Original Article

Effect of *Commiphora myrrha* Extract on Biofilm Formation of *Lactobacillus* Isolated from Teeth Caries

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Abstract

Background: Dental caries starts, progresses, and results in tooth decay due to biological factors such as cariogenic bacteria. *Streptococcus mutans* and *Lactobacillus* are considered to be the main microorganisms involved in this condition. **Objectives:** To study the effect of Myrrh extract as an antibacterial and anti-biofilm formation of *Lactobacillus* isolated from teeth caries. **Materials and Methods:** Approximately 200 clinical swabs were collected from various schools and hospitals in Baghdad, Iraq, and culture and biochemical tests were used for detection. The VITEK2 compact system was used to confirm identification. The drug and Myrrh extract susceptibility tests were performed using the macrodillution method. The MTP method was used for the biofilm formation assay. Clevenger apparatus used for Myrrh extract, its chemical composition was identified by GC mass method. Detection of *LuxS* and *pfs* in 12 isolates (moderate in biofilm formation) by conventional polymerase chain reaction. **Results:**Approximately 65 isolates were identified as *Lactobacillus* (*L. fermentum* and *L. plantarum*). 58 (89.23%) isolates have the ability to form biofilm. All *L. fermentum* isolates were intermediate resistant to ceftriaxone and cefotaxime, but sensitive to ampicillin, meropenem, and amoxicillin-clavulanic acid. 9 (81.82%) isolates of *L. plantarum* revealed resistance to both ceftriaxone and cefotaxime, but 2 (18.18%) isolates were intermediate to these drugs, and all *L. plantarum* were sensitive to ampicillin, meropenem, and amoxicillin-clavulanic acid. Myrrh extract consists of 35 chemical compounds. All of these 12 isolates, which are most productive in biofilm formation, became nonproducers after processing with Myrrh extract. All of these 12 isolates have *LuxS* and *pfs*. **Conclusion:** Myrrh extract has an antibacterial effect and is effective against biofilm formation to prevent bacterial attachment on the teeth and teeth caries formation.

Keywords: *Lactobacillus*, *luxS*, myrrh extract, *pfs*, teeth caries

Introduction

Lactobacillus is a Gram-positive, without spores, rods or coccobacilli, short or extended, found in chains, anaerobic, microaerophilic, or facultative. Catalase, oxidase, and gelatinase negative.[1,2] Most *Lactobacillus* are non-motile, but *Lactobacillus ruminis* and *L. agilis* have flagella.[3]

Several *Lactobacillus* species are used as starter cultures to regulate the production of yogurt, wine, beer, cheese, and other fermented foods and animal feeds.[4] The mouth contains a variety of microorganisms, some of which produce acid through carbohydrate fermentation, leading to demineralization and plaque formation. The formation of acid from fermentable carbohydrates is the primary property of the genus *Lactobacillus*.[5,6] Dental caries can develop due to *Lactobacillus* ability to adhere and produce acid.[7] They convert the neutral to acidic pH. They connect with dental plaque during the colonization and maturity stages for teeth caries production and begin to dematerialize tooth enamel.[8] In order for quorum sensing to occur, a type of molecule-mediated communication used for biofilm development, a certain amount of chemicals must be produced outside the cells. Without these chemicals, biofilms are unable to develop into mature structures, grow, or release planktonic cells.[9,10] The mechanism of response

