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Anti-Bacterial Effects of *Ziziphus Spina-Christi* and *Commiphora Myrrha* Leaves Extracts against *Streptococcus Oralis* (*In vitro Study*)

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ABSTRACT

Dental biofilm considered as the primary etiology for periodontal diseases and its early colonizers are of great importance in the succession stages of biofilm formation such as *Streptococcus oralis*. Nowadays there is a need to find natural substances from plants with antibacterial activity as an alternative to Chlorohexidine. To determine *in vitro* antibacterial effects of alcoholic *Commiphora Myrrha* and *Ziziphus spina-christi* leaves extracts alone and in Combination against *Streptococcus oralis*. Supragingival dental plaque samples were taken from 15 subject with dental plaque induced gingivitis then morphological, microscopical examination, biochemical tests and Vitek 2 were used to confirm identification of *Streptococcus oralis*. The *Commiphora Myrrha* and *Ziziphus spina-christi* leaves extracted by using ethanol alcohol. The susceptibility of bacteria against the extracts, the minimum bactericidal concentration and the minimum inhibitory concentration were determined separately and in Combination compared with chlorhexidine gluconate 0.2% and deionized water. *Commiphora Myrrha* and *Ziziphus spina-christi* leaves ethanol extracts had considerable antibacterial effects against *Streptococcus oralis* with various degrees of growth inhibition zones. It was shown that Combination extracts was the most effective compared to Chlorohexidine then *Myrrha* and lastly *Ziziphus* leaves extracts. The minimum inhibitory concentrations of the extracts ranged from (0.2-0.6 g/ml). The minimum bactericidal concentration of alcoholic extracts ranged from (0.4- 0.8 g/ml). The antibacterial activity of Alcoholic combination extracts was the highest antibacterial activity with all concentration against *Streptococcus oralis* than *Myrrha* and *Ziziphus* leaves extracts were even higher than Chlorohexidine when used at higher concentration, so it can be used as an alternative to Chlorohexidine.

1. INTRODUCTION

The periodontal disease can be defining as an inflammatory disease with a destructive ability to the tissues that surrounding the teeth and is caused by a group of specific microorganisms, [1].

Periodontal diseases are one of the major dental pathologies that affect human populations worldwide at high prevalence rates, [2] and affecting more than 30% of the human race, [3].

Dental biofilm-induced gingivitis is the most common form of periodontal diseases, It is initiated by accumulation of Dental plaque at or near the gingival sulcus [4], gingivitis affect more than 90% of the population [5]. Inflammation of the gingiva is considered the risk factor for the initiation of periodontitis, thus control of gingivitis is essential for the primary prevention of periodontitis [6]. The early colonizers of dental plaque are of great importance in the succession stages of dental biofilm formation and maturation. [7]. *Streptococci Species* (S.S) can colonize tooth surface early and initiate dental biofilm formation. The S. oralis is a facultative anaerobe and α-hemolytic reaction can be detected when colonies are grown on blood agar plates, [8]. Brushing and using the other forms of mechanical plaque control cannot completely remove all dental biofilm and its periodontal pathogens from.

the tooth surface [9], therefore the chemical control of supragingival dental plaque is important, chlorhexidine gluconate (CHX) can be used as first agent. But it has many side effects like brown staining of teeth and tongue, alteration in the taste and oral desquamation and others [10]. Plant derived products have been used for medicinal purposes for centuries as they are a reservoir of chemical agents with antimicrobial properties, [11], hence herbal medicines are increasingly used for treatment against different human disorders with their safety and efficacy [12], [13].

Herbal extracts of great importance and have received attention because of being natural, non-synthetic, non-chemical and they have been long used in traditional medicine [14]. Many Plants used in traditional medicine contain substances, these includes flavonoids, polyphenols. and alkaloids such as, *Commiphora Myrrha* (*C. Myrrha*) which used as an antiseptic in toothpastes and mouthwashes. It can be used as an analgesic for toothaches (powders) [15]. The antimicrobial activity showed by these, plants are potential sources for new antibiotics preparation [16- 18]. *Ziziphus spina-christ* (ZSC) is one of the most widespread native plant that provides wide and cheaply available source for new antimicrobial agents [19- 21], and used for toothaches and as a mouth wash [22]. This study investigate the possible anti-bacterial effects of herbs on *Streptococcus Oralis*, the primary colonizers of dental plaque. Considerably, it has been interested to determine the effects of *C. Myrrha* and *Ziziphus spina-christi* leaves (ZSCL) extracts as antibacterial agents against.

