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Study of Antibacterial Activity against *Streptococcus Mutans* of Lemon Seed Extract

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ABSTRACT

In this study, the antimicrobial efficacy of lemon seed extract against the growth and development of Streptococcus mutans was thoroughly investigated; specifically, in a comparative way with antibiotics such as Penicillin. Different concentrations and ratios of the aqueous extract of lemon seed were well prepared and poured into both medium broth and the agar plates with certain amount of inoculum, of which ranging from 5% to 20% to determine the Minimum Inhibitory concentration (MIC), the Minimum Bacterial Concentration (MBC), and the zone of growth inhibition against S. mutans. The obtained results of this study have indicated that the extract of lemon seed showed not only the statistically significant reduction of S. mutans colony formation at 8% in agar test and 15% at broth test with the ratio of 1:4 v/v but also the improved medicinal advantages of aqueous extracts of lemon seed usage against the growth and development of S. mutans. In summary, the lemon seed extract could effectively inhibit the growth of S. mutans at the selectively applied concentration.

Keywords: *Lemon seed extracts, streptococcus mutans, antibacterial activity*

INTRODUCTION

Oral diseases have been becoming one of the most popular health issues in many countries around the world, especially dental caries and gingivitis [1]. Reportedly, Viet Nam is a typical example of these concurrent diseases as up to 90% of population get an oral disease of which, 85% children has had but it cannot be completely cured [2] . Therefore, dental caries is a major chronic condition of a different range of ages, in which resulting from two main factor interactions such as lazy to brush the teeth and do not take care of the oral health properly; thus, increasing the growth of pathogenic bacteria in the mouth [3] . Also, tooth decay is an interaction between teeth, diet, oral environment and microorganism that is an active process of tooth destruction. The process of caries is directly related to the microorganism ability to colonize onto the tooth surface and form dental plaque.

One of the bacteria actively participated in the mentioned process is *Streptococcus mutans*, belonging to *Streptococcaceae* family [4]. *Streptococcus mutans* is a facultative anaerobe, plus gram - positive bacteria with cocci shaped. *Streptococcus mutans* can grow rapidly at 37°C. In addition, *Streptococcus mutans* can ferment carbohydrates, of which mainly sucrose, glucose or a byproduct in saliva to produce weak organic acid [5] that lead to the demineralized process on the tooth surface and the loss of tartar on the dental tissue [6]. *Streptococcus mutans* is the predominant microorganism discovered in dental plaque associated with a caries lesion. Therefore, dental caries prevention should be directly towards the reduction of *S. mutans* [7]

Dental caries is a disease that have been a great trial in the prevention and controlled studies. Conventional antibiotic has been increasing in resistance pathogens; Penicillin is an antibiotic which has been effective against many bacterial infections caused by *S. mutans*, but some patients are found allergic to them. Moreover, some undesirable side effects of existing therapies that make the herb like an attractive source to antimicrobial agents. Recently, special attention in studying of therapeutic agent of some of medical herbs and natural compounds have been especially paid, and as a result, it has

contributed to investigate the antibacterial activity for treatment of cancer and viral infections. Of about 250,000 flowering plants in the world and more than 50,000 plants, seeds have been used for medicinal purposes [8]. In addition, the antimicrobial effects of compounds derived from plants have especially been paid to the roles of biologically active compounds in human health and disease treatment instead of nutritional value only. Among many known phyto-constituents, several polyphenolic compounds of flavonoids and some limonoids have recently been considered to be the most promising biomolecules. Among many others, limonoids are naturally occurring plant secondary metabolites which can prevent bacterial dental plaque and can enhance remineralization of dental enamel [9]. It has been reported that the presence of polyphenolic compounds and some limonoids in citrus plants can be considered, responsible for activity against many clinically symptoms and disease [10]

Citrus is an origin in South East Asia and Citrus belongs to the genus of flowering tree and shrubs in the Rutaceae family, subfamily Aurantoidease and tribe Citrae. Based on different taxonomic in the genus Citrus have been suggested that contain between 16 and 162 species. In addition, some plants in citrus is acknowledged for being the samples for several results in field of testing the antimicrobial activity [11]. It has been proven that polyphenolic compounds [12] can prevent the oral Streptococci that adhere to the surface of tooth [13]. Also, the citrus secondary metabolites contributed to antimicrobial, antifungal [14], antiviral, and other beneficial activity [15]. Furthermore, lemon is one of the major plant in Citrus groups which contains many biological active compounds such as polyphenolic compounds of flavonoids and some limonoids.

Different part of lemon fruit contains different biologically active compounds, and the seeds are being the primary stage of the life cycle of plant, containing phyto-constituents which have been proven to contribute to antimicrobial activity by owing a strong defense mechanism [16]. Lemon seeds contain many secondary metabolites (Bentley et al., 1990) and prominently high concentration of limonoids [17] . There are three forms of limonoids in citrus seeds: monolactone, dilactone and glucosides by 36 different variations of limonoid skeleton. The first limonoids was identified mainly limonin as the bitter constituent of citrus seed which was following by accumulated nomilin, obacunone, and deacetylnomilin as well as dilactones, nomilinic acid, deacetylnomilinic acid and their glucosides. These compounds also contributed to plant defense by demonstrating anti- infection pathogenic bacteria.