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to the external signal is triggered by adverse conditions during bacterial development and leads to the production of signaling molecules that regulate specific behaviors.[11] Depending on the type of bacterium, *luxS* produces AI-2 and then associates with its receptor.[12] According to a new study, *Lactobacillus luxS* has a key metabolic function in the formation of biofilms. The ability to form biofilms was reduced in *Lactobacillus* by deleting *luxS*. The mutant *luxS* strain had lower biofilm adherence and generation than non-mutant strain. They also investigated its role by adding AI-2 that was made in the lab, which caused the altered *luxS* strain to form biofilms and adhere again.[13] The expression or repression of genes relevant to biofilms is connected to the stimulation or inhibition of QS signaling. Biofilm is essential for the continued growth of microorganisms in many environments.[14] The ability of probiotics to create biofilms in the host's gut is likewise regulated by the QS mechanism.[15] Other research revealed that adding various amounts of AI-2 to *Lactobacillus plantarum* considerably increased the quantity of EPS.[16] Numerous genes whose transcription was impacted by deletion of *luxS* contributed to the creation of several biofilm elements. As a result, altering the expression of certain of these genes may potentially impact EPS secretion. Overall, AI-2/LuxS is crucial for the development of biofilm.[17] An essential signaling component for this system in LAB was AI-2. As a result, it is able to investigate its impact on biofilm development and the mechanism underlying it, which involves the control of the synthesis of these components. Therefore, for optimal utilization of LAB, proper biofilm management is essential. Exogenous addition of AI-2 or *luxS* deletion, which are involved in the manufacture of these molecules, have been the major methods used to investigate the impact of QS on the development of biofilms.[18,19] In this way, the methyl donor S-adenosylmethionine (SAM) triggers the production of S-adenosylhomocysteine (SAH), which is then quickly metabolized to adenine and S-ribosylhomocysteine (SRH) via the function of S-adenosyl homocysteine nucleosidase (pfs). The *luxS* produces the enzyme S-ribosylhomocysteinase, which converts SRH to homocysteine and the unstable compound 4,5-dihydroxy-2,3-pentandione (DPD), which rearranges to produce AI-2.[20,21] Biological effects of myrrh on bacteria and viruses have been documented in the literature. Myrrh extracts have been shown to have impacts on viruses, and as a result, they exhibit antibacterial and antiviral properties.[22] Myrrh extracts have been shown to have bactericidal, fungicidal, and antiviral properties, indicating their ability to stop the spread and inhibit the growth of different types of bacteria and viruses.[23]

Materials and Methods

Isolation and identification

A total of 200 clinical samples were gathered from different schools and hospitals in Baghdad, Iraq, through the period of October 2022 to April 2023, and then transferred into tubes containing De Man, Rogosa, and Sharpe (MRS) broth.

Then, it was inoculated anaerobically on De Man, Rogosa, and Sharpe (MRS) agar (37°C for 48–72 h) prepared previously. Colonies that appeared as convex, opaque, and smooth without pigment were isolated from MRS agar and subcultured for 2–3 times at 37°C for 24–48 h to get isolated colonies.

Then carry out further tests such as oxidase, catalase, Gram-stain, endospore staining, and Vitek technique which confirmed the bacterial identification.[24-27]

Biofilm formation of lactobacillus by microtiter plate assay

Micro-titer plate involved 96 polystyrene wells:

- MRS broth was used to culture each isolate at 37°C for 24–48 h in anaerobic conditions. Thereafter, 100 µL of bacterial growth was transferred into a tube of 2 mL NaCl.
- Volume of 180 µL of MRS broth containing 1% glucose was added to microtiter plates.
- Volume of 20 µL of bacterial suspension (from normal saline) put inside three wells in microtiter plates. Three wells with sterile MRS broth were considered as a negative control.
- Plates were covered with their lid and incubated under anaerobic conditions (37°C for 72 h), gently washed thrice with distilled water, and then dried.
- The plates were stained with 200 µL of 1% crystal violet solution for 15 min at room temperature. Further, wells were washed and dried at 50–60°C for 30 min (completely dry and fixation).
- Then, add 200 µL ethanol 96% to wells for 15 min to the extraction of the stained adherent.
- By employing microtiter plate reader, OD for every well measured at 630 nm.

The findings were categorized into four groups based on their optical density as shown in Table 1.[28,29]

Antibiotic sensitivity test

Two-fold dilutions from antibiotics were prepared in sterile De Man, Rogosa, and Sharpe broth, and 15 test tubes labeled 1 to 15 were used to create different drug concentrations. One milliliter of De Man, Rogosa, and

Table 1: Biofilms categories according to optical density

Optical density	Results
OD ≤ ODc	Non-producer
ODc < OD ≤ 2 × ODc	Weak-producer
2 × ODc < OD ≤ 4 × ODc	Moderate-producer
4 × ODc < OD	Strong-producer

Table 2: Extent of resistance for each antibiotic and their MIC (μg/mL)[33]

Antibiotics	Sensitive (S)	Intermediate (I)	Resistance (R)
Ceftriaxone	≤16	32	≥64
Ampicillin	≤0.5	1	≥2
Cefotaxime	≤16	32	≥64
Meropenem	≤4	8	≥16
Amoxicillin-clavulanate	≤4	8	≥16