2. MATERIALS AND METHODS

The present *in vitro* study conducted at the Laboratory Unit at AL Shaheed Alsader Hospital in Baghdad. The study protocol was approved by the Medical Ethical Committee, College of Dentistry, University of Baghdad.

Preparation of culture media according to manufacturer's instructions include; Blood agar (Oxoid, England), Brain Heart Infusion Broth (BHI-B) (Himedia, India), Mitis Salivarius Agar (MSA) (Himedia, India), Nutrient Broth (Himedia, India), MuellerHinton Agar(MHA) (Neogen, England). The dental biofilm samples were collected from subjects with supragingival dental biofilm, subjects should informed about purpose of the study, should not take antibiotics or mouth wash medications within at least one month before the study and the consent form and approvals were obtained from each sujectss prior to collecting the samples. Samples were taken from supragingival dental plaque by a sterilized Gracy curette, after the tooth was isolated by cotton roll and dried by air spray to prevent contamination from saliva and other tissues, the collected samples of dental plaque were immediately transferred to 3 ml of (BHI-B) then immediately transporting to laboratory and incubated anaerobically for 4 hrs., at 37°C [23], then inoculate the bacteria from BHI-B to blood agar and culturing sample by streaking method and incubated for 48 hrs. at 37°C. in the anaerobic incubator after that each isolated colony was subcultured on the selective agar media for *Streptococci* which is MSA to be

inoculated under anaerobic conditions using anaerobic gas pack and anaerobic jar at 37°C for 48 hrs,[24].

The colonies of *S. Oralis* were identified and diagnosed according to their morphological characteristics on the agar plates [25], Gram stain [26], biochemical test (catalase test) [27], hemolytic ability [28], antibiotic sensitivity test [28], and Vitek 2 test [30].

The present study involved two *in vitro* experiments,

2.1 The first experiment

Concerning to test the effects of alcoholic *Commiphora Myrrha (C. Myrrha*) and *Ziziphus spina-cristi* leaves(*ZSC*L) extracts separately and in Combination on the sensitivity of *S. oralis.* isolated from supragingival plaque.

2.2 Second experiment

Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) [28] of alcoholic *C. Myrrha* and ZSCL extracts separately and in Combination extracts against *S. Oralis*, in comparison to 0.2% CHX. As a positive control and deionized water as a negative control.

Test tubes were labelled by the No. of the different conc. of the *Myrrha* and ZSCL extracts separately and together and arranged in a rack, after that 1 ml of bacterial suspension (5th dilution) were added to each tube then 0.5 ml of the tested agents were added to its designated tube. Then tubes anaerobically incubated for 48 hrs at 37ºC. Then tubes were examined to see if there was any turbidity (turbidity indicated bacterial growth), the tubes that lack the turbidity were identified as the MIC.

Swab was taken from each tube and spread on a blood agar plate using a sterile spreader and incubated anaerobically for 48 hrs. at 37ºC, then examined for bacterial growth, the plates that showed no growth were identified as MBC [34].

2.3 Extraction procedures

The extraction procedure of alcoholic ZSCL *and C. Myrrha was* conducted in the Ministry of Industry and Minerals, Corporation of research and Industrial Development, Ibn- AL-Betar research Centre, in Baghdad – AL-Iraq. The oleo-gum-resin of *C. Myrrha* was collected from local herbal market. Plant materials were cut into smaller pieces and washed with distilled water, dried in incubator at 37°C and then grinded into fine powder using electric blinder [31].

Plant Material of ZSCL were collected from the farms in Baghdad city and prepared according to previous procedure [21] as follows: The leaves of ZSC were washed under tap water followed by distilled water and air- dried at room temperature. Dried leaves were grinded into coarse powder using electric blinder and packed in clean and dry containers for further use. The 100 gm of ZSC leaves were dissolved in 500 ml of 70% ethanol concentration (conc.). The solution was shaken for 8 hrs at room temperature using shaker and then filtered by using Whitman ™ no.1 filter paper. The remaining solvent traces were evaporated by leaving the filtrate at room temperature until completely dry, the resulted powder was collected and kept in tightly closed dark glass container at room temperature.

The ground resin (100 gm) of C. Myrrha was extracted by percolation in 70% ethanol at (40–60°C) using sonic bath at room temperature for 8 hrs. and filtered by using Whitman™ no. 1 filter paper.

The solvent was removed under vacuum using rotary evaporator device, then placed in hot air oven at 40ºC to complete the dryness and the resulted thick sticky paste preserved in a refrigerator [32].