Though, numerous studies on the evaluation and utilization of lemon seeds extracts for testing the antimicrobial activity so far, there is no information about how to utilize and apply the extracts from lemon seeds for evaluating and testing the antimicrobial activity on the Streptococcus mutans. So, it is scientifically and economically important to know whether the extracts from lemon seeds can be used as antimicrobial activity against Streptococcus mutans. Also, consequence of choosing a suitable concentration of the lemon seeds extracts in order to meet the proposed works need to be determined.

In summary, this study has been conducted to determine as well as evaluate the possible and highly suitable concentration of lemon seeds extracts against the growth of Streptococcus mutans. The aim of this study was to evaluate the effect of the lemon seeds extracts against the Streptococcus mutans.

MATERIALS AND METHODS

Materials

Lemon fruits were collected from the South Western province of Ben Tre province, Vietnam. The lemon seeds were dried in drying oven at 105oC to get the consistent moisture content of 5% and then store in Desiccator ready for further use and analysis.

Streptococcus mutans ATCC 25715 with freeze-dried format was purchased from Lan Oanh Company, Ho Chi Minh, Vietnam.

Brain Heart Infusion Broth and Blood Agar were purchased from Nam Khoa Bioteck Company, Ho Chi Minh City. Brain Hear Infusion Agar was purchased from Gen Lab Company, Ho Chi Minh City, Vietnam

Methods

Preparation of Lemon Seed Extract

The well-prepared lemon seeds were well ground into fine powder by using electric grinder.

To prepare the aqueous extract, 10g of lemon seed powder was added to 100 ml deionized water (ratio 1:10 w/v) at 90^{o}C for 2-3 hours and cool down to 50^{o}C-55^{o}C. The collected mixture

was filtered using standard funnel and filtering paper to collect the filtrate (aqueous phase). The filtrate was then centrifuged at the speed of 1500rpm and at room temperature for 15 minutes to remove the insoluble and debris in order to have a purified lemon seed extract at a certain degree.

The following process of antimicrobial activity tests were performed with different concentrations of dilution from the total lemon seed extract; computed by the formula:

$$\text{w/v (\%)} = \frac{mass\ of\ solute(g)}{volume\ of\ solution(mL)}\ x\ 100$$

Each different final concentration was diluted using deionized water. The finalized prepared solution was autoclaved at 121°C and 15lbs pressure. After that, each solution was labeled and stored at 40C for further antimicrobial tests.

Preparation of the Testing Microorganisms

The commercial *Streptococcus mutans* ATCC 25715 is in freeze-dried form, which needed to be cultured on Blood agar (BA) before culturing in Brain-Heart infusion broth (BHI). The inoculation of *Streptococcus mutans* in BA was performed at 35°C for 24-36h. After checking without contamination on Agar Base, one colony was randomly taken for culturing on BHI broth medium for 24-36 hours at 37°C and used for testing the antibacterial activity.

To ensure the bacteria appeared in BHI broth medium was *Streptococcus mutans* only, the taken bacteria in BHI was then cultured again in BA from 24-36 hours at 37°C for gram stain and catalase test. This process was weekly performed in order to check the bacterial as well as to safely keep the bacterial for a such long time. It is worthy to mention that before using the bacteria for antimicrobial test, it was necessary to measure OD to make sure the same number of bacteria poured into the sample.

Testing Antibacterial Activity

The test based on culturing of *Streptococcus mutans* on the medium containing the lemon seed extracts.

Agar Plate Test

This test was performed by culturing the inoculum on BHI agar containing specific concentration of lemon seed extract. There are three ratios 1:4 v/v, 1:2 v/v, 1:1 v/v and repeated 5 times for each ratio with each concentration.

In details, 10 ml BHI agar medium was autoclaved at 121°C, 15 minutes and cooled down to about 60°C before transferring the lemon seed extract into the medium, vortex well and pour into the petri dish. A sterile cotton swab is used for spreading out 20 µl inoculum on the surface plates and incubated at 370C, 24 hours. There are several treatments which distinct in added the sample into medium.

Table1. *Experimental design for culturing the Streptococcus mutans for each treatment.*

	No. of replicated	Added materials		
		Medium	Inoculum	Lemon seed extract
Negative control	1	√	X	x
Negative control 2	1	√	X	√
Positive control1	1	√	√	x
Sample	5	√	√	√

(√: with; x: without)

Table2. *Experimental design for culturing Streptococcus mutans.*

Treatment No.	Concentrate of sample	No. of replicates
1	20%	5
2	15%	5
3	10%	5
4	9%	5
5	8%	5
6	7%	5
7	6.5%	5
8	6%	5
9	5%	5

Colony counter is a technique that determine the Minimal Bacterial Concentration and also the Minimal Inhibitory Concentration of lemon seed extract.