Sharpe broth was added to each tube. One milliliter of antibiotics was placed in tube number 15; after mixing, 1 µL of the content in each tube was added to the subsequent tube to create successive two-fold dilutions. The procedure was continued in tube 1. Another three tubes were used to prepare three controls: two negative controls (antibiotic + De Man, Rogosa and Sharpe broth, only De Man, Rogosa, and Sharpe broth) and one positive control (10 µL bacterial inoculums + De Man, Rogosa, and Sharpe broth). The experiment was performed in triplicate for each bacterial isolate.[30-32] The minimum inhibitory concentration (MIC) was determined using the macrodilution method. The MIC was the lowest dose of an antibiotic with an inhibitory impact on microbes that was not visible after being cultured at 37°C for 48 h. Ten microliters of the new bacterial culture were added to each dilution and then incubated at 37°C for 48 h.[30,32] Finally, the results were expressed as sensitive, intermediate, and resistant according to CLSI 2020 [Table 2].[33]

Myrrh Extraction

Myrrh extraction of *Commiphora myrrha* using hydrodistillation method by Clevenger apparatus in Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq, 250 g of Myrrh [Figure 1] was boiled with 1.2 L of distal water, the extract was kept at 4°C until used in other experiments.[34-38]

Chemical composition of *Commiphora myrrha* extract

The gas chromatography (GC) mass technique was used to determine the chemical makeup of the myrrh extract[37] using a gas chromatograph (Agilent 5977E MSD, USA; GC-Mass Spectrometer).

Determination of the minimal inhibitory concentration of Myrrh extract

Macro-dilution method in test tubes was used to determine the minimal inhibitory concentration (MIC) of Myrrh extract according to CLSI 2012. In sterile MRS broth, two-fold dilutions from Myrrh extract were made. Ten test tubes, labeled between 1 to 10, were used to prepare different concentrations of this extract. One milliliter of sterile MRS broth was added to each tube in sterile conditions. One milliliter of Myrrh extract was added inside tube number 10: after

Figure 1: Myrrh of *Commiphora myrrha*

mixing, one milliliter of the contents in each tube was transferred to the subsequent one to create successive two-fold dilutions. The procedure was continued to tube 1. Another three tubes were used to prepare three controls: two negative controls (one containing extract + MRS broth, and one containing only MRS broth) and one positive control (10 µL of bacterial inoculum + MRS broth). The experiment was performed in triplicate for each bacterial isolate.[32] The MIC was determined as above.[30-32]

Anti-biofilm activity of Myrrh extract against *Lactobacillus*

The assay was performed in a microtiter plate,[34] 50 µL of MRS broth containing 1% glucose added into microtiter plates, 50 µL of sub-MIC 62.5 mg/mL of extract in MRS broth was added to the wells and 10 µL out bacterial inoculum were added. A total of three wells with sterile MRS broth were considered as a negative control. The plates were covered with their lids and incubated under anaerobic conditions at 37°C for 72 h to allow enough time for bacterial growth and biofilm formation and to know the effect of Myrrh extract on the biofilm forming capacity of *Lactobacillus*. Then gently washed thrice with distilled water and then dried. The plates were stained with 200 µL of crystal violet solution (1%) for 15 min at room temperature. Furthermore, wells were washed and dried at 50–60°C for 30 min (complete drying and fixation). Then add 200 µL of 96% ethanol to the wells for 15 min to extract the stained adherent. The second microtiter plate was processed and inoculated using the same method but without treatment with extract. This was to compare the results for each isolate before and after processing with

Myrrh extract to identify the anti-biofilm-like activity of the Myrrh extract in preventing biofilm formation by *Lactobacillus*.

By using a microtiter plate reader, the OD for each well was measured at 630 nm. Based to the absorption of the medium control, the findings of initial adhesion inhibition were determined on microtiter plates.[28,29]

DNA molecular assay

DNA extraction and purification based on ABIOpure™ Total DNA Kit (ABIOpure, USA).

Genomic DNA was extracted from all 12 *L. fermentum* isolates (which exhibit moderate biofilm formation). Two pairs of primers are the following: For *luxS* forward primer AAACGGTGAGCAGTCAATCA, reverse primer AAGGTCCTAAGGGTGACAAGA. For *pfs* forward primer TGGTCCTGGTGAAATCCG, reverse primer CGTCAACATCGTGGTAAGC.[39]

Reaction setup and thermal cycling protocol are listed in Tables 3 and 4, using Thermal Cycler, Thermo Fisher Scientific, USA.

