Available online at *www.ujpronline.com*
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)
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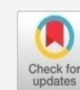

RESEARCH ARTICLE

IN VITRO EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF FIVE HERBAL EXTRACTS AGAINST *STREPTOCOCCUS MUTANS*

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Article Info:

Article History:
Received: 9 December 2021
Reviewed: 6 January 2022
Accepted: 11 February 2022
Published: 15 March 2022

Cite this article:
Gylan EMA, Muharram BA, Al-Kholani AIM, AL-Haddad KA, Al-Akwa AAY, Al-Shamahy HA, Al-Hamzi MA, Al-labani MA. *In-vitro* evaluation of the antimicrobial activity of five herbal extracts against *Streptococcus mutans*. Universal Journal of Pharmaceutical Research 2022; 7(1):37-44.
https://doi.org/10.22270/ujpr.v7i1.721

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Abstract

Background: The emergence and extend of antibiotic resistance, together with the development of new strains of illness causes, are of enormous alarm to the global health community. Successful treatment of a illness involves the development of new pharmaceuticals or some promise source of novel drugs. Universally make use of medicinal plants of our community could be an outstanding source of drugs to fight off dental caries. This study is focused on discovering the antibacterial properties of the plants that are frequently being used as traditional medications.
Methods: Five methanol extracts from *Salvia officinalis, Commiphora myrrha, Boswellia carteril, Saussurea lappa* and *Dracaena cinnabari* were examined for their antibacterial activities against most common bacterial oral pathogen, *Streptococcus mutans.* The antibacterial testing was carried out by using the disc diffusion and broth micro-dilution assays.
Results: Methanol extracts of the five plants were effective against *Streptococcus mutans* with diameter zone of inhibition ranging from 63.6 to 21.0 mm. The results of the microdilution assay confirmed that the *Salvia officinalis, Commiphora myrrha, Saussurea lappa* and *Dracaena cinnabari* were effective against the *Streptococcus mutans*, exhibiting MIC values, ranging from 0.310 to 0.156 mg/ml.
Conclusion: The results of our study indicate that the methanol extracts of plants used in this study have an antibacterial effect even at low concentration against the carcinogenic *Streptococcus mutans* bacteria, and they may be possible to combat *Streptococcus mutans* to increase the effectiveness of oral hygiene practices by incorporating the extracts of these plants into anti-caries such as Toothpastes and mouthwash.
Keywords: Antibacterial activities, *Boswellia carteril, Commiphora myrrha, Dracaena cinnabari*, *Salvia officinalis*, *Saussurea lappa*, *Streptococcus mutans.*

INTRODUCTION

Dental caries is one of the most prevalent oral diseases worldwide and the main cause of tooth loss in the population and dental caries is a post-eruptive infectious bacterial disease characterized by a gradual demineralization process that affects the mineralized dental tissues[1-4]. There are a variety of virulence factors unique to the isolated bacteria that play an important role in caries formation but *Streptococcus mutans* is an effective initiator of caries formation[3,4]. Whereas caries is a polymicrobial disease, selective targeting of *S. mutans* in dental biofilms is observed as a suitable method for its prevention. This is mostly for the reason that the production of insoluble glucans from sucrose by *S. mutans* is vital for the development of a steady biofilm matrix that make possible bacterial colonization of the tooth surface and, at the same time, operates as a diffusion obstacle helping to maintain the acidic milieu within which cariogenic bacteria thrive[3,4]. For the prevention and treatment of safe, effective and economical oral diseases, from increased incidence of