Broth Test

Broth test was performed by culturing *Streptococcus mutans* on broth medium containing specific concentration of lemon seed solution. To determine the minimal inhibitory concentration of lemon seed extract against Streptococcus mutans, the lemon seed solution was added directly into the broth medium with three different ratios 1:4 v/v, 1:2 v/v, 1:1 v/v (lemon seed solution/medium).

Broth medium was autoclaved at 121°C, 15 minutes and cooled down to approximately 60°C, then transferred the sample to medium which was followed by adding 20µl of the inoculum before incubated at 37°C for 24 hours

Table3. *Experimental design for culturing Streptococcus mutans.*

Treatment No.	Concentrate of sample	No. of replicates
1	20%	5
2	15%	5
3	10%	5
4	9%	5
5	8%	5
6	7%	5
7	6.5%	5
8	6%	5
9	5%	5

The result of this test was collected by counting the colony with "liquid serial dilution" method. Bacterial counting was determined to the effect of lemon seed to the pathogen and calculated by the following the formulas:

$$\% \text{ Reduction (D-value)} = \frac{[(Mean\ CFU)control - (Mean\ CFU)sample]x\ 100}{(Mean\ CFU)control}$$

$$\text{Log (Reduction)} = \text{Log}_{10}[(Mean\ CFU)control - \text{Log}_{10}\ (Mean\ CFU)sample$$

This method was intentionally used to determines minimum inhibitory concentration (MIC) as the minimum concentration of the extract solution that completely inhibits the growth of visible bacterial.

Antibiotic Test

In this part, the antibiotic test was performed by disk diffusion following protocol established by Baron (1994), of which indicated the bacteria resistant or sensitivity with the antibiotic used or the applied lemon seed extract.

The lemon seed solution with different concentration of 5%; 6%; 6.5%; 7%; 8%; 9%; 10%; 15% and 20% was fixed on four marked areas in BA surface by sterilized cotton-swab and incubated 37°C until the lemon seed solution had been up-taken by the BA surface. Then, the inoculum from BHI was streaked on the surface of plates in four different planes by a sterile cotton swab. After incubation, the diameters of inhibition zones were formed and measured by a standard ruler in mm. As the consequence, the results were compared with positive control (penicillin, incubated at 37°C, 24 hours) and were determined following the performance standards for antimicrobial susceptibility testing (Ronald, N. Jones; 2001) [18]

Data Analysis

All data were treated and presented as means ± standard deviation of representatives of similar test carried out in 5 times of replication. Statistical differences in colony forming unit (CFU) were determined by student's t-test in which, P-values less than 0.05 was considered statistically significant.

RESULTS

Preparation of Lemon Seed Extract

After the dried powder of lemon seed was added to deionized water, then sonicated in water batch at 90°C for 2-3 hrs. with the ratio of 1:10 w/v followed by filtering, using filter paper, the aqueous phase of lemon seed was obtained. It had a dark brown colored solution. The formula below was used to calculate the obtained yield:

$$\text{Yield of extraction(w/v \%)} = \frac{\text{dissolved powder in solutionx 100}}{\text{intitial biomass of powder}}$$

The initial biomass of lemon seed powder = 30 grams

The insoluble weight= 15 grams

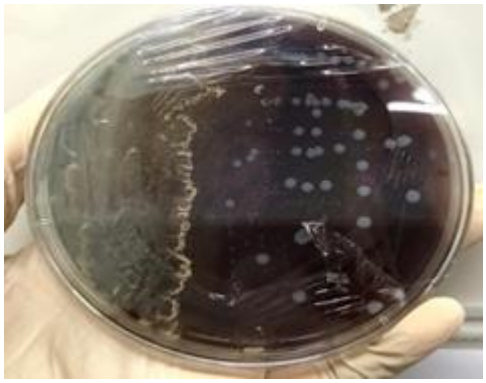
The powder that dissolved in the solution = 15 grams

Figure1. *The aqueous phase of Lemon seed extract*

PREPARATION OF MICROORGANISMS

Identification of the cultured Streptococcus mutans

The formation of colony on blood agar after incubated at 37^{o}C, 24 hours as opposed to the negative control is shown in the Figure 2.