Statistical analysis

Findings were processed statistically according to SAS (SAS Institute Inc., Cary, North Carolina, USA) 2018.[40]

Ethical approval

The study was conducted in accordance with the ethical principles. It was carried out with patient's verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by the University of Baghdad, College of Science, a local ethics committee according to document number CSEC\1022\0128 on October 17, 2022 to get this approval.

Table 3: Polymerase chain reaction component of reagents

Master mix components	Volume for 1 sample (μL)
Master mix	10
Forward primer	1
Reverse primer	1
Nuclease free water	6
DNA	2
Total volume	20

Table 4: *luxS* and *pfs* detection program

Steps	Temperature (°C)	Time (m: s)	Cycle
Initial denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	60	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Results

Isolation and identification

According to their culture characteristics and other biochemical tests presented in Table 5, only 65 (32.5%) isolates were diagnosed as *Lactobacillus* and then confirmed by the VITEK 2 compact system.

Lactobacillus isolates were distributed into two species, *L. fermentum* was represented as the dominant species in 83.08% (54 isolates) and *L. plantarum* was represented in 16.92% (11 isolates) of the total number of *Lactobacillus* isolates [Figure 2]. These findings were consistent with the results of Zhang and Zhang,[31] which revealed that 54.83% of *Lactobacillus* species isolated from dental caries had *L. fermentum* as the dominant species and 22.58% of *Lactobacillus* isolates represent *L. plantarum*.

Biofilm formation assay of *lactobacillus*

The present research showed different biofilm capabilities. The findings showed that 58 (89.23%) of the isolates produced biofilm, while 7 (10.77%) of the isolates did

Table 5: Culture characteristics and biochemical reactions

Tests	Results
Oxidase test	Negative (no change in color)
Catalase test	Negative (no bubbles)
Growth on MRS medium with $CaCO_3$	Clear zone
Gram staining	Gram-positive (purple)
Endospore staining	Non-spore former (pink)

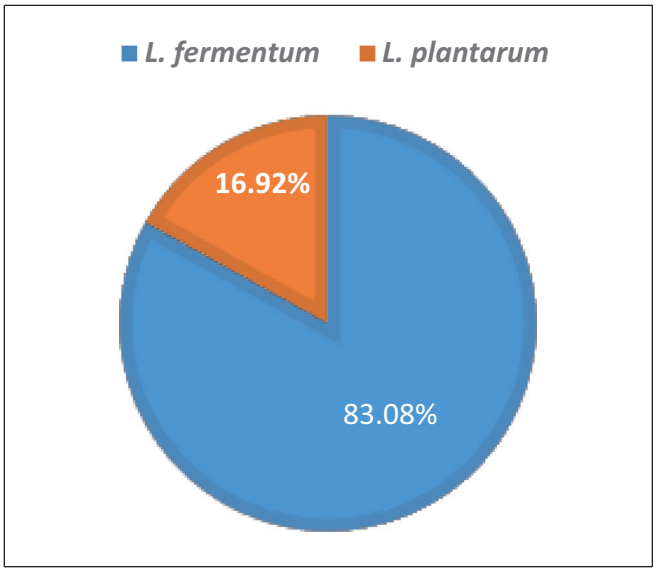

Figure 2: Prevalence of *Lactobacillus* species from teeth caries

not produce biofilm of *Lactobacillus* isolates as shown in Tables 6 and 7 and Figure 3]. Furthermore, there is no isolate with strong production for the biofilm, but 12 (18.46%) isolates showed moderate production and 46 (70.77%) were weak.

Detection of antibiotic sensitivity and MICs

The macro-dilution method was used to identify the antibiotic sensitivity of all *Lactobacillus* isolates from dental caries to five antimicrobial drugs.[32]

According to the results presented in Figure 4, all *L. fermentum* are sensitive to ampicillin in penicillin class (β-lactams group), meropenem in carbapenem class (β-lactams group), and amoxicillin-clavulanic acid (penicillin-like antibiotic and beta-lactamase inhibitor). But all isolates of *L. fermentum* show intermediate resistance to both ceftriaxone and cefotaxime (cephalosporin antibiotics).