2.4 Mixing procedure of alcoholic C. Myrrha with ZSCL extracts

According to method demonstrated previously [33], the mixing procedure for 20% conc. started by the addition of 1ml from 20% conc. of alcoholic Myrrha extract and 1ml from 20% conc. of ZSC leaves extract, then vortex mixer was used to obtain homogenous solutions and the same procedure followed for every conc.

Plant sample deionized water was evaluated by the disc diffusion method. Sterile filter paper discs (6 mm in diameter) impregnated with 0.1 ml of different conc.

from extracts were then sited on the surface of MHA plates. Plates were incubated anaerobically for 72 hrs. Zone of inhibition was measured by using ruler.

Statistical analysis; was done using mean(mm), standard deviation S.D., One-way Analysis of Variance test ANOVA test least significant difference LSD and Independent sample t-test. Significance of all the statistical tests were determined by using SPSS (Statistical Package for Social Science).
Non-significant (NS): $P>0.05$,
Significant (S): $0.05 \geq P>0.01$ and Highly
significant (HS): $P<0.01$.

3. THE RESULTS

The *S. oralis* colonies were examined and diagnosed in reference to their morphological characteristics on MSA plates, and appeared as spherical or ovoid in shape with raised or convex smooth surfaces, light blue in color, and about 1-2µm in diameter, The characteristics of *S. oralis* colonies were shown under stereomicroscope as concentric circle structure inside colonies of *S. oralis*, circular slightly raised surfaces and non-adherent agar surface.

The cells of *S. oralis* were Gram-positive, spherical, and arranged in short chains. Catalase negative, with Alpha hemolytic ability and it was resistant to Optochin, also was Arginine dihydrolase test negative. According to report of Vitek 2 test, results identified *S. oralis*

For *S. oralis* sensitivities, the diameter of inhibition zones were found to increase as the conc. of extracts increased and no inhibition zones at 20% and 40% conc. of ZSCL extract.

Myrrha extract at all conc. showed higher mean values of inhibition zones than CHX except at 20% conc. which revealed mean value less than CHX. The *ZSC*L extract showed that mean values of all conc. less than for CHX, except at 100% conc. that showed almost comparable mean values of inhibition zones but Combination extracts showed higher mean values of inhibition zones than CHX at all conc., hence, 100% conc. of Combination mixture extracts revealed a maximum mean value of inhibition zone was (10.27 mm), while D.W. showed no inhibition zones. One-way ANOVA test revealed highly significant differences among different conc. of extracts separately and in combination with CHX and D.W. All conc. of combination extracts revealed the highest mean values of inhibition zones against *S. oralis* followed by *Myrrha* then *ZSC*L extracts as shown in table (1). By using LSD test between each pair of different conc. of *Myrrha, ZSC*L and Combination extracts, table (2), the following results were demonstrated: Highly significant differences were found with all conc. of extracts except for *ZSC*L between 20% and 40% conc. there was a non- significant difference, while Combination extracts revealed a significant difference between 20% with 40% conc.

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Using LSD test to compare each conc. of *Myrrha*, *ZSC*L and Combination extracts with CHX and D.W., table (3). Highly significant differences were found between CHX, D.W. with each concentration of *Myrrha*, *ZSC*L and Combination extracts, except between CHX with *Myrrha* at 40% conc. there was non-significant difference, also between CHX with *ZSC*L at100% conc. and between CHX with Combination extracts at 20% conc. there were non-significant differences, hence between D.W. and *ZSC*L extract. there were non-significant differences at 20% and 40% conc.

Generally, by using t-test the comparisons between mean values of *S. oralis* inhibition zones for the same conc. of each pair of extracts as shown in tables (4), (5) and (6) demonstrated that highly significant differences presented at all conc. of extracts.

The MIC and MBC of alcoholic *C. Myrrha* were 40% (0.4 g/ml) and 60% (0.6 g/ml) respectively, while for ZSCL extract were 60% (0.6 g/ml) and 80% (0.8 g/ml) conc. respectively.

The MIC and MBC of the combination extract were 20% (0.2g/ml) and 40% (0.4g/ml) respectively.

Table (1): The statistical analysis of *S. oralis* inhibition zones by different conc. of alcoholic Myrrha, ZSCL, Combination extracts, CHX and D.W.

