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Effect of methanolic Cymbopogoncitratus extract on bacteria Streptococcus. mutans and Strertococcus. Sorbinus

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Abstract

Strep.sobrinus and Strep.mutans are among the most common species in the mouth. Dental rot, gum aggravation, and tooth rot are medical conditions predominant in the Iraqi people group. Due to their antimicrobial resistance, treating bacteria has become challenging. The focal point of this study is to analyze the impact of the methanolic concentrate of lemongrass leaves on Strep.sobrinusand Strep.mutansbacteria.

Objectives : The review plans to examine the impact of methanolic concentrate of lemongrass on Streptococcus. mutans and Streptococcus. sorbinus. In addition, it aims to determine the minimum inhibitory concentration (MIC) of the methanolic plant extract of lemongrass and investigate its anti-biofilm activity.

Materials and Methods : Samples of lemongrass Cymbopogoncitratus were collected from the Sulaymaniyah area in northern Iraq (Kurdistan), and the Herbal House (Alma'sheb) at the University of Baghdad / Al-Jadiria, for the purpose of studying their effect on Streptococcus. mutans and Streptococcus. sorbinus. The Soxhlet apparatus and Rotary evaporator were used for extraction purposes. High-performance liquid chromatography (HPLC) was used to identify the compound components in the methanolic extract of lemongrass. Enrichment and differential media were used for bacterial isolation and identification. The anti-biofilm activity of the methanolic plant extract of lemongrass was also studied using the Microtiter method. In addition to determining the minimum inhibitory concentration (MIC) of the methanolic extract of lemongrass.

Results : The results demonstrated the presence of seven phenolic compounds in the methanolic extract of Cymbopogon citrates. The study also showed that the MIC for the C. citrates methanolic extract was at a concentration of 1 mg/ml, which was inhibitory for isolates of S. sobrinus and S. mutans, except for some isolates where inhibition occurred at concentrations of 2 and 16 mg/ml. The biofilm formation of Strep. mutans and Strep. sobrinus was inhibited at 8 mg/ml, with the exception of one isolate from Strep. mutans, which was at 16 mg/ml.

Conclusions: The methanolic extract of Cymbopogoncitratus lemongrass leaves is rich in phytochemical compounds. Therefore, it contributes to its medical properties in treating various diseases. The methanolic extract also has an excellent effect in inhibiting the growth of bacteria that cause tooth decay.

Keywords: Cymbopogoncitratus،S. mutans،S. sorbinus،MIC ،HPLC

Introduction

Dental caries is a microbial disease that affects teeth. It damages tooth surfaces where dental plaque is present, leading to localized decay of tooth tissues, which in turn leads to the dissolution of elements on the tooth's surface. Caries affects the inorganic part of the tooth, destroys organic materials, and progresses from the exterior to the interior, due to the secretions of bacteria present in the mouth. One such bacterium is Streptococcus mutans, an anaerobic or facultatively anaerobic Gram-positive bacterium that arranges itself in chains and pairs of spherical cocci, usually non-motile, non-sporulating, and able to grow between 18-40 degrees Celsius. It is one of the most widely recognized reasons for dental caries. This kind of microbes endures acids and can go through physiological transformations to work really in acidic conditions, like dental plaque. They are major contributors to the formation of biofilms and produce lactic acid. Furthermore, Streptococcus sobrinus is one more supporter of human dental caries. Notwithstanding its critical job in oral wellbeing, concentrates on Sterp. There are few sobrinus. It is liable for tooth rot paces of 10 to 14%. It is Gram-positive, round in shape, catalase negative, non-motile, and anaerobic.

Recently, bacteria's ability to resist many antibiotics has been observed, leading current studies to highlight the importance of turning towards medicinal plants. These are generally safe, easily available, and relatively inexpensive. Thousands of plant species are estimated to be beneficial for treatment, one of which is Cymbopogoncitratus (C. citratus), also known as lemongrass. It is known to contain many non-toxic compounds with antimicrobial activities. Lemongrass oil is a common essential oil used for oral health care, especially for chronic periodontitis, which has led to the widespread use of lemongrass extract in many countries. It is used as a tea additive, to make ointments, and others, thus it is safe for use. For years, it has been proven that lemongrass extracts are added to mouthwash used in the treatment and prevention of various oral diseases, especially gum diseases caused by bacteria. It also has numerous biological properties; it is antifungal, antiviral, and antioxidant .