While all isolates of *L. plantarum* are sensitive to ampicillin, meropenem, and amoxicillin-clavulanic acid, 81.82% (9 isolates) of *L. plantarum* showed resistance to ceftriaxone and cefotaxime antibiotics, and 18.18% (two isolates) of *L. plantarum* showed intermediate resistance to both ceftriaxone and cefotaxime antibiotics, and all these results of the antibiotics sensitivity test for *L. fermentum* and *L. plantarum* are consistent with findings of Anisimova *et al.*, which revealed that *L. fermentum* and *L. plantarum* are sensitive to ampicillin and meropenem, and all isolates of *L. fermentum* showed intermediate resistance had ceftriaxone and cefotaxime antibiotics, while 72.73% of *L. plantarum* isolates showed resistance against ceftriaxone, 90.91% had resistance to cefotaxime antibiotic, 27.27% with intermediate resistance to ceftriaxone, and 9.09% showed intermediate resistance to cefotaxime antibiotic.[41] Also agree with the findings of Asan-Ozusaglam and Gunyakti, which showed that all isolates of *L. fermentum* were sensitive to ampicillin antibiotic.[42] The findings of Kamel *et al.* revealed that all *L. fermentum* and *L. plantarum* are sensitive to amoxicillin-clavulanic acid.[43]

Table 6: Results of biofilm formation of *Lactobacillus*

Categories of biofilm formation	No. of isolates (%)
Strong-producer	Non
Moderate	12 (18.46%)
Weak	46 (70.77%)
Non-producer	7 (10.77%)

Extraction and chemical composition of myrrh extract

Myrrh extract was extracted using Clevenger apparatus (hydro-distillation method) and kept at 4°C until use.[34-38] To determine the chemical structure of myrrh extract, GC mass method was carried out[37] using gas-chromatograph spectrometer. GC/MS findings showed 35 components when comparing their chemical structure with those in the library. According to Table 8, the largest proportion of components included benzofuran (22.13%), then cyclohexane (9.00%), and these findings were consistent.[44]

MIC of Myrrh extract against *Lactobacillus*

Macro-dilution method in test tubes was used to determine the MIC of Myrrh extract and the results revealed the MIC of Myrrh extract equal to 125 mg/mL against all 12 *L. fermentum* (moderate in biofilm-formation) isolates, so this extract has antibacterial activity at a concentration of 125 mg/mL and higher. The findings of this study were consistent with other studies showing an antibacterial effect of Myrrh extract[22,45] and with the results of Izzeldien *et al.*, which revealed an antibacterial effect of Myrrh extract against both *Lactobacillus* species and *S. mutans*.[44]

Anti-biofilm activity of Myrrh extract against *Lactobacillus*

The microtiter plate technique was used to determine the impact of myrrh extract on *L. fermentum* capacity to produce biofilm. All *L. fermentum* exhibiting moderate biofilm formation were processed with Myrrh extract at

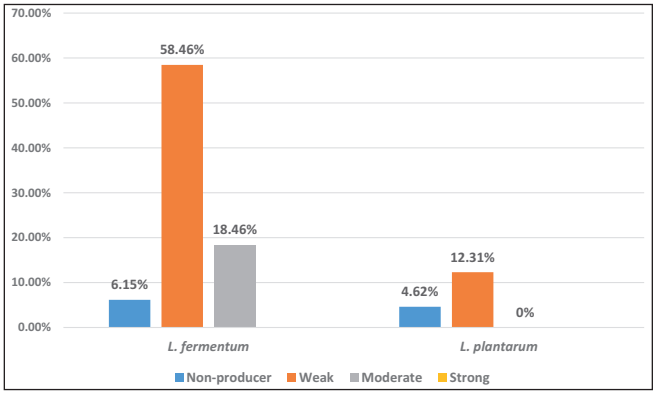

Figure 3: Finding of biofilm formation assay

Table 7: Distribution of biofilm in *Lactobacillus* isolates according to the species

Lactobacillus species	Non-producer no. (%)	Weak producer no. (%)	Moderate producer no. (%)
L. fermentum	4 (6.15%)	38 (58.46%)	12 (18.46%)
L. plantarum	3 (4.62%)	8 (12.31%)	0
Total	7 (10.77%)	46 (70.77%)	12 (18.46%)