Streptococcus mutans

Negative control

Figure2. *Streptococcus mutans was culture on blood agar medium (on the left).*

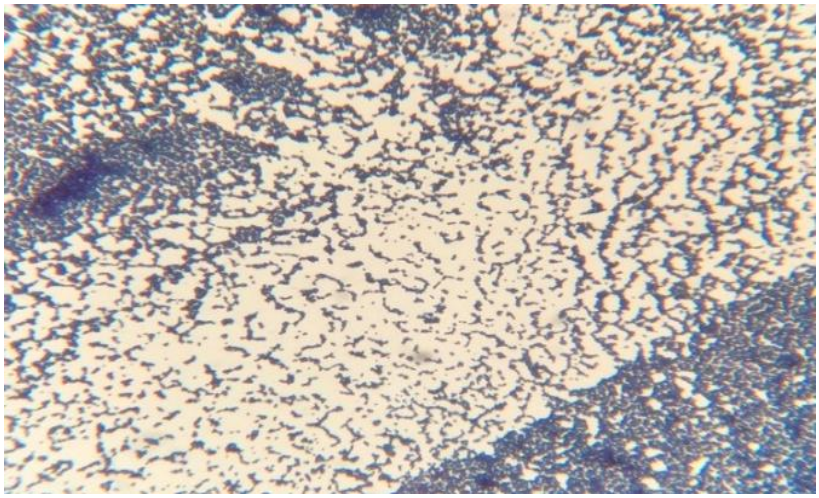


Figure3. *Gram stain of Streptococcus mutans*

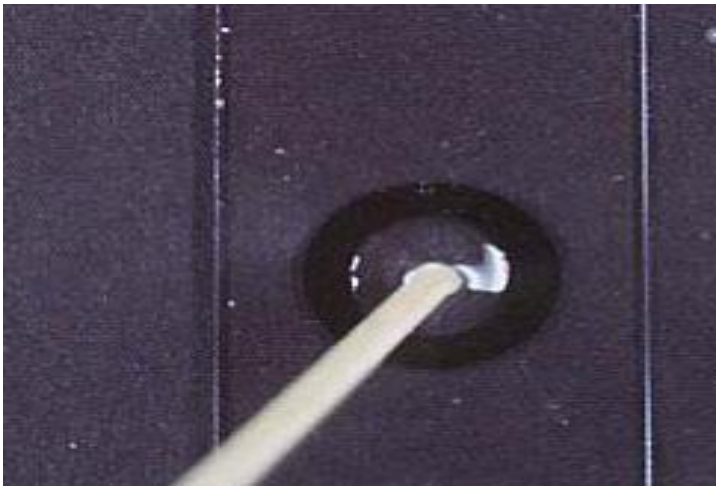
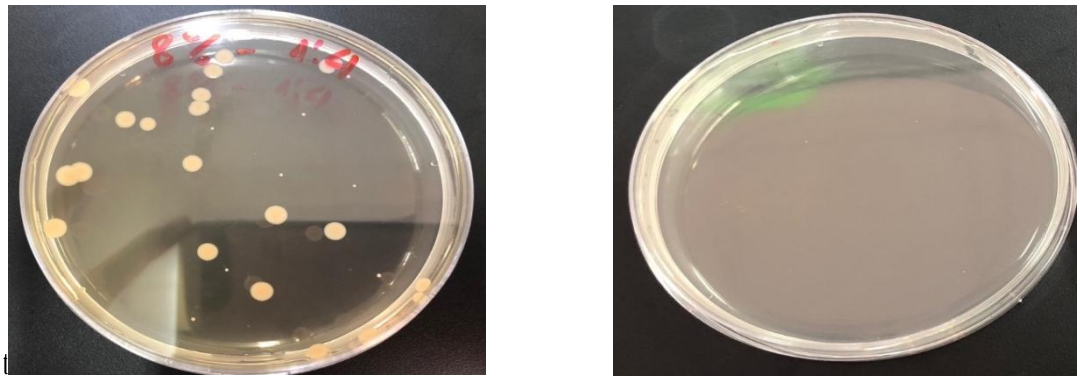


Figure4. *Catalase test of Streptococcus mutans*

DETERMINATION OF ANTIMICROBIAL ACTIVITY

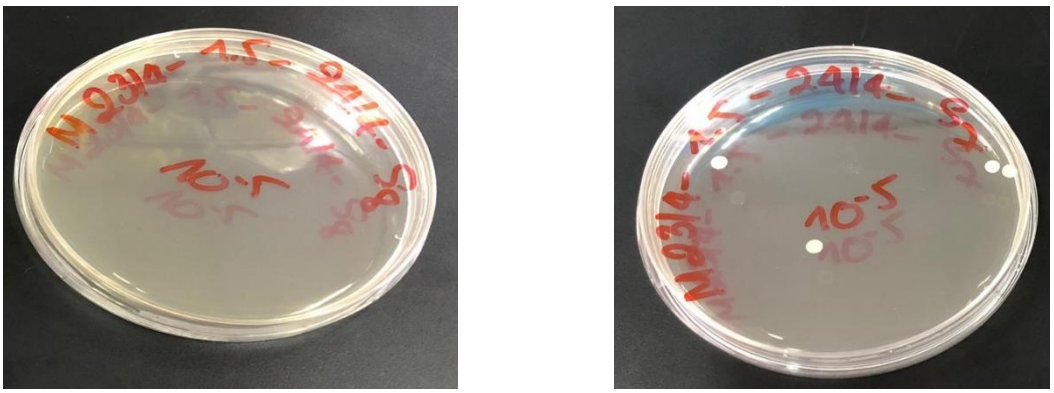
Agar Plate Test

The agar plate test was conducted using two types of control samples (positive sample and negative one) and thee testing samples with nine different concentrations of lemon seed extract (5%, 6%, 6.5%, 7%, 8%, 9%, 10%,15% and 20% (w/v)). Consequently, three ratios of the cultured medium(ml) to the lemon seed extract (ml) of 1:4 v/v; 1:2 v/v; and 1:1 v/v were applied and named as test 1, test2 and test 3, respectively. The experiment was repeated five times. The all tested samples were collected after adding lemon seed solution and then incubating at 37°C, for 24hours.