Thusly, the goals of this exploration are: to concentrate on the impact of the methanolic concentrate of lemongrass on Streptococcus. mutans and Streptococcus. sobrinus. Additionally, to determine the minimum inhibitory concentration (MIC) of the methanolic extract of lemongrass and investigate its effectiveness against biofilms.

Materials and Methods:

.1 Sample Collection

Oral swabs were gathered from 27 patients experiencing tooth rot, depressions, and gum aggravation at the Clinical City Medical clinic in Baghdad. The patients' ages ran somewhere in the range of 6 and 70 years of age, and they were of the two sexes.The sample collection occurred from 1st September 2022 to 1st

November 2022. The samples were immediately placed in sterilized tubes containing transport medium, namely Brain-Heart Infusion Broth. Afterward, the samples were immediately transferred to the laboratory for the inoculation process on enriching and selective media. Then, they were incubated for 24 hours at a temperature of 37°C to perform diagnostic tests subsequently.

Moreover, samples of Cymbopogoncitratus (lemongrass) plant were collected from the Sulaymaniyah region in northern Iraq (Kurdistan), and the Herb House at the University of Baghdad/Al-Jadriya. The plant was classified by specialists in plant taxonomy at the University of Baghdad/ Department of Life Sciences.

.2 Cultivation Media:

-Blood Agar: Used to isolate Streptococcus mutans and Streptococcus sobrinus and to test their ability to break down blood and identify the type of breakdown [16

-Urea Agar: Used to test the bacteria's ability to produce the Urease enzyme [17.[

-Motility Test Medium: Used to test the bacteria's ability to move [18.[

-MitisSalivarius Bacitracin Agar (MSBA): A selective medium for Strept. mutans and Strep. sobrinus, prepared according to [19,20.[

-Brain-Heart Infusion Broth: Used as a carrier medium for samples to ensure bacteria preservation until reaching the laboratory [21.[

-Voges-Proskauer and Methyl Red Media: Used to detect complete decomposition of sugars and production of organic acids after incubation for 24 hours (MR test), or partial decomposition of sugars and production of Acetoin after incubation for 48 hours (VP test) [21.[

-Biochemical tests: Tests (Oxidase Test, Catalase Test, IMViC Test, Urease Test, Motility Test, Clotting Enzyme Production, Lactose Fermentation) were performed according to [22,23.[

.3 Preparation of Alcoholic Extract of Cymbopogon Citrates:

The alcoholic concentrate was ready as indicated by [24,25] utilizing a Soxhlet gadget. In a thimble, 100 grams of lemongrass leaves from Cymbopogon citrates were added, 700 milliliters of methanol (70 percent concentration) were added, and the mixture was left at 40-60 degrees Celsius for six hours. The filtrate was then focused utilizing a Revolving evaporator under diminished tension at a temperature of 40 - 45°C to dispose of the dissolvable. Before being used, the alcoholic extract was obtained and stored in a sterile test tube.

.4 Phytochemical Tests of Cymbopogon Citrates:

Various tests were performed to identify the different components present in the lemongrass extract, which include tests for Flavonoids, Phenols, Alkaloids, Glycosides, Tannins, Resins, Terpenes, and Saponins.

.5 High-Performance Liquid Chromatography (HPLC:(

Used to identify components of the methanolic extract of Cymbopogon citrates as per the instructions [31.]

.6 **Finding the Cymbopogon Citrates' Minimum Inhibitory Concentration:**

The prepared plant extracts' minimum inhibitory concentration (MIC) was determined using the broth microdilution method. On a microtiter plate, the concentrations of 0.5 to 64 mg/ml were directly prepared [32].

.7 **Study of the Antibiofilm Activity of Cymbopogon Citrates**:

A 96-well microtiter plate was utilized to decide the antibiofilm movement of the plant removes under study. The plant extricates were ready at a centralization of 256 mg/ml in the supplement medium from which two-overlay weakenings were ready, straightforwardly on the plate, at groupings of 128-1 mg/ml. The weakening series was arranged utilizing a micropipette in a consecutive way [32].