Agents	Conc.	NO.	Mean	±S.D.	ANOVATest
CHX	0.2%	4	4.02	0.81	
D.W.		4	0.00	0.00	
Myrrha extract	20%	4	2.08	0.099	
	40%	4	4.30	0.408	F= 286.402
	60%	4	5.10	0.07	P=0.000 H.S.
	80%	4	7.24	0.245	d.f.= 21
	100%	4	9.26	0.12	
CHX	0.2%	4	4.02	0.81	
D.W.		4	0.00	0.00	
Ziziphus extract	20%	4	0.00	0.00	
	40%	4	0.00	0.00	F=111.938
	60%	4	1.02	0.05	P=0.000 H.S.
	80%	4	2.77	0.45	d. f.=21
	100%	4	4.05	0.10	
CHX	0.2%	4	4.02	0.81	
D.W.		4	0.00	0.00	
Combination extracts	20%	4	4.10	0.08	F=212.150
	40%	4	5.04	0.04	P=0.000 H.S.
	60%	4	7.05	0.06	d.f.=21
	80%	4	9.30	0.29	
	100%	4	10.27	0.92	

Table (2) Comparisons of mean values of *S. oralis* inhibition zones between each pair of different conc. for alcoholic *Myrrha*, *Ziziphus* and Combination extracts by LSD test.

Conc.	Myrrha extract			Ziziphus extract			Combination extracts		
	Mean difference	P-value	Desc.	Mean difference	P - value	Desc.	Mean difference	P - value	Desc.

20 %	40 %	-2.21	0.000	H.S	0.00	1.00	N.S	0.94	0.012	**S**
	60 %	-3.01	0.000	H.S	1.02	0.001	H.S	2.95	0.000	H.S
	80 %	-5.16	0.000	H.S	2.77	0.000	H.S	5.20	0.000	H.S
	100%	-7.17	0.000	H.S	4.05	0.000	H.S	6.17	0.000	H.S
40 %	60 %	-0.80	0.005	H.S	1.02	0.001	H.S	2.01	0.000	H.S
	80 %	-2.94	0.000	H.S	2.77	0.000	H.S	4.26	0.000	H.S
	100%	-4.96	0.000	H.S	4.05	0.000	H.S	5.23	0.000	H.S
60 %	80 %	-2.14	0.000	H.S	1.75	0.00	H.S	2.25	0.000	H.S
	100%	-4.16	0.000	H.S	3.02	0.00	H.S	3.22	0.000	H.S
80 %	100%	2.01	0.000	H.S	1.27	0.00	H.S	0.96	0.010	H.S

Table (3): Comparisons of mean values of *S. oralis* inhibition zones between each conc. of Myrrha, *Ziziphus* and Combination extracts with CHX and D.W. by LSD test.

Extract		**CHX 0.2%**			**D.W.**		
	Conc.	Mean differences	P-value	Desc.	Mean differences	P- value	**Desc.**
Myrrha	20%	1.94	0.000	H.S	-2.08	0.00	H.S
	40 %	-0.27	0.302	N.S	-4.30	0.00	H.S
	60 %	-1.07	0.000	H.S	-5.10	0.00	H.S
	80 %	-3.21	0.000	H.S	-7.24	0.00	H.S
	100 %	-5.23	0.000	H.S	-9.26	0.00	H.S
Ziziphus	20 %	4.02	0.000	H.S	0.00	1.00	N.S
	40 %	4.02	0.000	H.S	0.00	1.00	N.S
	60 %	3.00	0.000	H.S	-1.02	0.001	H.S
	80 %	1.25	0.000	H.S	-2.77	0.00	H.S
	100 %	-0.02	0.930	N.S	-4.05	0.00	H.S
Combination	20 %	-0.07	0.834	N.S	-4.10	0.00	H.S
	40 %	-1.01	0.007	H.S	-5.04	0.00	H.S
	60 %	-3.02	0.000	H.S	-7.05	0.00	H.S
	80 %	-5.28	0.000	H.S	-9.30	0.00	H.S
	100 %	-6.24	0.000	H.S	-10.27	0.00	H.S

Table (4): Descriptive statistics and comparisons between mean values of *S. oralis* inhibition zones for the same conc. of *Myrrha* and *Ziziphus* extracts by using t- test.

Conc.	**Descriptive statistics**		**Descriptive statistics**		**Mean differences**		
	Myrrha extract		Ziziphus extract				
	Mean	±S.D.	Mean	±S.D.	t-test	P-value	Desc.
20 %	2.08	0.09	0.00	0.00	41.877	0.000	H.S
40 %	4.30	0.40	0.00	0.00	21.066	0.000	H.S
60 %	5.10	0.07	1.02	0.05	149.632	0.000	H.S
80 %	7.24	0.24	2.77	0.45	13.388	0.001	H.S

100 %	9.26	0.12	4.05	0.10	74.555	0.000	H.S

Table (5): Descriptive statistics and comparisons between mean values of *S. oralis* inhibition zones for the same conc. of *Myrrha* and Combination extracts by using t- test.