disease (particularly in developing countries), increased resistance of pathogenic bacteria to currently used antibiotics and chemotherapies, opportunistic infections in immunocompromised individuals and financial considerations in developing countries; for all of these factors comes the global need for alternative options and products[5-16]. In current years, researchers provided awareness to use plant extracts against cariogenic bacteria observing their result on growth[17-22]. *Dracaena cinnabari* fit into Agavaceae family, which is frequently known as Damm Alakhwain in Yemen. *D. cinnabari* is endemic to the island of Socotra in Yemen. "Dragon's blood" is a deep red resinous exudate that is obtained from cut stems of quite a lot of plant species[19]. *D. cinnabari* (resin) has also been traditionally used in Socotra as a therapeutic agent for the treatment of dental, gastrointestinal tract, skin, and eye diseases[19,23]. There are a lot of studies that have been performed on *D. cinnabari* balf resin and approved its effectiveness as antitumor, antiviral, antimicrobial, and cytotoxic[19]. It is also a potent analgesic, antioxidant and anti-inflammatory[19-25]. *Saussurea lappa* belongs to *Asteraceae* family, is commonly known as costus in English and has different language names in Asian countries. It has been traditionally used for relieve pain in tenesmus, indigestion, abdominal distention, nausea, anorexia, dysentery and vomiting. Diverse pharmacological tests in an amount of *in vitro* and *in vivo* models have realistically confirmed the capability of *S. costus* to exhibit anti-ulcer, anti-inflammatory, anticancer and hepatoprotective activities, providing support to the rationale behind several of its traditional uses[26,27].

Boswellia carteril as well known as Olibanum or Frankincense is the dried juice of trees in the *Boswellia* genus, specifically *Boswellia sacra*. These trees grow in Yemen and Oman; also grow in the Horn of Africa, including Ethiopia and Somalia. Modern studies have shown that olibanum certainly has sedative and analgesic effects and alcohol extracts from olibanum inhibit the growth of fungi and bacteria. The anti-inflammatory activity is mainly approved to the existence of major ingredient of pentacyclic triterpene namely β-boswellic acids and α-boswellic acids[27].

Salvia officinalis also called common sage is the principal genus of the *Lamiaceae* family, which is Mediterranean area inhabitant and comprises about 900 species. From its Latin name "*Salvia*", meaning to cure and the most important components of *S. officinalis*, are phenolic components. Sage has antiviral, antimicrobial, immunosuppressive effects and antioxidant, so its therapeutic and aromatic use is important[28].

Commiphora myrrha also called common *Myrrh* is a member of the *Commiphora* family, is an original tree inhabitant to northern Kenya, Somalia and Ethiopia. *Myrrh* has been traditionally used in balms for mummification, perfumes, skin disease treatments and for healing wounds. *Myrrh* is also utilized as an antimicrobial agent and anti-inflammatory for example it has been used for the treatment of sinusitis, kidney infections, oral ulcers, gingivitis, brucellosis and parasitic infections. The vital oil of *Myrrh* has different chemical constituents, comprising sesquiterpenes, monoterpenes, and aromatic compounds. Additionally, other chemical compounds present in *Myrrh* resins include steroids, lignans, triterpenoids and diterpenoids[29]. Recently, dental research on oral clinical problems and oral infections has been conducted in[30-32], but no antibacterial sources from plant sources were searched for the treatment of oral infections. For this reason the current study selected five plants of D. *cinnabari, S. lappa, B. carteril, C. myrrha* and *S. officinalis* which are known for their medical applications to evaluate their antibacterial effect against *S. Mutans* and determine the antibacterial combination effects of these plants extracts and chlorhexidine.

MATERIALS AND METHODS

Plant materials

The selected plants of (*B. carteril, C. myrrha and S. lappa*) were collected from a local market in Sana'a-Yemen. The *S. officinalis* was collected from Ibb Governorate in Nov-2020. Methanol extract of *D. cinnabari* was obtained from Dr. Nahed Al-Baoqai, Department of Pharmacology and Therapeutics, Faculty of Medicine and Health Sciences, Sana'a University, Yemen. All plants were approved by the department of Pharmacognosy, Faculty of pharmacy, Sana'a University. All collected plants were dried and ground and stored in airtight bottles.

