobial and antioxidant activities, use in medicinal and cosmetic purpose. Journal of Agronomy, Technology and Engineering Management (JATEM), 2(6), 344-360.

[19] Li, X., He, T., Wang, X., Shen, M., Yan, X., Fan, S., ... & She, G. (2019). Traditional uses, chemical constituents and biological activities of plants from the genus Thymus. Chemistry & biodiversity, 16(9), e1900254. https://doi.org/10.1002/cbdv.201900254

[20] Park, Y. U., Koo, H. N., & Kim, G. H. (2012). Chemical composition, larvicidal action, and adult repellency of Thymus magnus against Aedes albopictus. Journal of the American Mosquito Control Association, 28(3), 192-198. https://doi.org/10.2987/12-6250R.1

[21] Mathur, V. P., & Dhillon, J. K. (2018). Dental caries: a disease which needs attention. The Indian Journal of Pediatrics, 85, 202-206. https://doi.org/10.1007/s12098-017-2381-6

[22] Mosaddad, S. A., Tahmasebi, E., Yazdanian, A., Rezvani, M. B., Seifalian, A., Yazdanian, M., & Tebyanian, H. (2019). Oral microbial biofilms: an update. European Journal of Clinical Microbiology & Infectious Diseases, 38, 2005-2019. https://doi.org/10.1007/s10096-019-03641-9

[23] Hughes, T. L., Dela Cruz, G. G., & Rozier, R. G. (2003). Oral Health, Early Childhood. In Encyclopedia of Primary Prevention and Health Promotion (pp. 756-767). Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-0195-4_111

[24] Peres, M. A., Macpherson, L. M., Weyant, R. J., Daly, B., Venturelli, R., Mathur, M. R., ... & Watt, R. G. (2019). Oral diseases: a global public health challenge. The Lancet, 394(10194), 249-260. https://doi.org/10.1016/S0140-6736(19)31146-8

[25] Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. (2023, July). Antimicrobial resistance: a growing serious threat for global public health. In Healthcare (Vol. 11, No. 13, p. 1946). https://doi.org/10.3390/healthcare11131946

[26] Gaba, J., Bhardwaj, G., & Sharma, A. (2020). Lemongrass. Antioxidants in vegetables and nuts-properties and health benefits, 75-103.

[27] Taher, M. S., Salloom, Y. F., Al-Asadi, R. A. U. H., Al-Mousswi, Z. J., & Alamrani, H. A. (2021). The medicinal importance of Thyme plant (Thymus vulgaris). Biomedicine, 41(3), 531-534. https://doi.org/10.51248/.v41i3.708

[28] Cheesbrough, M. (2005). *District laboratory practice in tropical countries* (2 nd Ed.). Cambridge University Press, Cambridge, UK., ISBN-13: 9781139449298.

[29] Maru, M., Teklemariam, Z. and Ayana, D. A. (2023). Magnitude, associated factors, and antimicrobial susceptibility pattern of bacterial isolates among adult dental caries patients attending Hiwot Fana comprehensive specialized university hospital, Harar, Eastern Ethiopia. *PLOS ONE*, *18*(2), e 0278829. https://doi.org/10.1371/journal.pone.0278829

[30] Mohammed, T. K., Aquel, N. and Al-Dujalli, E. S. (2020). Antimicrobial activity of liquid residues of *Cymbopogon citratus* oil extracts. *Journal of Physics*, *1660*(1): 012006. https://www.doi.org/10.1088/1742-6596/1660/1/012006

[31] Shaban, N. S., Ma, T. and Banana, H. E. (2015). Phytochemical and pharmacological studies of ethanolic extract of *Thymus vulgaris*. *World Journal of Pharmacy and Pharmaceutical Sciences*, *4*(10).

[32] Han ákov á, Z., Hosek, J., Kutil, Z., Temml, V., Landa, P., Venek, T., Schuster, D., Dall' Acqua, S., Cvacka, J., Polansky, O. and Smejkal, K. (2017). Anti-inflammatory activity of natural geranylated flavonoids: cyclooxygenase and lipoxygenase inhibitory properties and protein analysis. *Journal of Natural Products*, *80*(4): 999-1006. https://www.doi.org/10.1021/acs.jnatprod.6b01011

[33] Lanzotti, V., Romano, A., Lanzuise, S., Bonamoni, G. and Scala, F. (2012). Antifungal saponins from bulbs of white onion, *Allium cepa L. Phytochemistry*, *74*: 133-139. https://doi.org/10.1016/j.phytochem.2011.11.008

[34] Fartha, A. K., Yang, Q., Kim, G., Li, H., Zhu, F., Liu, H., Gan, R. and Corke, H. (2020). Tannins as an alternative to antibiotics. *Food Bioscience*, *38*: 100751. https://doi.org/10.1016/j.fbio.2020.100751

[35] Ma, C., He, N., Zhao, Y., Xia, D., Wei, J. and Kang, W. (2019). Antimicrobial mechanism of hydroquinone. *Applied Biochemistry and Biotechnology*, *189*(4): 1291-1303. https://doi.org/10.1007/s12010-019-03067-1

[36] Escobar, A. M., Perez, M. E., Romanelli, G. P. and Blustein, G. (2020). Thymol bioactivity: a review focusing on practical applications. *Arabian Journal of Chemistry*, *13*(12): 9243-9269. https://doi.org/10.1016/j.arabjc.2020.11.009

[37] Fani, M. M. and Kohanteb, J. (2017). In vitro antimicrobial activity of *Thymus vulgaris essential* oil against major oral pathogens. *Journal of Evidence-Based Complementary & Alternative Medicine*, *22*(4): 660–666. https://doi.org/10.1177/2156587217700772

[38] Ajijolakewu, K. A., Kazeem, M. O., Ahmed, R. N., Zakariyah, R. F., Agbabiaka, T. O., Ajide-Bamigboye, N. T., Ayoola, S. A., Balogun, A. O., and Sani, A. (2021). Antibacterial efficacies of extracts of lemongrass (*Cymbopogon citratus*) on some clinical microbial isolates. *Fountain Journal of National Ad Applied Sciences*, *10*(1). https://doi.org/10.53704/fujnas.v10i1.333