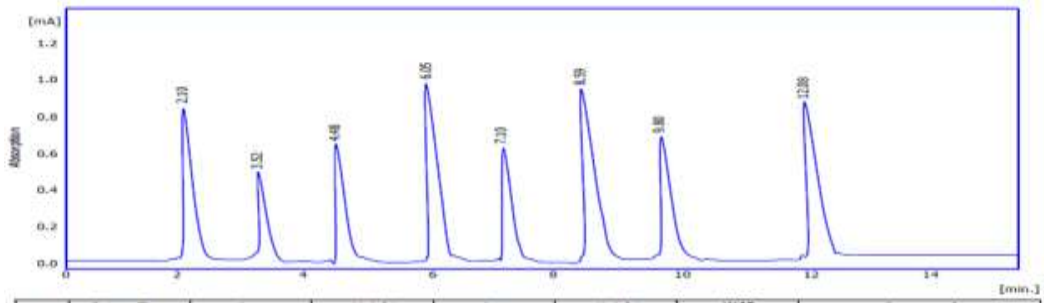
Results and Discussion:

Bacterial Isolation Rate :

The results indicated that seven isolates, accounting for 2.54%, belong to Strep. mutans and Strep. sobrinus. Out of these, six isolates (2.18%) were identified as Strep. mutans, whereas only one isolate (0.36%) was classified as Strep. sobrinus.

High-Performance Liquid Chromatography (HPLC:(

In the current study, seven phenolic compounds were detected (Gallic Acid, Sinapinic Acid, Catechin, Chlorogenic Acid, Caffeic Acid, Kaempferol, and Ferulic Acid) in the methanolic extract of Cymbopogon citrates (See figures 1, 2, 3, 4, 5, 6, 7, 8).



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	2.10	6524.70	850.12	12.24	12.25	0.25	Gallic acid
2	3.52	3654.28	584.15	8.22	8.30	0.15	Ellagic acid
3	4.48	4123.69	611.25	10.65	10.65	0.20	Quercetin
4	6.05	9741.02	980.12	15.97	15.44	0.25	Hydrobenzoic acid
5	7.10	4210.08	611.32	11.48	11.46	0.20	*P*-Coumaric acid
6	8.59	8821.64	940.25	12.44	12.36	0.25	Chlorogenic acid
7	9.80	7530.12	799.58	10.55	10.41	0.20	Caffeic acid
8	12.08	9652.33	860.22	11.98	11.85	0.25	Ferulic acid
	Total	54572.16	6237.49	100.00	100.00		

Figure 1: HPLC chromatogram of phenolic compounds inCymbopogoncitratus methanolicextract