Preparation of methanolic extract

Plants extraction was been performed at the Pharmacy faculty, Sana'a university, Republic of Yemen. The weight of the ground powder was taken. The crude methanol extract of the plants was been prepared by soaking of the air dried plants for at least 3 days (135.25g *S. lappa*, 146.16g *B. carteril,* 168g *C. myrrha* and 366.54g *S. officinalis*) with 250 ml methanol by using maceration method. The extracts were been filtered with Whatman No.1 filter paper and evaporated under reduced pressure in a rotary evaporator, at 40°C for 20 min to yield the crude extract. Samples were stored in bottles for further studies. The percentage yield obtained for each extract from different plants was calculated using the following formula[16].

$$\text{Percentage yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}} \times 100$$

Bacterial sample

The collection of samples was conducted in the Department of Dentistry and Oral Health in Republican Teaching Hospital Authority, Sana'a, Yemen. Before inclusion in the study, approval was obtained from the Medical Research and Ethics Committee of the Faculty of Dentistry, Sana'a University by document with reference number (129) dated 12 February 2021. A sixty five active dental caries samples were collected from patients attending the operative clinics of Republican Teaching Hospital. The specimens then transported to the laboratory without delay after collection use Thioglycollate broth then processed on same day. Prior to inoculation, specimens were vortexed (15 sec) and diluted 1:1000 in isotonic saline solution. Then a loop ($1/1000^{th}$ ml) of each sample was

inoculated onto Mitis *salivarius* agar with potassium tellurite medium, bacitracin and 20% sucrose. The plates were incubated at 37 °C anaerobically. After 72-hour, colony characteristics were investigated and identified[1-3].

Antibacterial assays

Disc diffusion assay

Anti-bacterial activity of the methanol extracts of five plants *against S. mutans* were evaluated using the disc diffusion method[33,34]. Chlorohexidine 0.1% was be used as positive control to determine the sensitivity of the tested bacteria. Sterile paper discs (Whatman No.3) of 6mm in diameter was been impregnated with 15 ml of the MeOH extracts (500 mg/ml). The discs then air dried under sterile condition. Chocolate agar plate (90 mm) was been inoculated with the stock bacterial suspension (1-2×108 CFU/ml) by streaking the surface in three different directions to ensure a proper distribution of the inoculum with a sterile cotton swab, which has been dipped into the bacterial suspension. Using an alcohol-flamed forceps, the prepared discs then evenly placed and distributed onto the inoculated plated. The plates then covered; inverted and incubated at 35°C for 24hr. Discs impregnated with only MeOH serve as negative control for the plant extract. The anti-bacterial activity was been demonstrated be measuring the diameter of the zone of inhibition for each test compound against the tested bacteria. The mean diameter of the inhibition zone was recorded from triplicate test. The anti-bacterial activity was defined as strong when the inhibition zone diameter was ≥15 mm, moderate for diameter of 10-15 mm and weak for diameter of < 10mm[33].

Broth micro dilution assays: MIC AND MBC

The quantitative assay of anti-bacterial activity of plants extracts was performed according to the reference method recommended by NCCLS[34]. Sterility condition was maintained throughout experimental. Figure 1 summarizes the micro-dilution assay. This assay was done only for the extracts that have inhibition zone more than 15 mm. Firstly, stock solutions were prepared by dissolving the plant methanol extracts in MeOH– except for the *D. cinnabari* that was dissolved in DMSO- to a final concentration of 500 mg/ml Serial dilutions of MeOH extract was prepared in Eppendorf tubes labeled 1 to 6. Tube1 was filled with 30µl of stock solution of the test material (500mg/ml) with 1470µL nutrient broth to obtain a concentration of (10mg/ml).

