Journal of Research in Medical and Dental Science
2020, Volume 8, Issue 3, Page No: 57-64

Available Online at: www.jrmds.in
eISSN No.2347-2367: pISSN No.2347-2545

Anti-Bacterial Effects of *Commiphora Myrrha* and *Ziziphus Spina-Christ* Leaves Extracts Against *Streptococcus Mitis* (Primary Colonizer of Dental Plaque) *In vitro* Study

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ABSTRACT

Background: Dental plaque plays a major role in the etiology of periodontal diseases and the early colonizers of dental plaque are of great importance in the succession stages of biofilm formation such as Streptococcus mitis. Nowadays there is a need to find naturally occurring substances from plants with antimicrobial activity as an alternative to available used Chlorohexidine.

Aims: To investigate in vitro antibacterial effects of alcoholic Commiphora Myrrha and Ziziphus spina-christi leaves extracts alone and in Combination against Streptococcus mitis.

Materials and Methods: At first tacking plaque samples from 15 patients with gingivitis-dental biofilm-induced then morphological, microscopical examination, biochemical tests and Vitek 2 were used to confirm identification of Streptococcus mitis. The Commiphora Myrrha and Ziziphus spina-christi leaves extracted by using ethanol alcohol. The susceptibility of bacteria against the extracts, the minimum inhibitory concentration and the minimum bactericidal concentration were determined separately and in Combination compared with chlorhexidine gluconate 0.2% and deionized water.

Results: The ethanol extracts exhibited considerable antibacterial effects against Streptococcus mitis with various degrees of growth inhibition zones. It was shown that Combination extracts was more antibacterial effects 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).

Conclusion: Alcoholic Combination extracts showed higher antibacterial activity with all concentration against Streptococcus mitis 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.

Key words: Antibacterial, Chlorhexidine, *Streptococcus mitis*, *Myrrha* and *Ziziphus spina-christi* leaves

HOW TO CITE THIS ARTICLE: Zainab Mohsen Obaid, Maha Abdul-Aziz Ahmed, Anti-Bacterial Effects of *Commiphora Myrrha* and *Ziziphus Spina-Christ* Leaves Extracts Against *Streptococcus Mitis* (Primary Colonizer of Dental Plaque) *In vitro* Study, J Res Med Dent Sci, 2020, 8(3): 57-64

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Received: 14/02/2020
Accepted: 27/04/2020

INTRODUCTION

Gingivitis is initiated by the noxious substances resultant from the accumulation of microbial plaque at or near the gingival sulcus [1], therefore, about more than 90% of the population have gingivitis-dental biofilm-induced which is one of the most frequent periodontal diseases [2]. Gingival inflammation in response to bacterial plaque accumulation (microbial biofilms) is considered the key risk factor for the onset of periodontitis, thus control of gingival inflammation is essential for the primary prevention of periodontitis [3]. The early colonizers of dental plaque are of great importance in the succession stages of biofilm formation and its overall effect on the oral health of the host [4]. *Streptococci* have their prime habitat in plaque, can colonize tooth surface and initiate plaque formation [5]. Nevertheless, it has been demonstrated that mechanical removal of biofilm cannot completely remove all periodontal pathogens from the tooth surface [6], so chlorhexidine gluconate (CHX) seems to be an agent of choice in chemical control of supragingival dental plaque. However, due to some secondary side effects [7]. The global need for safe, effective and economical preventive and treatment options for oral diseases arises from the increase in disease incidence, resistance of pathogenic bacteria to antibiotics and chemotherapeutics, opportunistic infections and financial considerations in developing countries. Herbal extracts have received special attention because of being non-chemical, non-synthetic, and they have been long used in traditional medicine [8].

Plants used in traditional medicine contain a wide range of substances, these includes flavonoids, polyphenols. and alkaloids such as, *Commiphora Myrrha* (*C. Myrrha*) which used as an antiseptic in mouthwashes, and toothpastes, and may be applied to abrasions and other minor skin

ailments. *Myrrha* has also been recommended as an analgesic for toothaches (as common ingredient of tooth powders) [9]. The antimicrobial activity showed by these plants or their extracts are potential sources for new antibiotics [10-12]. *Ziziphus spina-christ* (ZSC) is one of the most widespread native plant that provides vast and cheaply available source for finding new antibacterial agents [13-15], and has been used in folk medicine as relaxing, emollient, stomach-ache, for toothaches and as a mouth wash [16].