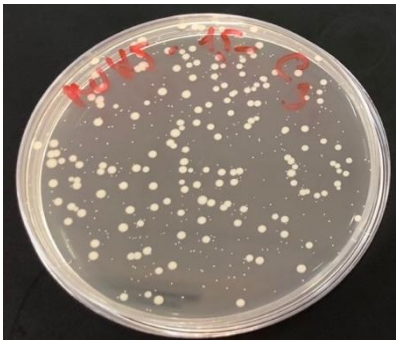
8%(w/v) *9% (w/v)*

Figure5. *Sample concentration testing on agar plate with ratio 1:2 (v/v) with the minimal inhibitory concentration (on the right) in comparison with 6.5% of sample (on the left).*

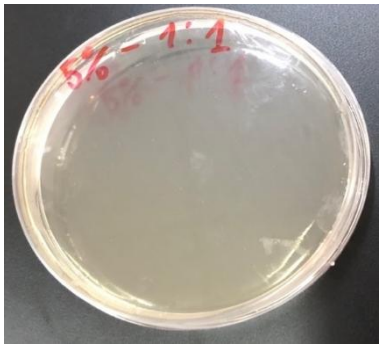


7%(w/v) *8%(w/v)*

Figure6. *Sample concentration testing on agar plate with ratio 1:2 (v/v) with the minimal inhibitory concentration (on the right) in comparison with 6.5% of sample (on the left).*



Control



6.5 %(w/v)

Figure7. *Sample concentration testing on agar plate with ratio 1:1 (v/v) with the minimal inhibitory concentration (on the right) in comparison with 6.5% of sample (on the left).*

Table1. *The antimicrobial activity result of lemon seed extract testing on agar plate.*

	Concentration (%)	Mean (CFU/ml)	Standard deviation	Reduction (%)	Log Reduction
Test 1 (1:4)	Control	104.80	9.011		
	5%	25.60	1.342	75.57	0.612121
	6%	18.60	1.140	82.25	0.750848
	6.5%	14.40	1.342	86.26	0.861999
	7%	7.60	0.894	92.75	1.139548
	8%	2.20	0.837	97.90	1.677939
Test 2 (1:2)	Control	104.80	9.011		
	5%	20.40	1.140	80.53	0.710731
	6%	10.40	1.140	90.07	1.003328
	6.5%	3.20	1.095	96.95	1.515211
	7%	1.40	1.140	98.66	1.874233
Test 3(1:1)	Control	104.80	9.011		

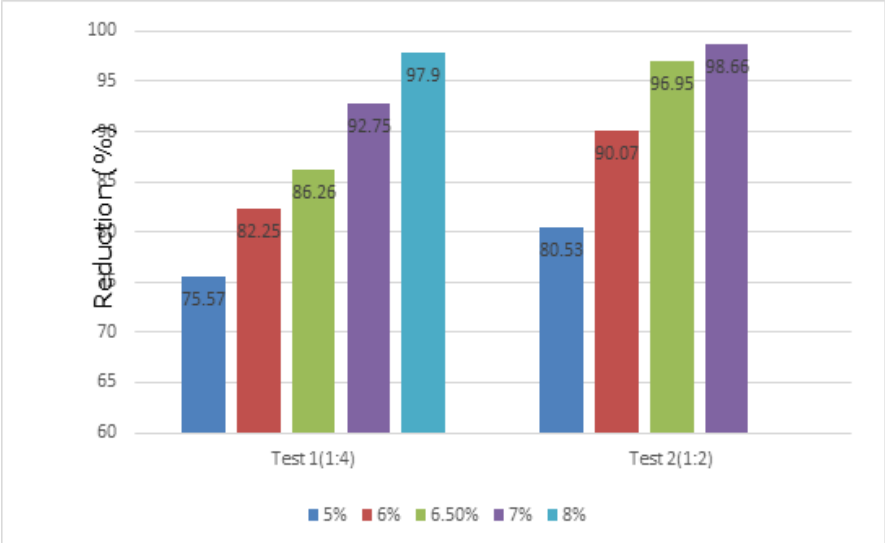


Figure8. *The percentage of bacterial reduction (%) at different concentration lemon seed extract with different ratios in agar media.*

Broth Test

The broth test was conducted with 3 control sample and 9 testing samples with different concentration of lemon seed extract in 12 test tubes. The experiment was repeated five consecutive times. This test used liquid medium for indicating the bacterial growth based on the turbidity of medium. After 24 hours of incubation, dilution method was used for counting colony from broth medium into agar plates. Taking 0.1 ml of the suspension was added to 0.9 ml of sterile distill water. From this suspension, 1/100000 was done and 0.1 ml of lasted dilution is cultured on agar plated for 37^0C, 24hours.