About 750µL of nutrient broth was dispensed in to the tubes 2 to 6 then 750µL of the solution in tube 1 was transferred to tube 2. This was repeated sequentially for the solutions in tubes 2 to 6 in order to obtain intermediate concentrations ranging between 10 to 0.3125 mg/ml. Aliquots (100µL) of each resultant solution then transferred into 96-well micro titer plate. Each well later filled with 100µL of bacterial suspension (1-2×108 CFU/ml), which achieve the desired final concentration of the test materials 5, 25, 1.25, 0.63, 0.31 and 0.16 mg/ml. The concentration of the solvents not more than 1%. The microtiters were incubated for 24 hr at 35°C. Four controls were used for the broth microdilution assay including:

- **Negative control**: 100µL of 2% MeOH in nutrient broth was mixed with 100µL of bacterial suspension in the well giving the concentration of the solvents not exceeding 1% as recommended by NCCLS[34].
- **Growth control**: 100µl of bacterial suspension was mixed with 100µL of nutrient broth.
- **Positive control**: 0.1% chlorhexidine was used to determine the sensitivity of tested bacteria.
- **Sterility control (purity control)**: 200µL of nutrient broth alone was used to confirm the sterility of the broth.

The minimum inhibition concentration (MIC) of the tested substance was determined optically to observe growth turbidity as recommended by the NCCLS [34]. It is the lowest sample concentration at which there is no bacterial growth after the time of incubation. The MIC value was recorded as the mean concentration of triplicated. The anti-bacterial activity categorized as strong if MIC < 1.00 mg/ml, moderate if 1.00 ≤ MIC ≤ 4.9 mg/ml and weak if MIC ≥5.00 mg/ml.

Antibacterial combination assay (Checkerboard assay)

In vitro antibacterial combination assay was carried out to examine the combined result of tested plants with chlorohexidine against *S. mutans*. by using the checkerboard technique, as described by Davidson and Parish[35]. The assay involved multiple dilutions of tested plant and chlorohexidine in concentrations equal to, above and below their MIC values for the bacteria being tested. The concentrations tested for each plant ranged from 4 to 5 dilutions below the MIC to twice the MIC, using two-fold dilution. Seven serial two fold dilution of each plant and chlorohexidine were prepared in MeOH solution as described in the broth microdilution procedure and then diluted with nutrient broth to obtain a series of dilutions at concentration 4 times higher than its final concentration in the reaction mixture.

Fifty microliter aliquots of *D. cinnabari* (0.02-1.25 mg/ml), *C. myrrha* (0.04-2.5 mg/ml), *S. lappa* (0.01-0.625 mg/ml), *Salvia officinalis* (0.01-0.625 mg/ml) and chlorohexidine (0.002-0.1 mg/ml) were dispensed in to the wells vertically down the 96-well microtitre plate and 50µl aliquots of each plants with the same pervious concentration was dispensed horizontally. A100 µl suspension [1-5 counting form unit/ ml (CFU/ml)] of *S. mutans* was added into each well. The final concentration in the reaction mixture ranged from (0.005-0.30 mg/ml) for *D. cinnabari,* (0.01-0.625 mg/ml) for *C. myrrha,* (0.0025-0.16 mg/ml) for *S. lappa,* (0.0025-0.16 mg/ml) for *S. officinalis* and (0.0005-0.025 mg/ml) for chloro-hexidine. The result was that each square in the checkerboard contained a series of combination of each plant and chlorohexidine being tested.

RESULTS AND DISCUSSION

Yields of the methanol extracts

The percentage yield of methanol extracts obtained from the five plants are as listed in Table 1. In general,

the percentage yields of the *B. carteril* extract was the highest among the five extracts, and the *C. myrrha* is the lowest. Methanol which was used in the extraction could be responsible for the high yields (>5%) of extracts for all the used plants. Cowan[36] found that MeOH has a high efficiency in extracting most of non-polar and polar phytochemicals from plant materials.

Table 1: The percentage yield of the methanol extracts of plants.