Conc.	Descriptive statistics		Descriptive statistics		Mean differences		
	Myrrha extract		Combination extracts				
	Mean	±S.D.	Mean	±S.D.	t- test	P-value	Desc.
20 %	2.08	0.09	4.10	0.08	-45.42	0.000	H.S
40 %	4.30	0.40	5.04	0.04	-3.527	0.009	H.S
60 %	5.10	0.07	7.05	0.06	38.526	0.000	H.S
80 %	7.24	0.24	9.30	0.29	14.474	0.001	H.S
100 %	9.26	0.12	10.27	0.92	-2.019	0.007	H.S

Table (6): Descriptive statistics and comparisons between mean values of *S. oralis* inhibition zones for the same conc. of *Ziziphus* and Combination extract by using t- test.

Conc.	Descriptive statistics		Descriptive statistics		Mean differences		
	Ziziphus extract		Combination extracts				
	Mean	±S.D.	Mean	±S.D.	t-test	P-value	Desc.
20 %	0.00	0.00	4.10	0.08	-100.4	0.000	H.S
40 %	0.00	0.00	5.04	0.04	-231.8	0.000	H.S
60 %	1.02	0.05	7.05	0.06	-253.3	0.000	H.S
80 %	2.77	0.45	9.30	0.29	-26.01	0.000	H.S
100 %	4.05	0.10	10.27	0.92	-12.19	0.001	H.S

4. Discussion

Herbal drugs are used by physicians for hundreds of years old as indigenous systems of medicine and about 80% of the world population still use them for primary health care, [35].

Among single herbal preparations, many studies [14], [31], have focused on *C. Myrrha* or *ZSCL* alone as antibacterial.

Myrrha has been approved in the United States of America by Food and Drug Administration as a safe natural flavoring agent in foods and beverages and as fragrance in cosmetics, [36].

Myrrha are effective in preventing and treating gingivitis. Topically, Myrrh was also applied to bacterial and fungal skin infections [37]. On the other hand, because of the biological benefits of ZSCL extract It was used as an anti inflammatory eye wash, and treat toothache [38], and in ethno medicine.

There were no previous studies that researched the antibacterial effect of alcoholic C. Myrrha and ZSCL

extracts on the primary dental plaque colonizer (*S. oralis*), thus it is often quite difficult to compare the results obtained also the herbal mixture introduced in this study, has not been previously prepared and investigated for its effect on primary colonizer of dental plaque thus the results of the present study may be considered as the first report. In this study, it was found that the diameters of the inhibition zone (IZ) were found to increase when the conc. of the extracts increased because of the increase in the amount of the active antimicrobial components of the extracts that are dissolved causing increased antimicrobial activity of the extracts

The mean values of IZ revealed that all conc. of Combination extracts against *S. oralis* was the most efficient antimicrobial agent followed by *Myrrha* (40%,60%, 80% and 100% conc.) as compared to CHX and lastly *Ziziphus*.

Sensitivity of *S. oralis* to different conc. of alcoholic *Myrrha* by disk diffusion method had been tested in this study and results showed that Myrrha extract were able to inhibit the growth of *S. Oralis*.

The results of comparisons about *Myrrha* in the present study revealed that the *S. oralis* at 40% conc. there were no significant differences in mean values of inhibition zones with positive control(CHX), hence, other study [39] proved that *Myrrha* extract caused inhibition of *Enterococcus faecalis* equal to 2% CHX, this might be attributed to the interaction of *Myrrha* extraction with the cell envelopes which in turn leads to the disruption of cell membranes and thereafter bacteriolysis.

Phytochemical analyses of the oleo-gum resins [40] showed the presence of Sesquiterpenes and Furanosesquiterpenes as major constituents of the *Myrrha*, these results confirmed the antibacterial activity of gum resins, since a sesquiterpenoid detected in the *Myrrha* resin is reported to exhibit significant role in antibacterial activities, [41], [42]. The antimicrobial effects of the sesquiterpene(T-cadinol) has a bactericidal rather than a bacteriostatic effect which interacted with the cell envelopes, causing bacterial lysis and subsequent fatal loss of intracellular material, [43], while phytochemical analysis [44] revealed the presence of the carbohydrates, Alkaloids and saponin detected in *C. myrrha* extracts may be responsible for the antibacterial activity of the plant species. Tannins is another important constituent, affect the microbial and enzyme activities [45]. Others proved the inhibitive effect of the *Myrrha* extract may be referred to the existence of the volatile oils that are large terpene single compounds, These oils have the ability to analyze the cell wall, also leads to the weakening of the biological activities in the cell through overlapping with the cytoplasmic membrane function represented by the process of synthesis of protein by inhibiting and stopping the process, this hindering the process of active transfer of the ions and salts through this membrane, [46].