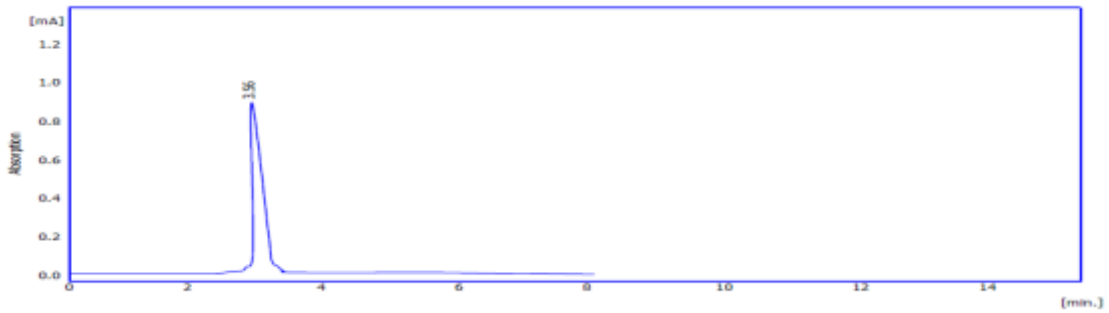


Figure 2: HPLC chromatogram of phenolic compound standard Gallic acid

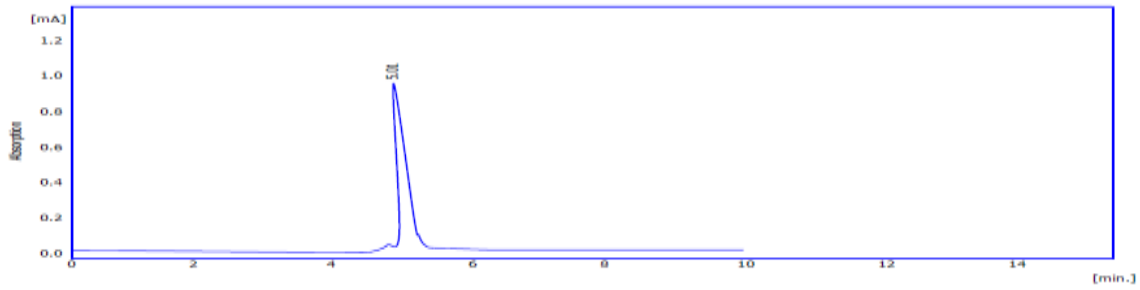


Figure 3: HPLC chromatogram of phenolic compound standard Sinapinicacid

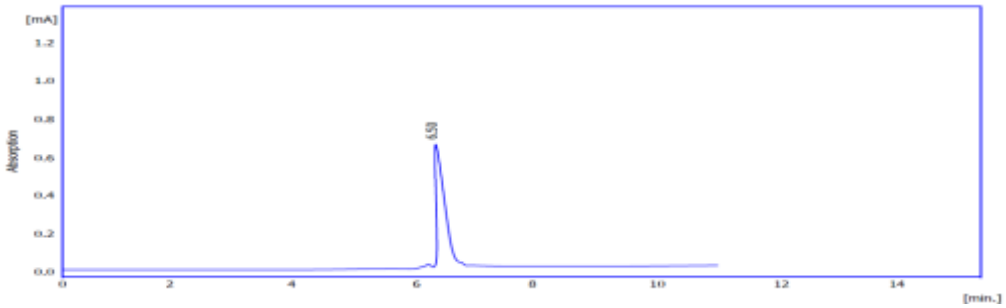


Figure 4: HPLC chromatogram of phenolic compound standard Catechin

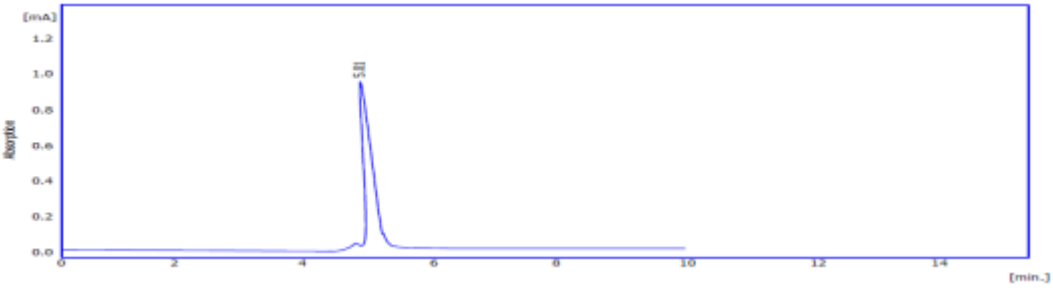
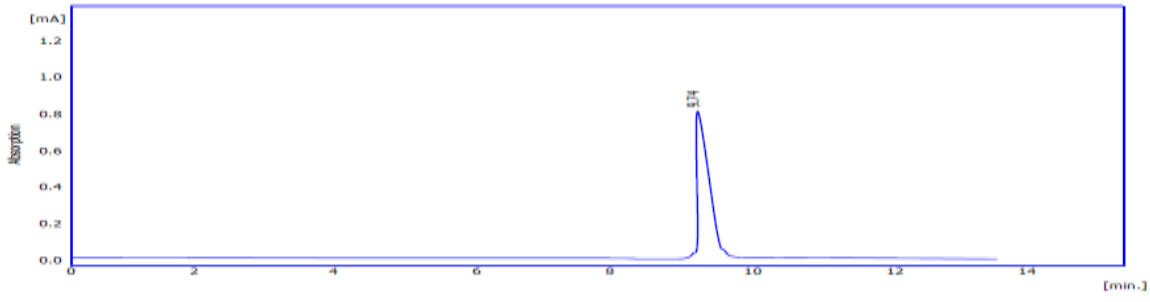