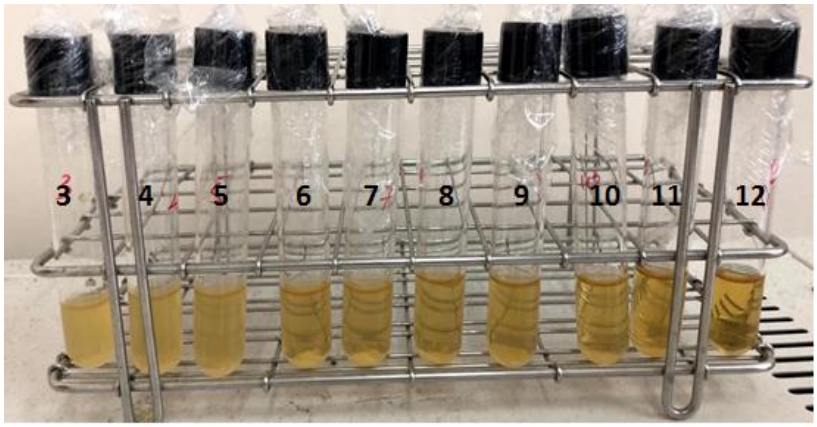


Figure9. *Concentration broth with the ratio 1:4(v/v) in test 1*

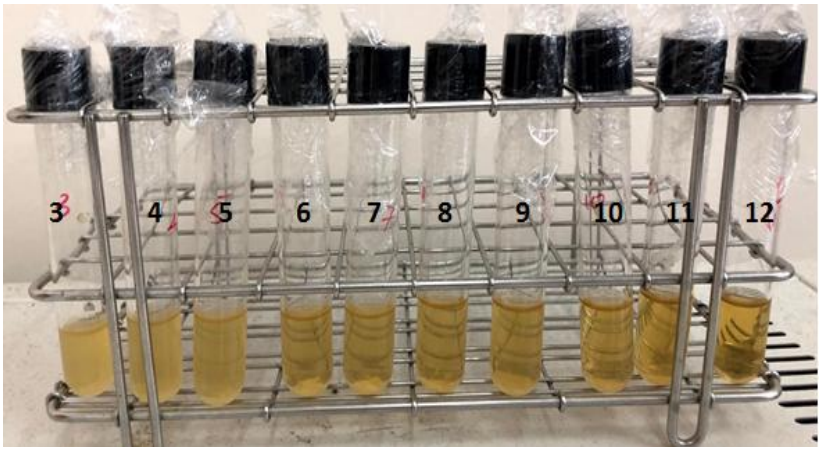


Figure10. *Concentration broth with the ratio 1:2(v/v) in test 2*

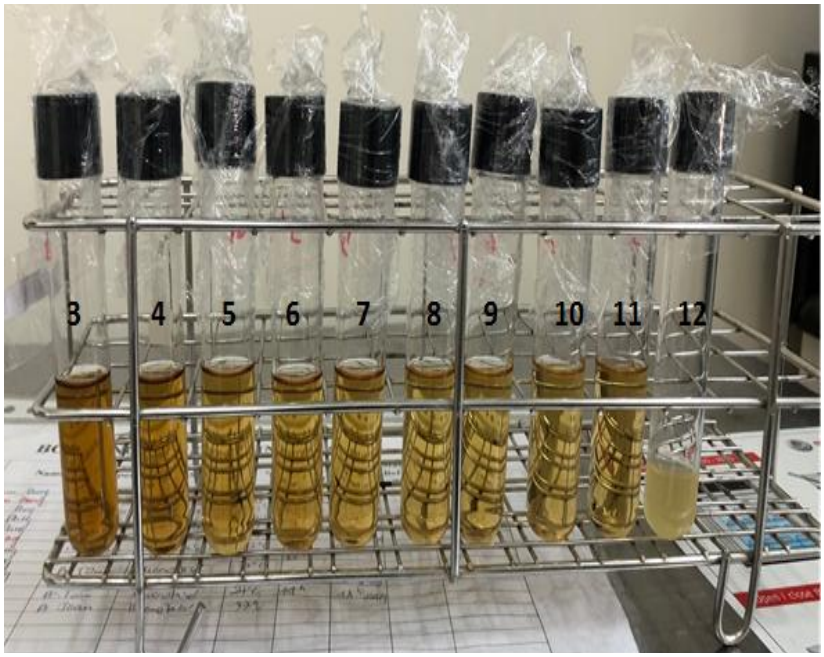


Figure11. *Concentration broth with the ratio 1:1(v/v) in test 3*

Test tube 1: Negative control 1 with 3ml BHI broth medium only.