Plants	Yield %
S. lappa	25.3
B. carteril	44.7
C. myrrha	6.4
S. officinalis	10.9
D. cinnabari	unknown

Yield based on dry weight plant

Disc diffusion assay

The antibacterial activity of MeOH extracts from *D. cinnabari*, *B. carteril*, *S. officinalis*, *C. myrrha* and *S. lappa* was carried out using the disc diffusion method. All of MeOH extracts used in this study showing strong antibacterial activity against of *Mutans streptococci* with varying sizes of zone of inhibition Figure 2. Also, the readings for the positive and negative controls were obtained. The results obtained are shown in Figure 1. *C. myrrha* had the highest inhibition diameter (36.6±5.1 mm) followed by *S. lappa* (35.6±3.6 mm), the lowest inhibition zone diameter was with *S. officinalis* (21±1.5 mm). No zone indicative of the lack of growth around the methanol which was used as negative control was detected and the inhibition zone around the chlorhexidine (0.1%) disc which was used as positive control was observed with inhibition zone (32.6±2.2 mm). The degree of susceptibility of the bacteria to the extracts varied according to the sensitivity of the bacteria, the nature or concentration of the chemical inhibitors in the plant materials and according to the relative solubility of the chemical components in aqueous media. The results of the phytochemical screening of methanol extract of *D. cinnabari, S. lappa, B. carteril, C. myrrha* and *S. officinalis* reveal that these natural products are rich in flavonoids and terpenoids. Flavonoids can inhibit the growth of both Gram positive and Gram negative bacteria and is highly active against the anaerobic bacteria pathogens in the mouth. Flavonoids also have anti-viral activity and play a vital role in the general health of a person[36].

The results in this study were consistent with previous studies that tested the effects of a number of medicinal plant extracts against *S. mutans*. One of these studies was carried out by Wannachot and Rattanakiat[37] in which they investigated the inhibitory effect against *S. mutans in vitro* of the 95% ethanol extracts from five herbs: *M. cochinchinensis* Spreng, *P. guajava* L, *G. glabra* L, *P. retrofractum* Vahl and *S. aromaticum* L, the largest inhibition zone 16.7±0.5 mm in diameter observed in *S. aromaticum* extract. The extract of *P. retrofractum* produced a small inhibition zone (6.7±0.5). In other study conducted by Elgamily *et al.*[38], methanolic extractions of five plants including Ginger, Clove, Black seed, Cinnamon and Turmeric were tested against the growth of the *S. mutans*, only Cinnamon and Clove produced inhibition zones against *S. mutans* with inhibition zones diameters of 14.00 mm and 12.67 mm respectively. *C. myrrha* and *B. carteril in our present study* showed strong result in the disc diffusion assay against *S. mutans*, the inhibitory zones were 36 m and 25 mm in diameter respectively, this result is higher than the results of previous studies that assessed the impaction of these plants against *S. mutans,* for example, study conducted by SB, *et al.*[39], in which anti-cariogenic properties of essential oil (E. Oil) and crude extracts obtained from *B. frereana* and *B. carterii* were investigated; the average microbial inhibition was 14.6 mm for *S. mutans.* Other study conducted by Barre et *al.*[40], showed that *C. myrrha* methanol extract showed inhibition zone (15±1.0 mm) against *S. mutans.* Study by Izzeldien, *et al.*[41], well and disk diffusion methods were used to investigate the result of four concentration (100, 50, 25 and 12.5 mg/ml) of Myrrh volatile oil, extracted by the technique of hydro-distillation, the results revealed that the four dilutions of oil were effective on *S. mutans* with the largest inhibition zone (18.7±0.6 mm) through the well diffusion method and inhibition zone of (14.00 mm).

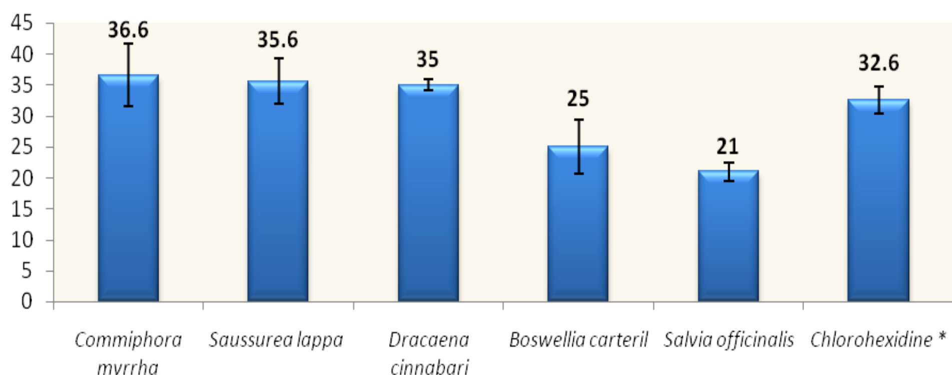

Figure 1 : The antibacterial activity of MeOH extracts and chlorhexidine against *S. mutans* as determined by disc diffusion assay.