This study mainly focuses on the possible anti-bacterial effects of herbs on the primary colonizers of dental plaque. Considerably, it has been interested to determine the effects of *C. Myrrha* and *Ziziphus spina-christ* leaves (ZSCL) extracts as antibacterial agents against *streptococcus mitis* (*S. mitis*).

MATERIALS AND METHODS

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

Preparation of culture media according to manufacturer's instructions include; Blood agar (Oxoid, England), *Mitis Salivarius Agar* (MSA) (Himedia, India), Brain Heart Infusion Broth (BHI-B) (Himedia, India), Mueller Hinton Agar(MHA) (Neogen, England), Nutrient Broth (Himedia, India).

The plaque samples were collected from15 persons with gingivitis-dental biofilm-induced, patients should not use antibiotics medications within at least one month before the study and should informed about purpose of the study and patients' consents and approvals were obtained prior to collecting the samples. Samples were taken from supragingival 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 were immediately transferred to 3 ml of (BHI-B) then immediately transporting to laboratory and incubated anaerobically for 4 hrs., at 37°C [17], 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 [18], 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.

The colonies of S. mitis were identified and diagnosed according to their morphological characteristics on the agar plates [19]. Gram stain [20], biochemical test (catalase test) [21], hemolytic ability [22], antibiotic sensitivity test [23], and Vitek 2 test [24].

Extraction procedures

The extraction procedure of alcoholic *C. Myrrha* and ZSCL was conducted in the Ministry of Industry and Minerals, Corporation of research and Industrial Development, Ibn-AL-Betar research Centre.

Plant Material of ZSCL were collected from the farms in Baghdad city and prepared according to previous procedure [15] 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 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 [25]. 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 [26].

Mixing procedure of alcoholic C. Myrrha with ZSCL extracts

According to method demonstrated previously [27], 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.

S. mitis 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.

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. mitis*.

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 [29].

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 ≥ P>0.01 and Highly significant (HS): P<0.01.

RESULTS

Isolation and identification of *S. mitis*: According to their morphological characteristics, *S. mitis* colonies appeared on MSA Plates as circular and even margin colonies about 0.6–0.8 µm in diameter with flat smooth surface, they were blue light in color and firmly adhered to the agar plates. Forms small broken glass-like colonies on sucrose blood agar plates. Microscopic examination showed cells were gram positive, spherical or ovoid in shape, arranged in short or long chains. The *S. mitis* were catalase negative, also with Alpha hemolytic ability and it was resistant to Optochin and the results of Vitek 2 identified S. mitis with 94% probability.

Sensitivity of *S. mitis*: The diameter of inhibition zones was found to be increased as the conc. of all extracts increased, Myrrha and Combination extracts at 60%, 80% and 100%conc. showed higher mean values of inhibition zones (IZ) than CHX, on the other hand, ZSCL extract showed that mean values of all conc. less than that CHX, hence, 100% conc. of Combination extracts revealed a maximum mean value of IZ was (12.50 mm), but, D.W. revealed no IZ. One-way ANOVA test revealed highly significant differences among different conc. of extracts separately and combination with CHX and DW table 1.

Comparisons between each pair of different conc. of *Myrrha*, *Ziziphus* and Combination extracts revealed highly significant differences with all conc. except for 20% with 40% conc. of ZSCL extract, since there was non-significant difference, table 2.

From table 3, Highly significant differences were found between CHX, D.W. with each conc. of *Myrrha*, ZSCL and Combination extracts, except the non-significant differences between CHX, with 60% conc. of Myrrha extract as well as between D.W. with 20% and 40% conc. of ZSCL extract, while between ZSCL extract at 100% conc. with CHX there was significant difference.

Generally, the comparisons shown in tables 4, 5 and 6 demonstrated that Combination extracts illustrated highest mean values of *S. mitis* IZ at all conc. then *Myrrha* and lastly ZSCL extracts. So, highly significant differences presented at all conc. except, the non- significant differences at 20% and 40% conc. between *Myrrha* and Combination extracts, while there were significant differences at 80% and 100% conc. The MIC and MBC of alcoholic *C. Myrrha* and Combination extracts were 20% (0.2g/ml) conc. and 40% (0.4 g/ml) conc. respectively, while for ZSCL extract were 60% (0.6 g/ml) and 80% (0.8 g/ml) conc. respectively also, CHX 0.2% showed bacteriostatic effect against *S. mitis*.