In addition, an important studies [47], [48] concluded that the presence of secondary metabolites as flavonoids, alkaloids, tannins, glycosides and presence of secondary metabolites as flavonoids tannins, glycosides and saponin in the Myrrha would be marked a good anti-bacterial effect. Other Study suggested the occurrence of phenolic compounds in the plants that these plants may be anti-microbial agent [50].

An important study (14) about ZSCL revealed that the highest activity was demonstrated by the ethanolic Extract of Sider leaves at a conc. of 128 mg/L with (13mm) IZ against gram positive bacteria *Streptococcus spp*) and lowest activity with IZ (8 mm) the results suggested that antibacterial activity of Sider ethanolic extract against tested bacteria increased when used in higher conc. others declared the action of ZSCL extracts was due to the presence of several active components like essential oils, alkaloids, flavonoids and phenolic compounds [44], these secondary metabolites may act on the cell membrane by altering its permeability or rupture the cell membrane of microorganisms causing its complete destruction [45].

The results suggest that the herbal Combination improves the antibacterial effects of 2 herb. However, the main limitation of the present study is the lack of identifying the main constituent of the *Myrrha* and *ZSC*L extracts to know the actual mechanism of herbal action

The MIC of alcoholic *C. Myrrha* extract was able to inhibit *S. oralis* growth in broth media, which represented the bacteriostatic effect against *S. oralis*. At 40% conc.

On the other hand, the MIC for *ZSCL* leaves extract was 60% conc. and the MIC for Combination extracts was 20% conc. needed to inhibit *S.oralis* growth in broth media (bacteriostatic effect), so MIC of *Myrrha* and Combination extracts less than *ZSCL* leaves extract against all *S.oralis.* Chlorohexidine gluconate 0.2% used in this experiment as a positive control also showed bacteriostatic effect against S. oralis

The MBC of alcoholic *C. Myrrha* extract was (0.6 g/ml) conc. needed to kill *S. oralis*, which indicated that the agent demonstrated bactericidal effect against *S. oralis*

The MBC of *ZSC*L leaves extract that kills *S.Oralis* isolates was 80%(0.8 g/ml) conc. which indicated that the agent exhibited bactericidal effect, while the MBC of Combination extracts that kills *S.Oralis* isolates growth was 40% (0.4 g/ml) conc. (that demonstrated bactericidal effect of Combination extracts).

The MBC of *C. Myrrha* and Combination extracts less than the MBC of *ZSC* leaves extract against *S.Oralis*. this could be due to active antibacterial compounds effects in the *ZSCL* extract may be appeared only in high conc. against tested bacteria.

In summary, this study confirms that many plant extracts possess *in vitro* antibacterial activity., *S. oralis* are sensitive to different conc. Of alcoholic *C. Myrrha* and Combination extracts starting from 20% to 100% conc. and to alcoholic *ZSC*L extract starting from 60% to 100% conc.

5. References

[1] Al-Jehani, Y.A. 2014. Risk Factors of Periodontal Disease: Review of the Literature. International Journal of Dentistry, Article ID 182513, page9.

[2] Petersen PE. 2003. The World Oral Health Report: Continuous improvement of oral health in the 21st century – The approach of the WHO Global Oral Health Programme. Community Dent Oral Epidemiol; 31:3-24.

[3] Arora N, Mishra A, Chugh S. 2014. Microbial role in periodontitis: Have we reached the top? Some unsung bacteria other than red complex Indian Soc Periodontol,18:9-13.

[4] Nair AA, Malaiappan S. The comparison of the antiplaque effect of aloe vera, chlorhexidine and placebo mouth washes on gingivitis patients. J Pharm Sci Res 2016; 8:1295-1300.

[5] Karim B, Bhaskar DJ, Agali C, et al. Effect of aloe vera mouthwash on periodontal health: Triple blind randomized control trial. Oral Health Dent Manag 2014; 13:14-19.

[6] Murakami S, Mealey BL, Mariotti A, et al. Dental plaque – induced gingival conditions. J Clin Periodontal 2018; 45:17–27.