Figure 5:HPLC chromatogram of phenolic compound standard Chlorogenic acid



Firure 6: HPLC chromatogram of phenolic compound standard Caffeicacid

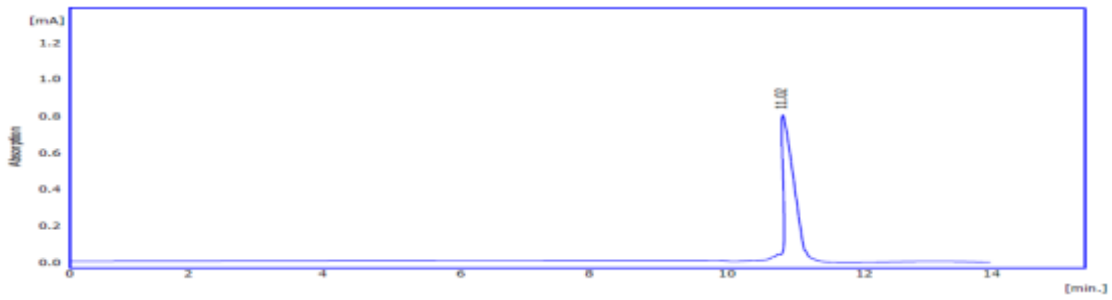


Figure 7: HPLC chromatogram of phenolic compound standard Kaempferol

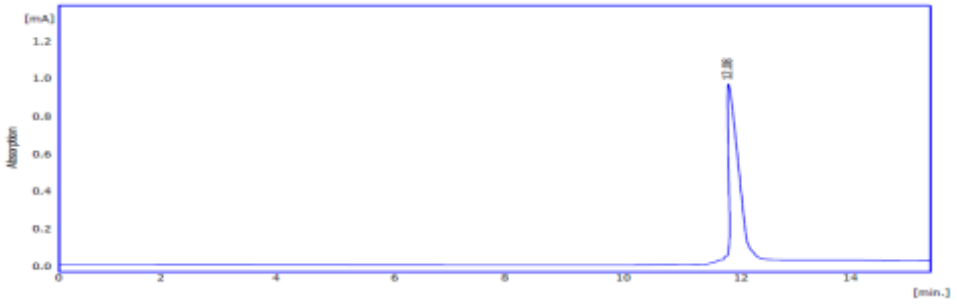
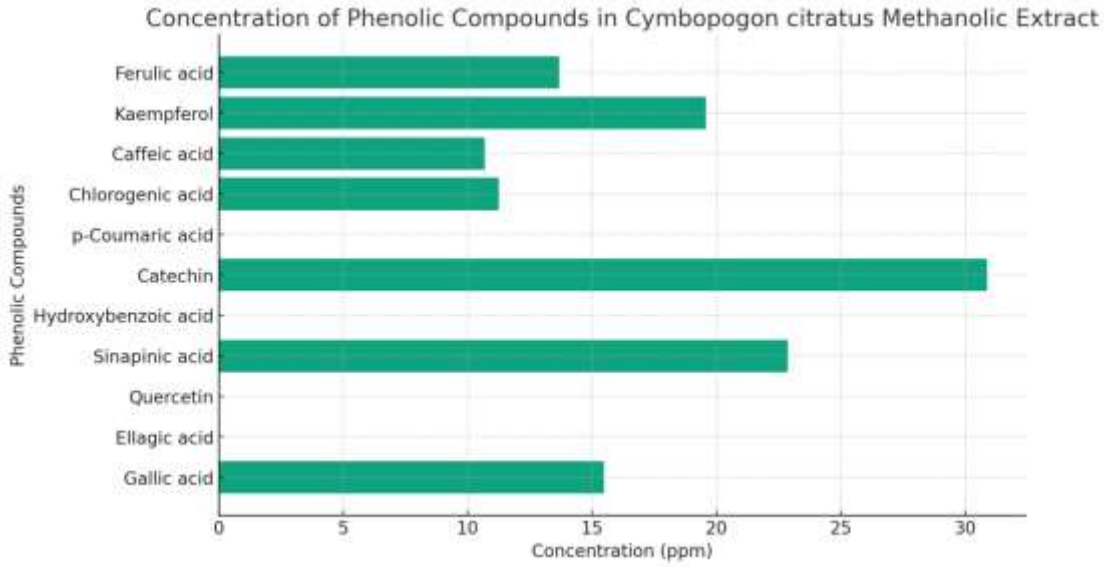


Figure 8: HPLC chromatogram of phenolic compound standard Ferulic acid

Chemical Quantitative Analysis of Methanolic Extracts of Lemongrass Plant:

Table 1: Quantitative analysis of Cymbopogoncitratus extracts

phenolic compounds	Cymbopogoncitratusmethanolicextract (ppm)
Gallic acid	15.48
Ellagic acid	------
Quercetin	------
Sinapinicacid	22.88
Hydroxybenzoic acid	------
Catechin	30.89
p-Coumaric acid	-----
Chlorogenic acid	11.25
Caffeic acid	10.69
Kaempferol	19.58
Ferulic acid	13.68



Isolation Rate of Bacteria: The results revealed that seven isolates, representing 2.54%, belong to Strep. mutans and Strep.sobrinus. It was found that six isolates (2.18%) were of the Strep. mutans type, while only one isolate (0.36%) was of the Strep.sobrinustype.