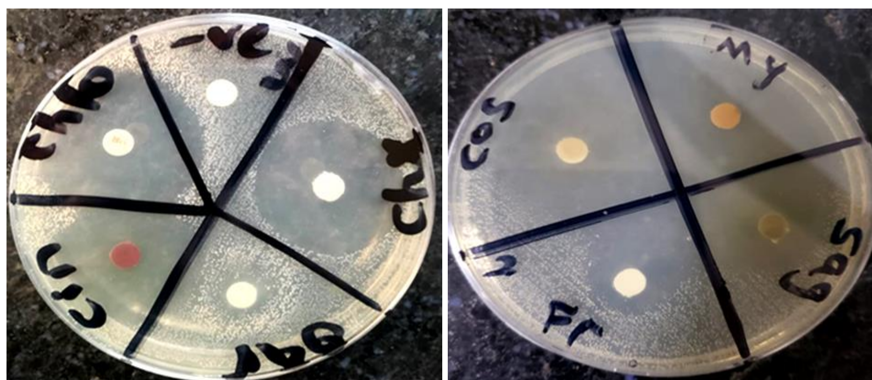

Figure 2: Plates showing the inhibitory zone of MeOH extracts of plants.
Cin=*Dracaena cinnabari*, -ve=negative control) MeOH (, chx=chlorohexidine, chlo=Chloramphenicol. myr=*Commiphora myrrha*, cos=*Saussurea lappa*, Sag=*Salvia officinalis*, fra=*Boswellia carteril*, against *Streptococcus mutans*

S. officinalis in our current study showed strong result against *S. mutant* with inhibitory zone (21±1.5 mm), this result was in agreement with the result of previous studies that tested antimicrobial activity of *S. officinalis* against *S. mutans* and is not in agreement with others. For example, Krumin *et al.*[42], tested the impaction of ethanol extract of *Salvia officinalis* against *S. mutans* and showed a result similar to that we obtained in current study. In contrast, Dalirsani, *et al.*[43], studied antibacterial activity of ten medicine plants including *S. officinalis* against *S. mutans* and showed very week antimicrobial activity of *S. officinalis* against tested bacteria with inhibitory zone (0.6 mm). *S. lappa* showed inhibition against *S. mutans* with inhibition zone of 35.6±3.6 mm, this result was in agreement with study conducted by Yu, *et al.*[44], that examined the effects of ethanolic extract on the growth and acid production of *S. mutans*, as well as the adherence and synthesis of water-insoluble glucans, their result showed that ethanolic extract (0.5 mg/ml to 4 mg/ ml) inhibits the growth and acid production of *S. mutans*, reduces the adherence of *S. mutans*, and inhibits the synthesis of water insoluble glucans. These results proved that *S. lappa* remarkably inhibits the cariogenic activity of *S. mutans*. Although few studies showed that *D. cinnabari* collected from Soqotra Island, Yemen, has antimicrobial activity our study is the first -to our knowledge- to investigate antimicrobial activity of *D. cinnabari against S. mutans*. *D. cinnabari* in present study showed strong antimicrobial activity against these bacteria with inhibition zone (35±0.9). There are few studies that have tested the effect of *D. cinnabari* on different types of bacteria, for example, Ansari, *et al.*[45], investigated the antibacterial activity of the of *D. cinnabari* resin on equally on poly-microbial culture and on antibiotic multi-resistant human pathogens; the results of this study illustrated that ethanolic extract of *D. cinnabari* resin has a significant antibacterial activity against Gram-positive and Gram-negative human pathogens and fungi. The difference in the results between our study and results in other studies for all used plants might be attributed to the difference in the extraction method and concentration. The MeOH has a high efficiency in extracting most of non-polar and polar phytochemicals from plant materials. Also, these differences might be attributed to the fact that Yemeni strains of *C. myrrha*, *B. carteril*, *S. officinalis*, and *D. cinnabari* have more antibacterial properties than other strain in other countries[33,37].