Test tube 2: Negative control 2 with 3ml BHI broth medium and the lemon seed extract.

Test tube 3: Positive control with 3ml BHI broth medium and 20ul Streptococcus mutans inoculum.

Test tube from 4 to 12: contains 20ul bacterial inoculum with three ratio of the lemon seed extract with different concentration 5%, 6%, 6.5%, 7%, 8%, 9%, 10%, 15%, and 20%.

Table2. *The antimicrobial activity result of lemon seed extract testing on broth media*

	Concentration (%)	Mean (CFU/ml)	Standard deviation	Reduction (%)	Log Reduction
Test 1 (1:4)	Control	104.8	9.011		
	5%	89.8	2.387	14.31	0.067085
	6%	78.0	4.000	25.57	0.128266
	6.5%	61.4	3.715	41.41	0.232193
	7%	41.8	3.564	60.11	0.399185
	8%	35.2	3.962	66.44	0.473819
	9%	16.2	2.168	84.54	0.810846
	10%	3.2	1.304	96.95	1.515211
Test 2 (1:2)	Control	104.8	9.011		
	5%	76.6	2.966	26.91	0.136132
	6%	5t6.2	3.033	46.37	0.270624
	6.5%	34.8	1.924	66.79	0.478782
	7%	10.2	1.789	90.26	1.001176
	8%	6.6	1.140	93.70	1.200817
Test 3 (1:1)	Control	104.8	9.011		
	5%	3.2	1.304	96.95	1.515211

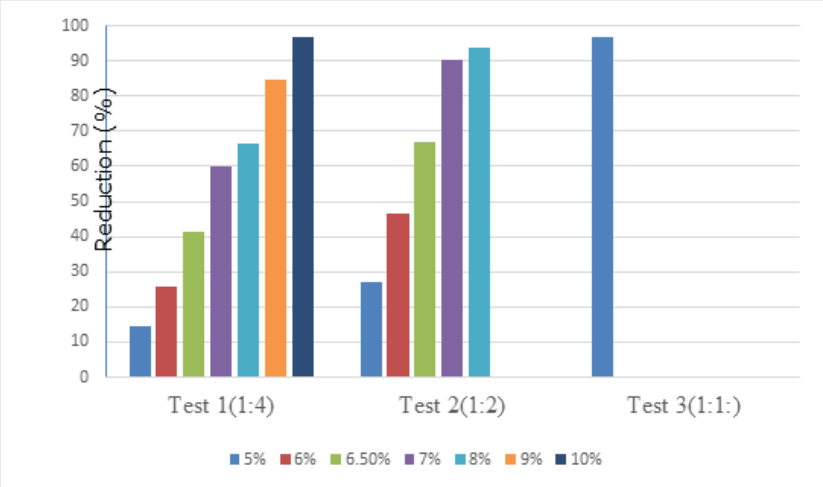


Figure12. *The proportion of bacterial reduction (%) at different concentration lemon seed extract with different ratios in broth media*

Antibiotic Test

Table3. *Comparison of inhibitory growth zone diameter of Streptococcus mutans with difference concentration of lemon seed extract and penicillin by independent t test analysis, df: degree of freedom.*

Concentration	Zone diameter (mm)of Sm	df	Mean Difference	T	P
Control (pencicillin)	29.80	4		15.5012	0.0001
7%	24.10	4	5.30	0.4226	0.3472
8%	25.48	4	3.92	6.2879	0.0016
9%	26.30	4	3.50	7.6667	0.0008
10%	26.62	4	3.18	8.5274	0.0005
15%	28.20	4	1.60	11.2250	0.0002
20%	29.40	4	0.40	12.5547	0.0001

DISCUSSION

A lemon seed extract is a crucially traditional herbal medicine which has been used for the treatment of inflammatory conditions of the respiratory system [16]. Analytical differences provoke the difficulty in comparison in-between studies' results. Such factors would alter the obtained results include the type of the material used, manufacturers, and the extraction technique implied during the experiment. Additionally, during the extraction preparation processes, some or all the active extraction components might be inactivated, or the active ingredients' concentrations might be different in terms of the geographical location, seasonal, and cultivation processes and all these factors affect the efficacy of lemon seed extract [19].

Streptococcus mutans belongings to the group of Viridans streptococci, henceby the agar under the colony formation on blood agar is dark and greenish by "alpha" hemolysis or "green" hemolysis. Since the mentioned bacteria can produce hydrogen peroxide, oxidizing hemoglobin into methemoglobin for generating the green oxidized derivative product is called alpha hemolysis [20]. The formation of colony on blood agar after incubated at 370C, 24 hours as opposed to the negative control is shown in the Figure 2.