Table 1: The statistical analysis of S. mitis IZ by different conc. of alcoholic *Myrrha*, ZSCL, Combination extracts, CHX and D.W.

Agents	Conc.	No.	Mean	± S.D.	ANOVATest
CHX	0.20%	4	8.1	0.89	
D.W.		4	0	0	
Myrrha extract	20%	4	4.2	0.21	
	40%	4	6.43	0.42	F=184.022
	60%	4	8.16	0.12	P=0.000 HS
	80%	4	10.15	0.99	*d.f.=21
	100%	4	11.45	0.47	
CHX	0.20%	4	8.1	0.89	
D.W.		4	0	0	
Ziziphus extract	20%	4	0	0	
	40%	4	0	0	F=170.072
	60%	4	2.12	1.03	P=0.000 HS
	80%	4	4.12	0.18	d.f.=21
	100%	4	7.17	0.28	
CHX	0.20%	4	8.1	0.89	

D.W.		4	0	0	
Combination extracts	20%	4	4.9	0.55	F=221.998
	40%	4	6.55	0.59	P=0.000 HS
	60%	4	9.4	0.45	d.f.=21
	80%	4	11	0.56	
	100%	4	12.5	0.47	

*DF=Degree of freedom

Table 2: Comparisons of mean values of *S. mitis* IZ between each pair of different Conc. for alcoholic *Myrrha, Ziziphus* and Combination extracts by LSD test.

		Myrrha extract			*Ziziphus* extract			Combination extracts		
Conc.		**Mean difference**	**P-value**	***Desc.**	**Mean difference**	**P- value**	**Desc.**	**Mean difference**	**P- value**	**Desc.**
20%	40%	-2.23	0	HS	0	1	NS	-1.64	0	HS
	60%	-3.96	0	HS	-2.12	0	HS	-4.49	0	HS
	80%	-5.95	0	HS	-4.12	0	HS	-6.1	0	HS
	100%	-7.25	0	HS	-7.17	0	HS	-7.59	0	HS
40%	60%	-1.73	0	HS	-2.12	0	HS	-2.85	0	HS
	80%	-3.72	0	HS	-4.12	0	HS	-4.45	0	HS
	100%	-5.02	0	HS	-7.17	0	HS	-5.95	0	HS
60%	80%	-1.99	0	HS	-2	0	HS	-1.6	0	HS
	100%	-3.29	0	HS	-5.05	0	HS	-3.1	0	HS

*Desc=Description

Table 3: Comparisons of mean values of *S. mitis* IZ between each conc. of alcoholic *Myrrha, Ziziphus* and Combination extracts with CHX and D.W. by LSD test.

Extracts	**Conc.**	**CHX 0.2%**			**D.W.**		
		Mean Differences	**P- value**	**Desc.**	**Mean differences**	**P- value**	**Desc.**
Myrrha	20%	3.9	0	H.S	-4.2	0	H.S
	40%	1.67	0	H.S	-6.43	0	H.S
	60%	-0.06	0.883	N.S	-8.16	0	H.S
	80%	-2.05	0	H.S	-10.15	0	H.S
	100%	-3.35	0	H.S	-11.45	0	H.S
Ziziphus	20%	8.1	0	H.S	0	1	N.S
	40%	8.1	0	H.S	0	1	N.S
	60%	5.97	0	H.S	-2.12	0	H.S
	80%	3.97	0	H.S	-4.12	0	H.S
	100%	0.92	0.023	S	-7.17	0	H.S
Combination	20%	3.19	0	H.S	-4.9	0	H.S
	40%	1.55	0.001	H.S	-6.55	0	H.S
	60%	-1.3	0.004	H.S	-9.4	0	H.S
	80%	-2.9	0	H.S	-11	0	H.S

100%	-4.4	0	H.S	-12.5	0	H.S

Table 4: Descriptive statistics and comparisons between mean values of *S. mitis* IZ for the same conc. of *Myrrha* extract and *Ziziphus* extracts.