Table 2: The minimum inhibitory concentration (MICs)(mg/ml) of methanol extracts against *S. mutans* as determined by broth micro-dilution assay.

Plant	MIC	MBC
D. cinnabari	(0.3125)	(0.625)
C. myrrha	(0.625)	(0.625)
S. officinalis	(0.156)	> (0.156)
S. lappa	(0.156)	> (0.156)
B. carteril	> 5	nd
Chlorohexidine (+ve)	0.02	> 0.02
N broth/MeOH (-ve)	+	nd
N broth (100 µl) (g)	+	nd
N broth (200µl) (st)	-	nd

Each value is the main of triplicate. -=no growth, += growth, (+ve)=positive control. (-ve)=negative control, (g)=growth control, (st)=sterility control, nd=not determined

Table 3: Combination between chlorohexidine and *D. cinabarri, C. myrrha* and *S. lappa.*

Plants	Effect	FICI	Plants MIC (mg/ml)		Chlorohexidine MIC(mg/ml)	
			Alone	Combined	MIC alone	MIC combined
D. cinnabari	Synergistic	0. 131	0.313	0.002	0.02	0.002
C. myrrha	Synergistic	0.43	0. 63	0.006	0.08	0.006
S. lappa	Synergistic	0.28	0.313	0.08	0.02	0.006

Each value is the main of triplicate. (MICs)=Minimal inhibitory concentration, (FICI)=fraction inhibitory indices

Broth micro-dilution assay for MIC and MBC

This is determined by dilution of the broth for MIC tests by sub-culturing of agar plates that do not contain the test agent. MBC is determined by determining the lowest concentration of an antibacterial agent that reduces the viability of primary bacterial inoculums by a predetermined reduction such as ≥99.9%. The antibacterial activity of methanol extracts was quantified using the microdilution method. The MICs and MBCs were determined for all methanolic extracts those used in this study since they showed an inhibition zone more than 14 mm in diameter in the disc diffusion assay. The results are summarized in Table 2. The results of micro-dilution assay were consistent with the disc diffusion results confirming the antibacterial activity of used plants except for *B. sacra* which showed a strong result in the disc diffusion test, while it did not show inhibition for *S. mutans* in the broth microdilution test. In previous study by Bakhtiari, *et al.*[46], *B. sacra* did not show inhibition for *S. mutans* at the lowest concentration (5 mg/ml) used in their study while it exhibits antibacterial activity when used in higher concentration of organic and hydro-alcoholic extracts of *B. serrata* of (50 mg/ml). This may explain the absence of bacterial inhibition of *B. sacra* in current study, as the highest concentration that was used is (5 mg/ml), also the low solubility of it in water could be responsible for this result.