From the figure 2 and 3, it could be clearly indicated that the identification tests were conducted in appropriate ways since *Streptococcus mutans* is a gram-positive bacterium and the color of gram stain was a purple and in cocci shape. In addition, the catalase test showed a negative result. In the laboratory, many strains were cultured at the same time, so opportunistic bacteria contamination could not be avoidable. Therefore, each individual test should be performed singularly and separately in order to prevent and avoid any possible contaminants.

For the agar plate testing, there were significant differences in the bacterial reduction at different concentration of 5 %,6%, 6.5%, 7% of test 1 and 2. Comparisons of the *Streptococcus mutans* reduction at different concentrations of the extract showed no significant differences between the 8% in test 1 and 7% in test 2 ($p<0.005$). However, the differences between the other concentrations of the extract in each test were significant ($p<0.005$). Therefore, when the concentration of sample increasing, the probability of colony formation on agar plates would reduce. In contrast, the increasing volume of lemon seed solution in the agar medium in order to decrease concentration of lemon seed extract to inhibit the bacteria growth or kill them. Furthermore, the proportion of bacterial reduction underwent a rise as well as the increasing concentration of lemon seed extract (figure 8). In addition, at the concentration of 9%,8% and 5% of sample solution in test 1, test 2 and test 3, respectively, which showed 5 of 5 replicates had no colony formation after incubation 24 hours. There was demonstrated that the aqueous phase of lemon seed was able to inhibit the growth of *Streptococcus mutans* in 24 hours. Although it was found to be effective at a higher concentration and volume, it indicated a marked antimicrobial activity and should be considered to replace the synthetic medicine for dental caries treatment. To sum up, the lemon seed solution given their antimicrobial activity when it contributed at least 8%(MIC) accounted for a quarter per total solution.

In the case of broth testing, as showed in the Figure 9, the liquid inside the tube ranging from 11 to 12 was clear and slightly transparent which indicated no bacteria growth in the ratio 1:4(v/v). With the ratio of 1:2(v/v), the tube from 9 to 12 indicated the clear and transparency liquid that verify no bacteria growth in the figure 10. No bacteria growth in the test tube arrangement from 5 to 12 with the clear and transparency liquid (figure 11) with the ratio 1:1(v/v). In a short summary, the increased amount of lemon seed extract in the medium could inhibit the growth of *S. mutans* at a wider range.

Also, from the Table 5, it can be seen that the percentage of bacterial reduction performing in broth medium, after the broth culture was put on agar plate, and then incubated in 24 hours for counting the colony formation units as CFU. The CFU was calculated for the reduction's proportion to determine whether sample had antimicrobial activity or not. The MIC of lemon seed extract in broth testing was different and higher from agar plate testing because *S. mutans* can increasingly grow in the medium with high moisture contents. As presented in this table, comparison between bacterial reduction of different concentration shows significant difference between test 1, 2 and 3. On the other hand, comparison between the same extract concentration on *S. mutans* reduction determines a significant difference between three tests in broth medium. The difference of reduction for *S. mutans* at 10%(test 1), 9%(test 2) and 5%(test 3) was no significant.

Additionally, the experiment has proven that the sample concentrations of 15%, 9% and 6% in test 1, test 2, and test 3, respectively represented for MIC in each test. At the concentration of 10% in test 1, 8% in test 2 and 5% in test 3, the lemon seed extract starts their antimicrobial activity with the percentage of *S. mutans* reduction was higher 90%(Figure 12) due to the steadily low CFU. This concludes the growth of *S. mutans* was able to be inhibited by the lemon seed extract for at least 24 hours at the concentration of 15% with one-fourth per total solution.

In a comparative way with antibiotic test as presented in the Table 6, the inhibitory growth zone of S. mutans at different concentrations of

lemon seed extract and penicillin brought the significant differences (P<0.05), providing that the increase in concentration of lemon seed extract, the decrease in growth of S. mutans, simultaneously. The mean zone of inhibition test for S. mutans with 10 µm penicillin was 29.8mm in diameter when used as the positive control group and 24 mm in diameter when applied as the negative control group. Based on the collected data and statistical test, it could be proven at certain degree that means of growth inhibition zone diameters for all concentrations of the extract were less than those in positive control group excepted for the concentration of 20%. However, the difference of the growth inhibition zone of applied lemon seed extract showed a highly significant from the negative control group. In contrast, there were no significant differences in the mean inhibition zone of 20% and control group for S. *mutans*. Moreover, in reviewing the inhibition zone in diameters due to significant differences the inhibition zone means of 20% extract with other concentration in order to achieve the inhibition effect on bacteria that observed a subsequent increase altogether with the raised concentration of lemon seed extract. As a consequence, the extract had more potency on S. *mutans* that can be applied for further investigations.

CONCLUSIONS

In this study, the effects of the lemon seed extract against the growth and development of *S. mutans* was successful and thoroughly investigated. It was clearly found that the lemon seed extract at concentration of 9%(w/v) accounted for a quarter per total medium affected on the reduction percentage of *S. mutans* over 95%, and the most suitably found concentration of the extract was 9% of which showed the strongest effect on the growth inhibition of *S. mutans*

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