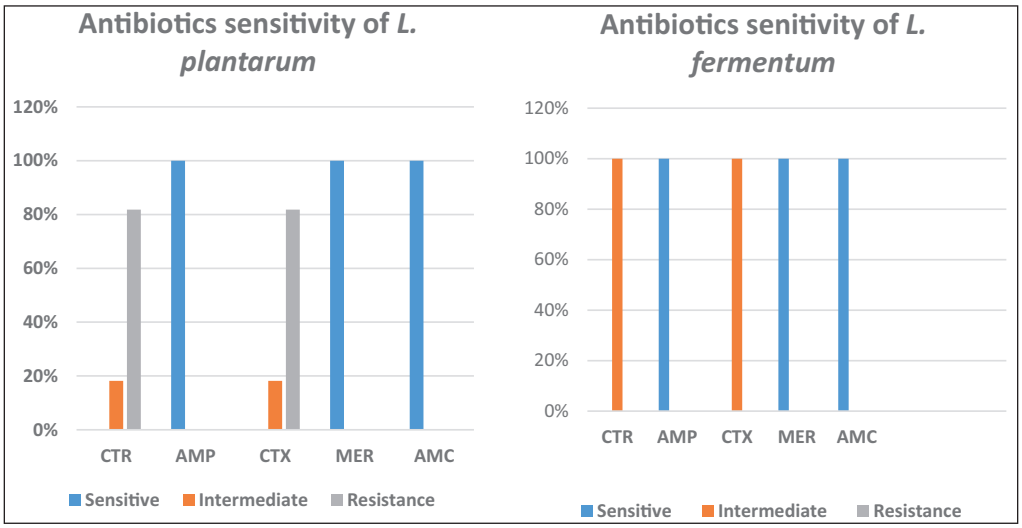

Figure 4: Results of antibiotics sensitivity test of *L. fermentum* and *L. plantarum*. CTR: ceftriaxone, AMP: ampicillin, CTX: cefotaxime, MER: meropenem, AMC: amoxicillin-clavulanic acid

a concentration of 62.5 mg/mL, which corresponds to a value below the MIC, after comparing their results of OD with positive and negative OD controls on non-producers. So, Myrrh extract is an effective extract against biofilm formation to prevent bacterial attachment on the teeth so prevention biofilm formation by these bacteria and teeth caries formation. This finding is consistent with Lee *et al.*[46] from 2014 that revealed the antibiofilm activity of Myrrh extract and with another study from 2011 that revealed antimicrobial activities of Myrrh.[47]

Molecular detection

The concentration ranged between 15 and 25 ng/µL. Detection of *luxS* and *pfs* by polymerase chain reaction (PCR) was conducted using the *luxS* and *pfs* primers to amplify these genes. *luxS* and *pfs* bands were confirmed by gel electrophoresis. Figures 5 and 6 show the results of the amplification of *luxS* and *pfs* from *L. fermentum*. The results revealed that all 12 (100%) *L. fermentum* isolates had both *luxS* and *pfs* with high significance ($P \leq 0.01$).