[7] Li J, Helmerhorst FJ, Leone CW, et al. Identification of early microbial colonizers in human dental biofilm. J Appl Microbiol 2004; 97:1311–1318.

[8] Xuedong Zhou Yuqing Li.2015. State Key Laboratory of Oral Diseases, Atlas of Oral Microbiology from Healthy Microflora to Disease.

[9] Adriaens PA, Adriaens LM. Effects of nonsurgical periodontal therapy on hard and soft tissues. Periodontal 2004; 36:121–145.

[10] Emilson C. Potential efficacy of chlorhexidine against mutans Streptococci and human dental caries. J Dent Res 2004; 73:682-691.

[11] Sharma A, Kolhapur S. 2005. Medicine update. 12(10): 39-45.

[12] Rajani M, Kanaki N. 2008. Phytochemical standardization of herbal drugs and polyherbal formulations: Bioactive molecules and medicinal plants.Springer: Berlin Heidelberg, 349-69

[13] Rai M, Chinkindas M. 2010. Natural antimicrobial in food safety and quality.CABI Publishing.

[14] Poureslami H. The effects of plant extracts on dental plaque and caries, contemporary approach to dental caries, Edited by Li MY, 2012; ISBN:978-953-51-0305-9.

[15] Mohamed A, Shahid SMA, Alkhamaiseh SI, et al Antibacterial activity of commiphora molmol in wound infections. J Diagnostic Pathol Oncol 2017;2:32-36.

[16] Omer SA, Adam SE, Mohammed OB. Antimicrobial activity of commiphora myrrha against some bacteria and candida albicans isolated from gazelles at king khalid wild life research centre. Res J Med Plant 2011; 5:65–71.

[17] Al-Sieni AI. The antibacterial activity of traditionally used salvadora persica L. (Miswak) and commiphora gileadensis (Palsam) In Saudi Arabia. Afr J Tradit Complement Altern Med 2014; 11:23-27.

[18] Hana DB, Kadhim HM, Jasim GA, et al. Antibacterial activity of commiphora molmol extracts on some bacterial species in Iraq. Scholars Academic J Pharm 2016; 5:406-412.

[19] Motamedi H, Seyyednejad M, Hasannejad Z, et al. Comparative study on the effects of ziziphus spinachristialcoholic extracts on growth and structural integrity of bacterial pathogens. Iranian J Pharm Sci 2014; 10:1-10.

[20] Aly SM, Al-Mutairi MH, Ali S, et al. Antibacterial activity of sider (ziziphus spina christi), Leaves extract against selected pathogenic bacteria. Euro J Pharm Med Res 2016; 3:138-144.

[21] Temerk HA, Salem WM, Sayed WF, et al. Antibacterial effect of phytochemical extracts from ziziphus-spina christi against some pathogenic bacteria. Egypt J Bot 2017; 57:595 – 604.

[22] Shahat AA, Pieters L, Apers S, Nazeif NM, et al. Chemical and biological investigations on zizyphus spina-christi l. Phytother Res 2001; 15:593-597.

[23] Jesper H, Reinholdt J, Kilian M. Population dynamics of streptococcus mitis in its natural habitat. Infection Immunity 2001; 69.

[24] Kumar S. Textbook of microbiology. First Edn. Jaypee Brothers Medical Publishers (P) LTD. New Delhi 2012.

[25] Bergey's manual of systematic bacteriology. Second Edn New York: Springer 3;2009.

[26] Koneman E, Schreckenberge P, Allens S, et al. Diagnostic microbiology. 4th Edn. J.B. Lippincott Co. USA. 1992.

[27] Reiner K. Catalases test protocol. ASM Micro Library 2013

[28] Buxton R. Blood agar plates and hemolysis protocols. ASM Micro Library 2013.

[29] Al-mohammadawy ZH, Aljarah AK, Saad AM. Isolation and Identification of streptococcus mutans from dental caries by using Sm479 gene. Indian J Public Health Res Develop 2018; 9.

[30] Garcia-Garrote F, Cercenado E, Bouza E. Evaluation of a new system, VITEK 2, for Identification and antimicrobial susceptibility testing of enterococci. J Clin Microbiol 2000; 38:2108–2111.

[31] Tiwari P, Kumar B, Kaur M, et al. Phytochemical screening and extraction: A review. Int Pharm Sci 2011; 1:98-106.

[32] Mekonnen Y, Dekebo A, Dagne E. Toxicity study in mice of resins of three commiphora species. Sinet Ethiop J Sci 2003; 26:151–153.