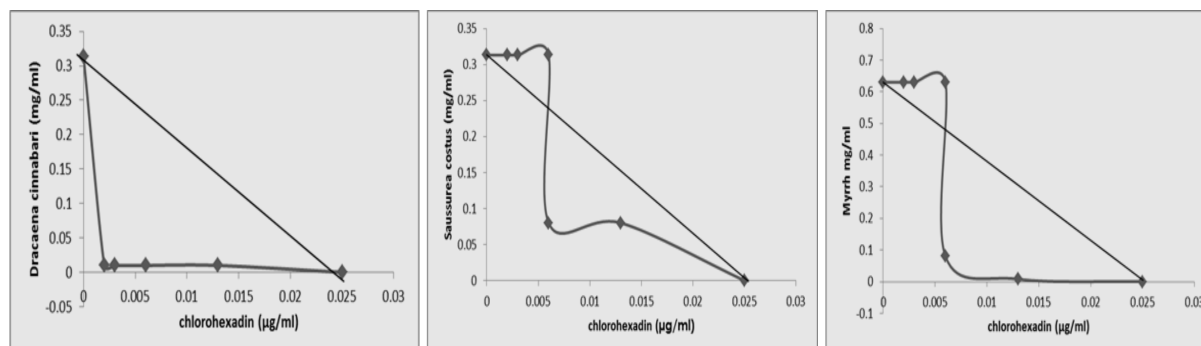

Figure 3: Isobologram showing the synergetic effect of the combining the chlorohexidine and *D. cinnabari*, *S. lappa* and *C. myrrha* against *S. mutans.*

Antibacterial combination

The checkerboard technique was performed to investigate antibacterial combination of chlorhexidine with *D. cinnabari, S. officinalis, C. myrrha* and *S. lappa* against *S. mutans.* The combination of chlorohexidine with *D. cinnabari, C. myrrha* and *S. lappa* showed synergistic effect as the FICI was less than 1. The combination of chlorohexidine with *Salvia officinalis* did not show any inhibition of bacteria. Table 3 and Figure 3 show the MICs and fraction inhibitory indices (FICI) of the chlorohexidine and *D. cinnabari, C. myrrha* and *S. lappa* combination against *S. mutans.*

To our knowledge, there is no study that examined the combination of plants used in this study with chlorhexidine, but there are other studies that examined the combination of chlorhexidine with other medicinal plants against *S. mutans*, for example, Filoche *et al.*[47], compared antimicrobial effects of essential oils of manuka, *Leptospermum morrisonii*, cinnamon, tea-tree, grapefruit, arnica, eucalyptus. The essential oil mouthrinse of Cool Mint Listerine and two of its components alone and in mixture with chlorhexidine gluconate against planktonic and biofilm cultures of *Lactobacillus* and *S. mutans* and concluded that; the amount of chlorhexidine required to achieve an equivalent growth inhibition against the biofilm cultures was reduced 4–10-fold in combination with *L. morrisonii*, *Thymol cinnamon*, manuka and Listerine. In a previous study conducted by Yoo *et al.*[48], the synergistic effect of chlorhexidine digluconate and protamine sulfate on the inhibitory activity of *L. japonica* and *R. officinalis* extracts against *S. mutans* was examined and concluded that; the use of sub-MIC of chlorhexidine digluconate with sub-MIC of *R. officinalis* extract and *L. japonica* extract produced synergistic inhibitory effects of these antibacterial agents except for chlorhexidine digluconate and *L. japonica* combination.

CONCLUSIONS

The results of our study indicate that the methanol extracts of plants used in this study have an antibacterial effect even at low concentration against the *S. mutans* bacteria, and they may be possible to combat *S. mutans* to increase the effectiveness of oral hygiene practices by incorporating the extracts of these plants into anti-caries agents such as Toothpastes and mouthwash. The study also successfully evaluated the antibacterial combination of *D. cinnabari, C. myrrha*, and *S. lappa* with chlorhexidine. The results showed a synergistic effect between the compounds. However, studies that closely simulate situations in vivo are required to obtain a clear understanding. Further studies such as the toxicological and pharmacokinetic properties of these plants need to be conducted to develop these plants into antibacterial agents for clinical use.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Department of Pharmacognosy, Faculty of pharmacy, Sana'a University Republic of Yemen for support.

AUTHOR'S CONTRIBUTION

The research is a master degree in the Department of Conservative Dentistry, Faculty of Dentistry, Sana'a University. **Gylan EMA:** writing original draft, methodology. **Muharram BA:** research design, data collection. **Al-Kholani AIM:** statistical analysis, conceptualization. **AL-Haddad KA:** editing, methodology. **Al-Akwa AAY:** investigation. **Al-Shamahy HA:** supervision. **Al-Hamzi MA:** formal analysis, conceptualization. **Al-labani MA:** research design, data collection. Final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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