Discussion

Medical microbiology laboratories have used a different method for microbial detection, *Lactobacillus* samples transferred into tubes contain MRS broth. It was then inoculated anaerobically on previously prepared MRS agar at 37°C for 48–72 h. Colonies that appeared as convex, opaque and smooth without pigment were isolated from MRS agar and subcultured 2–3 times at 37°C for 24–48 h to carry out other tests such as oxidase, catalase, Gran-stain, endospore staining and then Vitek technique that confirmed bacterial identification.[26,48-51] Sixty-five *Lactobacillus* isolates were isolated from swabs of dental caries, all of these isolates were oxidase and catalase negative, and these isolates gave a clear zone when grown on MRS agar with $CaCO_3$, Gram-positive (purple) and non-spore former, all these results are in agreement with.[26,50-52] *Lactobacillus fermentum* was represented as dominant species and followed by *L. plantarum*. These findings were consistent with Zhang *et al.*,[31] which revealed that *Lactobacillus* species isolated from dental caries were *L. fermentum* as the dominant species and followed by *L. plantarum*. *Lactobacillus* isolates showed different abilities to form biofilm and their results were consistent with different studies.[53,54] The macro-dilution method was used to determine antibiotic sensitivity of all *Lactobacillus* isolates from dental caries to five antimicrobial drugs.[32] All of these antibiotics sensitivity test results for ampicillin, meropenem, ceftriaxone, and cefotaxime for both *L. fermentum* and *L. plantarum* are consistent with Anisimova *et al.*,[41] and with the findings of Kamel *et al.*, that revealed that all *L. plantarum* and *L. fermentum* are sensitive to amoxicillin-clavulanic acid.[43] Myrrh extract was extracted using Clevenger apparatus (hydro-distillation method) and kept at 4°C until use. The GC mass method was applied to determine the chemical structure of Myrrh Eos[34-38] using the gas chromatograph spectrometer. According to Table 8, the largest proportion of components included benzofuran (22.13%), then cyclohexane (9.00%), and these findings were consistent with Izzeldien *et al.*[44] Myrrh extract have antibacterial activity at a concentration of 125 mg/mL and higher, consistent with other research that revealed antibacterial activity of Myrrh essential oil against different bacterial species such as *Lactobacillus* species.[22,44,45] Myrrh extract is effective extract against biofilm formation to prevent bacterial attachment on the teeth so prevention biofilm formation by these bacteria and dental caries formation. This result in agreement with the different results.[46,47] The *luxS* and *pfs* genes are responsible for the production of AI-2 signaling molecules, which are responsible for quorum sensing to form a biofilm. This explains the presence of

Table 8: Chemical composition of Myrrh essential oil

No.	Chemical name	RT MS	Perc. %	CAS no.
1	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1methylethyl)-, (3R-trans)	10.961	2.66	020307-84-0
2	Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)-, [1S-(1α,2β,4β)]-	12.052	9.00	000515-13-9
3	Unknown	12.355	0.42	-
4	Caryophyllene	12.597	1.55	000087-44-5
5	γ-Elemene	12.770	3.39	029873-99-2
6	Humulene	13.281	0.64	006753-98-6
7	1H-Cyclopropa[a]naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-, [1aS-(1aα,3aα,7aβ,7bα)]-	13.610	1.38	020071-49-2
8	Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans-	14.094	22.13	017910-09-7
9	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	14.441	1.89	000483-76-1
10	1,1,5-Trimethyl-1, 2-dihydronaphthalene	14.796	1.15	1000357-25-8
11	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-, [1R-(1α,3α,4β)]-	14.951	0.43	000639-99-6
12	Cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-	15.142	1.71	003242-08-8
13	2-Pyridinamine, 6-methyl-	15.445	1.67	001824-81-3
14	Phenol, 2,3-dimethyl-	15.886	2.01	000526-75-0
15	4-Benzyloxyaniline	16.371	8.90	006373-46-2
16	Benzene, 1,3-dimethyl	16.743	4.17	000108-38-3
17	Bicyclo [5.3.0] decane, 2-methylene-5-(1-methylvinyl)-8-methyl	16.994	3.97	1000159-39-3
18	Benzenamine, 2-methoxy-	17.228	2.51	000090-04-0
19	Cyclopentene, 3-ethylidene-1-methyl-	17.453	1.38	062338-00-5
20	3-Fluorobenzoic acid, 4-methoxy-2-methyl butyl ester	17.712	7.88	1000355-66-5
21	Ethane, 1, 2-dibromo-	18.526	0.94	000106-93-4
22	Agarospirol	18.708	0.96	001460-73-7
23	2-Methyl-2-bornene	18.976	1.87	072540-93-3
24	4-(Phenylmethyl) benzenemethanamine	19.210	0.71	100710-32-5
25	Phenol, 4-[(phenylmethyl)amino]-	19.409	2.88	000103-14-0
26	Ethane, 1,1-dibromo-	19.668	0.65	000557-91-5
27	1H-Pyrrole-2-carboxaldehyde, 1-methyl-	20.023	3.55	001192-58-1
28	Fumaric acid, 2-methylcyclohex-1-enylmethyl octyl ester	20.145	0.48	1000345-16-0
29	1H-Pyrrole, 2,3,5-trimethyl-	20.430	1.23	002199-41-9
30	(3S,4R,5R,6R)-4,5-Bis(hydroxymethyl)-3,6-dimethylcyclohexene	20.586	1.85	1000099-24-3
31	2-Hydroxy-4-phenyl-6-acetonylpyrimidine	21.408	1.05	050324-22-6
32	9-Propanoyl-1,2,3,4,5,6,7, 8-octahydrophenathrene	21.581	0.43	1000255-01-4
33	2,5-Dimethylbenzyl chloride	21.876	3.06	000824-45-3
34	10,12-Tricosadiynoic acid, methyl ester	22.075	1.16	1000333-59-4
35	Unknown	23.191	0.36	–