[33] Stephenson FH. Calculations for molecular Biology and biotechnology, a guide to mathematics in the laboratory 2nd Edn. Academic Press is an imprint Elsevier 2010

[34] Rollins DM, Temenak JJ, Shields P, et al. Microbial pathogenesis laboratory manual. 2nd Edn 2003.

[35] Ghada Alotaibi, Uma M. Irfan and Sarah Ali .2017. An In-Vitro Study to Test Antimicrobial Effects of Commiphora Myrrha in Comparison to Biocides. European Journal of Pharmaceutical and Medical Research, ejpmr,4(11), 449-453.

[35] Hall RL, Oser BL. 1965. Recent progress in the consideration of flavor ingredients under the Food Additives Amendment. 3. GRAS Substances. Food Technology, 19(2, Part 2): 151-197

[36] Dolara P, Corte B, Ghelardini C, et al. Local anaesthetic, antibacterial and antifungal properties of sesquiterpenes from Myrrh. Planta Medica 2000; 66:356-358.

[37] Jens ASS, Hammer GK, Buerkert A. Ziziphus spinachristi (L.) Willd: A multipurpose fruit tree. Genet Resour Crop Evol 2008; 55:929–937.

[38] Suresh Anand, Mathan Rajan, and Kalaiselvam Rajeswari.2016. Evaluation of the Antibacterial Efficacy of Azadirachta Indica, Commiphora Myrrha, Glycyrrhiza Glabra Against Enterococcus Faecalis

using Real Time PCR. Open Dent J. 10: 160–165.

[39] Alhussaini, M.S.; Saadabi, A.M.; Alghonaim, M.I and Ibrahim, K.E. 2015. An Evaluation of the Antimicrobial Activity of C. Myrrha Nees (Engl.) Oleo-gum Resins from Saudi Arabia. J. Med. Sci. 15(4): 198-203

[40] Dolara, P., Corte, B., Ghelardini, C., Pugliese, A.M., Cerbai, E., Menichetti, S. and Lonostro, A. 2000. Local anaesthetic, antibacterial and antifungal properties of sesquiterpenes from Myrrh. Planta. Medica., 66 (4): 356-358.

[41] Syed Rizwan Ahamad, Abdul Rahman, Al-Ghadeer, Raisuddin Ali, Wajhul Qamar ,Suliman Aljarboa .2017: Analysis of inorganic and organic constituents ofmyrrh resin by GC–MS and ICP-MS: An emphasison medicinal assets. Saudi Pharmaceutical Journal, 25, 788–794.

[42] Claeson, P.; Raadstroem, P.; Skoeld, O.; Nilsson, A.; Hoeglund, S. 1992.Bactericidal effect of the sesquiterpene T-cadinol on Staphylococcus aureus. Phytother. Res. Volume 6, Issue 2 Pages 94-98.

[43] Emad, M.A., Amna, S.K., Nazlina, I. 2009. Antibacterial activity of oleo-gum resins of Commiphora molmol and Boswellia papyrifera against methicillin resistant Staphylococcus aureus (MRSA). Sci. Res. Essays 4 (4), 351–356.

[44] Makkar, H.P.S., B. Singh and S.S. Negi. 1989. Relationship of rumen degradability with microbial colonization, cell wall constituents and tannin levels in some tree leaves. Anim. Prod., 49: 299-303

[45] Cowan MM. 1999. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 12(4):564–582

[46] Al-Qaysia, SA. 2008. Effect of Volatile Oil of Myrtus communis on growth and activities of some types of Pathogenic Bacteria and Candida albicans. Journal of Baghdad for Science. 5(1):8-13.

[47] Al-Marby, A., Ejike, C.E., Nasim, M.J., Awadh-Ali, N.A., Al-badani, R.A., Alghamdi, G.M.A. and Jacob, C. 2016. Nematicidal and antimicrobial activities of methanol extracts of 17 plants, of importance in ethnopharmacology, obtained from the Arabian Peninsula. J Intercult. Ethnopharmacol. 5(2): 114–121.

[48] Meison Abdulbary, Ahmed Abies Motar and Rajaa Ali Hussein. 2018. Antibacterial Effect of Some Medicinal Plants against Staphylococcus Aureus and Pseudomonas Aeruginosa., International Journal for Research in Applied Sciences and Biotechnology Volume-5, Issue-4 Page Number: 8-13.

[49] Kareem, S.O; Akpan, I and Ojo, O.P.2008. Antimicrobial activities of Calotropisprocera on selected pathogenic microorganisms. African Journal of Biomedical Research. 11: 105 –110.