	Descriptive Statistics		Descriptive Statistics		Mean differences		
	Myrrha extract		*Ziziphus* extract				
Conc.	Mean	±S.D.	Mean	±S.D.	t- test	P-value	Desc.
20%	4.2	0.21	0	0	38.884	0	HS
40%	6.43	0.42	0	0	30.458	0	HS
60%	8.16	0.12	2.12	1.03	8.769	0.003	HS
80%	10.15	0.99	4.12	0.18	14.65	0.001	HS
100%	11.45	0.47	7.17	0.28	12.309	0.001	HS

Table 5: Descriptive statistics and comparisons between mean values of *S. mitis* IZ for the same conc. of *Myrrha* extract and Combination extracts.

	Descriptive statistics		Descriptive statistics		Mean differences		
	Myrrha extract		Combination extracts				
Conc.	Mean	± S.D.	Mean	±S.D.	t-test	P-value	Desc.
20%	4.2	0.21	4.9	0.55	-3.412	0.071	NS
40%	6.43	0.42	6.55	0.59	-0.305	0.78	NS
60%	8.16	0.12	9.4	0.45	99	0	HS
80%	10.15	0.99	11	0.56	-1.183	0.022	S
100%	11.45	0.47	12.5	0.47	-2.566	0.033	S

Table 6: Descriptive statistics and comparisons between mean values of *S. mitis* IZ for the same conc. of *Ziziphus* extract and Combination extract.

	Descriptive statistics		Descriptive statistics		Mean differences		
	Ziziphus extract		Combination extract				
Conc.	Mean	±S.D.	Mean	±S.D.	t- test	P-value	Des.
20%	0	0	4.9	0.55	-17.78	0	HS
40%	0	0	6.55	0.59	-22.14	0	HS
60%	2.12	1.03	9.4	0.45	-15.31	0.001	HS
80%	4.12	0.18	11	0.56	-18.93	0	HS
100%	7.17	0.28	12.5	0.47	-16.92	0	HS

DISCUSSION

The standard Western medicine had only limited success in the prevention of periodontal disease, hence, the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are good alternatives to synthetic chemicals [30]. Furthermore, herbal elements are gaining attention as both preventive plaque formation approaches and as adjunctive treatments. 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 flavouring agent in foods and beverages and as fragrance in cosmetics [32,33]. Evidence suggested that toothpastes and mouthwashes which contain *Myrrha* are effective in preventing and treating gingivitis [34,35]. Topically, Myrrh was also applied to bacterial and fungal skin infections [34]. On the other hand, because of the biological benefits of ZSCL extract they were used as an anti inflammatory eye wash, and treat toothache [36], and in ethno medicine, the pastes of the roots and leaves of ZSC were used to treat boils, swollen glands, wounds and sores [37].

There were no previous studies that researched the antibacterial effect of alcoholic *C. Myrrha* and ZSCL extracts on the primary dental plaque colonizer (*S. mitis*), 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 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 Combination extracts (60%, 80% and 100% conc.) against *S. mitis* was the most efficient antimicrobial agent followed by *Myrrha* (60%, 80% and 100% conc.) in comparison to CHX and lastly *Ziziphus* also, the results showed that *C. Myrrha* extract was able to inhibit the growth of S. mitis at 100% conc. with the IZ was (11.45mm), another study [38] observed highest antibacterial activity of *Myrrha* extract against *S. pyogenes* was (12 mm) of IZ and other study [39], found that the extraction of *C. Myrrha* by ethanol showed the best antimicrobial activity, also the effect against *S. mutans* revealed IZ (32 mm). The result in the present study revealed that for S. mitis at 60% conc. of *Myrrha* there was no significant difference with CHX, hence a study [31] proved that Myrrha extract demonstrated IZ for *S. faecalis* equal to that of 2% CHX, this might be attributed to the interacts of *Myrrha* extract 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 Furan sesquiterpenes as major constituents of the *Myrrha*, thus confirmed the antibacterial activity of gum resins, since a sesquiterpenoid detected in the *Myrrha* resin is reported to exhibit significant role in antibacterial activities [34,41] .The antimicrobial effects of the sesquiterpene has a bactericidal rather than a bacteriostatic effect which interacted with the cell envelopes, causing bacterial lysis and subsequent fatal loss of intracellular material [42]. The positive control agent used in this experiment, CHX 0.2% revealed bacteriostatic effect against *S. mitis*.