High-Performance Liquid Chromatography (HPLC):

In the present study, seven phenolic compounds (Gallic acid, Sinapinic acid, Catechin, Chlorogenic acid, Caffeic acid, Kaempferol, and Ferulic acid) were discovered in the methanolic extract of Cymbopogon citrates (Figures 1,2,3,4,5,6,7,8).

Chemical Quantitative Analysis of Methanolic Extracts of Lemongrass Plant:

Table 1 presents the quantitative analysis of Cymbopogoncitratus extracts.

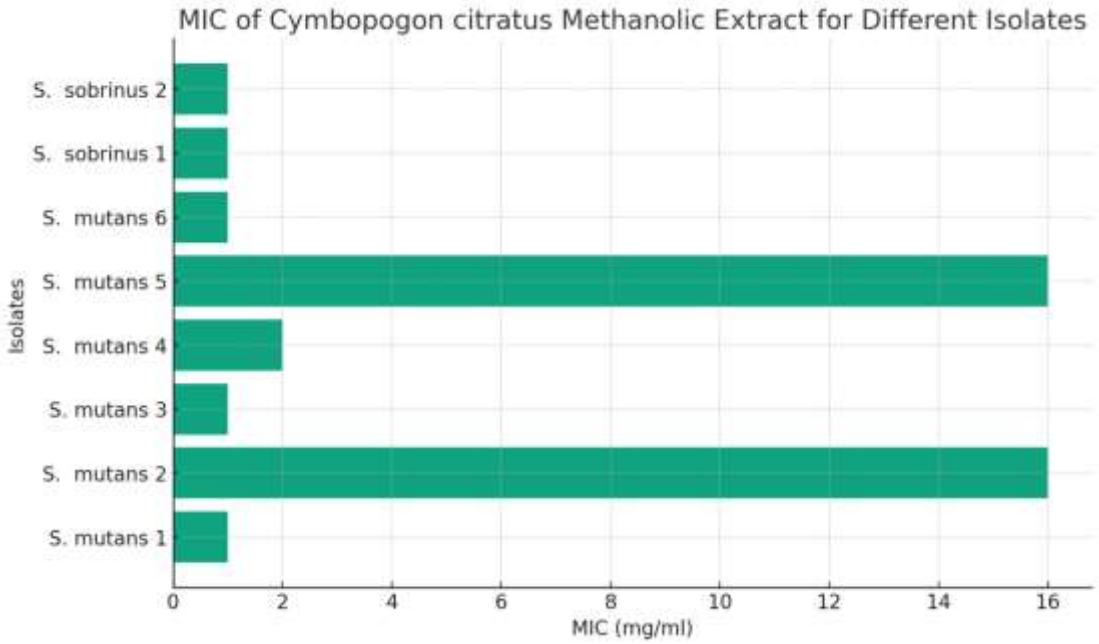
The outcome for the Cymbopogoncitratus extract in this study was consistent with a study conducted by Ali et al. in 2022, which revealed that the methanolic extract of Cymbopogoncitratus contains phenolic compounds such as Chlorogenic, Kaempferol acid, Ferulic acid, Catechin, Sinamic acid, Quinic acid, and Caffeic acid [33].

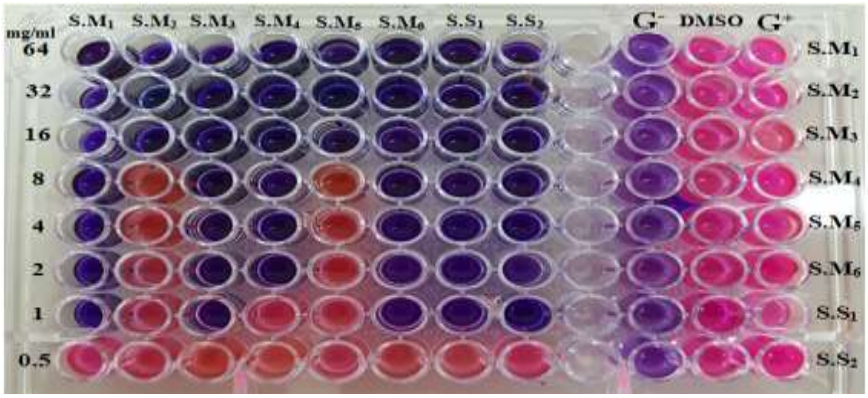
Determination of Minimum Inhibitory Concentration of Cymbopogoncitratus:

The ongoing review results for C. citrates extricates showed that the MIC for the methanolic remove at 1 mg/ml hindered the development of S. sobrinus and S. mutans detaches, aside from separates number (4, 2, and 5) that were inhibited at a concentration of 2 and 16, and 16 mg/ml, respectively, as shown in Table 2. It was mentioned by [34] that the leaves of C. citrates possess plant chemical components that play an essential role health-wise. In another method, Extraction can be in water, ethanol, and hexane, all of which possess antimicrobial activities. He pointed to the minimum inhibitory concentration (MIC) for the extracts that ranged between (<10 to >80) mg/ml.

Table 2: The Minimum Inhibitory Concentration (MIC) of the methanolic extract of lemongrass against Streptococcus mutans and Streptococcus sobrinus.

Isolates	Methanolic extract (mg/ml)
	MIC
S. mutans$_1$	1
S. mutans$_2$	16
S. mutans$_3$	1
S. mutans$_4$	2
S. mutans$_5$	16
S. mutans$_6$	1





(S.M): ***Streptococcus mutans,*** **(S.S):** ***Streptococcus sobrinuss*****,(DMSO):** Dimethyl sulfoxide, **(C^+):** Control positive (Bacteria + Media), **(C^-):** Control negative (Media only)

Study of the Antibiofilm Activity of Cymbopogon citrates:

The methanolic concentrate of Cymbopogoncitratus (100 percent) hinders the development of biofilms of Strep. mutans and Strep. sobrinus at 8 mg/ml, aside from Strep. mutans isolate (4), which had a concentration of 16 mg/ml, as depicted in Table 3.

Table 3: Thin biofilm formation of Streptococcus mutans and Streptococcus sobrinus before and after treatment with the methanolic extract of lemongrass.

Reference [34-49] reported that the essential oil of Cymbopogoncitratus exhibited antimicrobial effects on Streptococcus mutans and Candida albicans, and was able to inhibit the formation of multi-species biofilms in the laboratory.

Concentration (mg/ml)	Before treatment (Control)							
	S. mutans $_1$	*S. mutans* $_2$	*S. mutans* $_3$	*S. mutans* $_4$	*S. mutans* $_5$	*S. mutans* $_6$	*S. sanguinis*$_1$	*S. sanguinis*$_2$
	Strong	Strong	Strong	Strong	Strong	Strong	Strong	Moderate
	After treatment							
1	Strong	Strong	Strong	Strong	Moderate	Strong	Strong	Weak
2	Strong	Moderate	Moderate	strong	Weak	Moderate	Moderate	Weak
4	Moderate	Moderate	No Biofilm	Moderate	Weak	Weak	Weak	No Biofilm
8	No Biofilm	No Biofilm	No Biofilm	Moderate	No Biofilm	No Biofilm	No Biofilm	No Biofilm
16	No Biofilm	No Biofilm	No Biofilm	Moderate	No Biofilm	No Biofilm	No Biofilm	No Biofilm
32	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm
64	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm
128	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm
256	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm	No Biofilm

Conclusion:

The current study confirmed the antibacterial activity of Cymbopogoncitratus (lemongrass) against Streptococcus mutans and Streptococcus sobrinus, which are the common bacteria causing dental caries and tooth decay. The methanolic extract of lemongrass showed a significant inhibitory effect on these bacteria, along with a strong capability to prevent biofilm formation, a key factor in dental plaque accumulation and subsequent dental caries. Furthermore, the detected phenolic compounds in the methanolic extract might be responsible for the antibacterial and anti-biofilm properties, reflecting the potential application of this plant extract in dentistry, particularly in the prevention and treatment of dental caries.

Nonetheless, while these findings are promising, further research is required to validate these results in clinical settings and explore the detailed mechanism of action of lemongrass extract on oral bacteria. It would be interesting to conduct further studies involving human subjects to determine the optimal dosage and application method, and to explore any potential side effects.

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