RT: retention time, Perc: percentage, CAS: chemical abstracts service

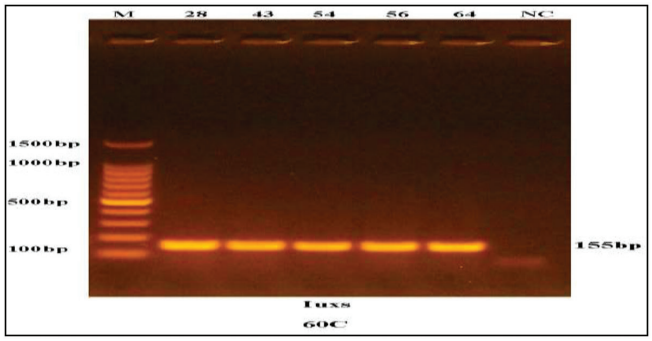

Figure 5: *luxS* amplification of *L. fermentum* isolates with 155bp polymerase chain reaction products on agarose gel (1.5%) electrophoresis stained by Eth.Br. M: 100bp ladder marker, 100 v/mA for 60 min

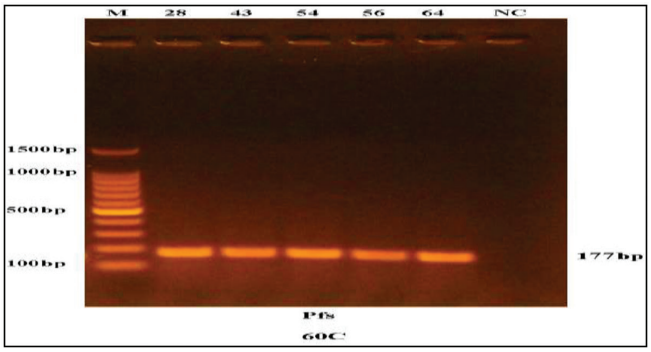

Figure 6: *pfs* amplification of *L. fermentum* isolates with 177bp polymerase chain reaction products on agarose gel (1.5%) electrophoresis stained by Eth.Br. M: 100bp ladder marker, 100 v/mA for 60 min

these genes *luxS* and *pfs* in all 12 *L. fermentum* isolates (which are moderate biofilm formation) tested to detect the presence of these genes by conventional PCR.[17-21]

Conclusion

Sixty-five (32.5%) *Lactobacillus* isolates were isolated from teeth caries patients. *Lactobacillus* isolates were distributed into two species, *L. fermentum* was represented as the dominant species in 83.08% (54 isolates) and *L. plantarum* was represented in 16.92% (11 isolates). All of these isolates showed different abilities to form biofilm extended between non-producers, weak and moderate, isolates that are moderate biofilm formation represented in only 12 *L. fermentum*. Molecular detection of *luxS* and *pfs*, which are responsible for biofilm formation in all these 12 *L. fermentum* isolates, showed that all of these isolates have these two genes, explaining the significant relation between the presence of these genes and biofilm formation. The GC mass method was applied to determine the chemical structure of Myrrh EOs using gas chromatograph spectrometer. GC/MS results showed that Myrrh extract consist of 35 compounds. The largest proportion of components included benzofuran (22.13%), then cyclohexane (9.00%). Myrrh extract revealed antibacterial activity at 125 mg/ml concentration equal to MIC and higher than it against all these 12 *L. fermentum* isolates. Myrrh extract has antibiofilm activity at a concentration of 62.5 mg/mL, which corresponds to a value below the MIC, since all *L. fermentum* that exhibit moderate biofilm formation became non-producer after processing with Myrrh extract at this concentration. Myrrh extract is an effective extract against biofilm formation to prevent bacterial attachment on the teeth so prevention biofilm formation by these bacteria and teeth caries formation.

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Conflicts of interest

Nil.

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