Although, the sensitivity of *S. mitis* to different conc. of alcoholic ZSCL (except 0.20 g/ml and 0.40 g/ml had no antibacterial effects), 0.60 g/ml, 0.80 g/ml and 1g/ml were shown lower IZ than CHX 0.2% conc., but it was considered better because it is a natural herb. It was found that the inhibitory effect of the Sider leaves extracts against *S. faecalis* was observed up to 50 mg/ml conc. and recoded (13.33 mm) IZ with conc. of (200 mg/ml) [43]. Another study (14) revealed that the highest activity was demonstrated by the ethanolic extractof Sider leaves at a conc. of 128 mg/L with (13 mm) IZ against gram positive bacteria (*Streptococcus spp*) and lowest activity with (8 mm) IZ was demonstrated conc.of results suggested that antibacterial activityof Sider ethanolic extractagainst 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].

For S. mitis, Combination extracts at 60%,80% and 100% conc. and for *Myrrha* at 80% and 100% conc. produced diameters of IZ larger than those produced by 0.2% CHX with highly significant differences which may be caused by the presence of active antimicrobial constituents in conc. sufficient enough to inhibit the growth and damage the isolates in a degree close to that of CHX, thus the use of Myrrha alone and in Combination with ZSCL extracts leads to antibacterial effects that may act as a good alternative to CHX.

The MIC which was 20% conc. of *C. Myrrha* and Combination extracts but at 60% conc. for ZSCL extract. According to previous study [46], plant extracts with MIC less than/or around 0.5 mg/ml indicated good antibacterial activity. Based on this, it is concluded that ethanol extracts of *Myrrha* and Combination extracts exhibited good antibacterial activity against *S. mitis*.

The MBC of *Myrrha* and Combination extracts that kills *S. mitis* was 40% conc. While the MBC of ZSCL extract was 80% conc. which indicated that the agents exhibited bactericidal effect. But, 0.2% CHX did not revealed bactericidal effect hence, CHX in low conc. (0.2%) did not demonstrate bacteriostatic effect against periodontal pathogens [47].

In summary, this study confirms that plant extracts possess in vitro antibacterial activity, However, if plant extracts are to be used for food preservation or medicinal purposes, issues of safety and toxicity will need to be addressed.

CONCLUSION

Alcoholic Combination extracts are effective as same as CHX against S. mitis than *Myrrha* and *Ziziphus* leaves extracts separately, so it can be used as an alternative to CHX, further in vitro and in vivo studies are essential to validate the use of *Myrrha* and *Ziziphus* leaves extracts as antimicrobial agent against primary colonizer microorganism.

REFERENCES

1. 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.
2. 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.
3. Murakami S, Mealey BL, Mariotti A, et al. Dental plaque – induced gingival conditions. J Clin Periodontal 2018; 45:17–27.
4. Li J, Helmerhorst FJ, Leone CW, et al. Identification of early microbial colonizers in human dental biofilm. J Appl Microbiol 2004; 97:1311–1318.

5. Hamada S, Slade HD. Biology, immunology and carcinogenicity of *streptococcus* mutan. Microbial Rev 1980; 44:331-384.
6. Adriaens PA, Adriaens LM. Effects of nonsurgical periodontal therapy on hard and soft tissues. Periodontal 2004; 36:121–145.
7. Emilson C. Potential efficacy of chlorhexidine against mutans *Streptococci* and human dental caries. J Dent Res 2004; 73:682-691.
8. 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.
9. Mohamed A, Shahid SMA, Alkhamaiseh SI, et al. Antibacterial activity of commiphora molmol in wound infections. J Diagnostic Pathol Oncol 2017; 2:32-36.
10. 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.
11. 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.
12. 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.
13. Motamedi H, Seyyednejad M, Hasannejad Z, et al. Comparative study on the effects of *ziziphus spina-christi* alcoholic extracts on growth and structural integrity of bacterial pathogens. Iranian J Pharm Sci 2014; 10:1-10.
14. 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.
15. 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.
16. 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.
17. Jesper H, Reinholdt J, Kilian M. Population dynamics of *streptococcus mitis* in its natural habitat. Infection Immunity 2001; 69.
18. Kumar S. Textbook of microbiology. First Edn. Jaypee Brothers Medical Publishers (P) LTD. New Delhi 2012.
19. Bergey's manual of systematic bacteriology. Second Edn New York: Springer 3;2009.
20. Koneman E, Schreckenberge P, Allens S, et al. Diagnostic microbiology. 4th Edn. J.B. Lippincott Co. USA. 1992.
21. Reiner K. Catalases test protocol. ASM Micro Library 2013.
22. Buxton R. Blood agar plates and hemolysis protocols. ASM Micro Library 2013.
23. 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.
24. 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.
25. Tiwari P, Kumar B, Kaur M, et al. Phytochemical screening and extraction: A review. Int Pharm Sci 2011; 1:98-106.
26. Mekonnen Y, Dekebo A, Dagne E. Toxicity study in mice of resins of three *commiphora* species. Sinet Ethiop J Sci 2003; 26:151–153.
27. Stephenson FH. Calculations for molecular biology and biotechnology, a guide to mathematics in the laboratory 2nd Edn. Academic Press is an imprint of Elsevier 2010.
28. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemotherap 2001; 48:5–16.
29. Rollins DM, Temenak JJ, Shields P, et al. Microbial pathogenesis laboratory manual. 2nd Edn 2003.
30. Torwane NA, Hongal S, Goel P, et al. Role of Ayurveda in management of oral health. Pharmacogn Rev 2014; 8:16–21.
31. Anand S, Rajan M, Rajeswari K. Evaluation of the antibacterial efficacy of *azadirachta indica, commiphora myrrha, glycyrrhiza glabra* against *enterococcus faecalis* using real time PCR. Open Dent J 2016; 10:160–165.
32. Hall RL, Oser BL. Recent progress in the consideration of flavor ingredients under the food additives amendment. 3 GRAS substances. Food Technology 1965; 19:151-197.
33. Ford RA, Api AM, Letizia CS. Monographs on fragrance raw materials. Food Chem Toxicol 1992; 30:1S-138S.
34. Dolara P, Corte B, Ghelardini C, et al. Local anaesthetic, antibacterial and antifungal properties of sesquiterpenes from *Myrrh*. Planta Medica 2000; 66:356-358.
35. Saeidi M, Azadbakht M, Semnani K, et al. Formulation of herbal toothpaste from *chamomile* and *myrrh*, a preliminary clinical evaluation on bleeding gum. J Mazandaran Univ Med Sci 2003; 13:61-69.
36. Jens ASS, Hammer GK, Buerkert A. *Ziziphus spina-christi (L.) Willd*: A multipurpose fruit tree. Genet Resour Crop Evol 2008; 55:929–937.
37. Olajuyigbe OO, Afolayan AJ. Evaluation of combination effects of ethanolic extract of *ziziphus mucronata willd subsp mucronata willd* and antibiotics against clinically important bacteria. Scientific World J 2013; 2013:1-9.
38. Al-Daihan S, Al-Faham M, Al-shawi N, et al. Antibacterial activity and phytochemical screening

of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. J King Saud University Sci 2012; 25:115-120.

39. Almekhlafi S, Thabit AAM, Alwossabi AMI, et al. Antimicrobial activity of Yemeni *myrrh* mouthwash. J Chem Pharm Res 2014; 6:1006-1013.
40. Alhussaini MS, Saadabi AM, Alghonaim MI, et al. An evaluation of the antimicrobial activity of *c. myrrha nees* (Engl.) oleo-gum resins from Saudi Arabia. J Med Sci 2015; 15:198-203.
41. Ahamad SR, Rahman A, Al-Ghadeer, et al. Analysis of inorganic and organic constituents ofmyrrh resin by GC – MS and ICP-MS: An emphasison medicinal assets. Saudi Pharm J 2017; 25:788–794.
42. Claeson P, Raadstroem P, Skoeld O, et al. Bactericidal effect of the sesquiterpene Tcadinol on *staphylococcus aureus*. Phytother Res1992; 6:44399.
43. Al-Bayatti KK, Aziz FM, Abdalah E. A study of antibacterial activity of *cidar (zizyphus spinachristi l.)* on bacterial pathogens isolated from skin infections. AJPS 2011; 9:1.
44. Kadioglu O, Jacob S, Bohnert S, et al. Evaluating ancient Egyptian prescriptionstoday: anti-inflammatory activity of *Ziziphus spina-christi*. Phytomed 2016; 23:293–306.
45. Sparg S, Light M, Van Staden J. Biological activities and distribution of plant saponins. J Ethnopharmacol 2004; 94:219–243.
46. Salvat A, Antonacci L, Fortunato RH, et al. Antimicrobial activity in methanolic extracts of several plant species from northern Argentina. Phytomed 2004; 11:2304.
47. Frentzen M, Ploenes K, Braun A. Clinical and microbiological effects of local chlorhexidine application. Int Dent J 2002; 52:32-59.