

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

ABSTRACT

Background: The emergence and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, are of great concern to the global health community. Effective treatment of a disease entails the development of new pharmaceuticals or some potential source of novel drugs. Commonly used medicinal plants of our community could be an excellent source of drugs to fight off this problem.

Aims: This study is focused on exploring the antimicrobial properties of the plants that are commonly being used as traditional medicines.

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. Checkerboard technique was performed to investigate the effects of combination of the *Salvia officinalis*, *Commiphora myrrha*, *Saussurea lappa* and *Dracaena cinnabari* with chlorohexidine.

Results: We have observed that, all methanol extracts of five plants were effective against *streptococcus mutans* with inhibition zone diameter ranging from 63.6 to 21 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 0.31 to 0.156 mg/ml. Whereas the *Boswellia carteril* showed great activity against *streptococcus mutans* disc diffusion assay, with inhibition zone 25 mm, the results of the microdilution assay not confirmed this antibacterial activity with MIC value of > 5 mg/ml. Isobologram and FIC indices indicated that these combinations produced synergetic effects against *streptococcus mutans*.

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 anticariogenic agents such as Toothpastes and mouthwash.

Keywords: antibacterial activities, *Boswellia carteril*, *Commiphora myrrha*, *Dracaena cinnabari*, *Salvia officinalis*, *Saussurea lappa*, *Streptococcus mutans*

INTRODUCTION

Dental caries consists in a post-eruptive bacterial infectious disease characterized by a progressive demineralization process that affects the mineralized dental tissues. It is considered to be the most prevalent oral disease worldwide and the main cause of tooth loss among the population¹⁻⁴. The *Streptococcus mutans* is a potent initiator of caries because there is a variety of virulence factors unique to the bacterium that have been isolated that play an important role in caries formation^{3,4}. While caries is a polymicrobial disease, selective targeting of *S. mutans* in dental biofilms is viewed as a suitable approach for its prevention. This is mainly because the synthesis of insoluble glucans from sucrose by *S. mutans* is central for the formation of a stable biofilm matrix that facilitates bacterial colonization of the tooth surface and, at the same time, serves as a diffusion barrier helping to maintain the acidic milieu within which cariogenic bacteria thrive^{3,4}. The global need for alternative prevention and treatment options and products for oral diseases that are safe, effective and economical comes from the rise in disease incidence (particularly in developing countries), increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics, opportunistic infections in immune-compromised individuals and financial considerations in developing countries⁵⁻¹⁶. In recent years, researchers gave attention to the use of plant extracts against cariogenic bacteria regarding their effect on growth¹⁷⁻²². *Dracaena cinnabari* belongs to Agavaceae family, which is commonly known as Damm Alakhwain in Yemen. It is endemic to the Socotra Island, Yemen. "Dragon's blood" is a deep red resinous exudate that is acquired from cut stems of several plant species¹⁹. In Socotra, *D. cinnabari* (resin) has also been traditionally used as a therapeutic agent for the treatment of GIT (gastrointestinal tract), skin, eye, and dental diseases^{19,23}. There are a lot of researches that have been worked on *Darceana cinnabari* balf resin and approved its effectiveness as antimicrobial, antiviral, antitumor and cytotoxic¹⁹. It is also found that, it is a potent analgesic, antioxidant and anti-inflammatory¹⁹⁻²⁵.

Saussurea lappa belongs to asteraceae family, is commonly known as costus in English and has different vernacular names in India. It has been traditionally used for alleviating pain in abdominal distention and tenesmus, indigestion with anorexia, dysentery, nausea, and vomiting. Different pharmacological experiments in a number of in vitro and in vivo models have convincingly demonstrated the ability of *Saussurea costus* to exhibit anti-inflammatory, anti-ulcer, anticancer and hepatoprotective activities, lending support to the rationale behind several of its traditional uses^{26,27}.

Boswellia carteril also known as Frankincense or Olibanum is the dried sap of trees in the *Boswellia* genus, particularly *Boswellia sacra*. These trees grow in Oman, Yemen and the Horn of Africa, including Somalia and Ethiopia. Contemporary studies have shown that olibanum indeed has analgesic, tranquilising, and alcohol extracts from olibanum inhibit the growth of fungi and bacteria. The anti-inflammatory activity is mainly attributed to the presence of major constituent of pentacyclic triterpene namely α -boswellic acids and β -boswellic acids²⁷.

Salvia officinalis also called common sage is the largest genus of the Lamiaceae family, which is native of the Mediterranean area and includes about 900 species. From its Latin name "Salvia", meaning to cure, The most important components of *S. officinalis*, are phenolic components. Sage has antimicrobial, antioxidant, antiviral, and immunosuppressive effects, so its medical and aromatic usage is important²⁸.

Commiphora myrrha also called common Myrrh is a member of the Commiphora plant family, is an indigenous tree native to Somalia, Ethiopia and northern Kenya. Myrrh has been traditionally used in perfumes, balms for mummification, skin disease treatments and for healing wounds. Myrrh is also used as an anti-inflammatory and antimicrobial agent for the treatment of oral ulcers, gingivitis, sinusitis, glomerulone-phritis, brucellosis and parasitic infections. The essential oil of Myrrh contains different chemical constituents, including monoterpenes, sesquiterpenes, and aromatic compounds. In addition, other chemical compounds present in Myrrh resins include triterpenoids, diterpenoids, steroids, and lignans²⁹.

Recently, dental research has been conducted in Yemen to discuss oral clinical problems and oral infections etc³⁰⁻³², but no antibacterial sources from plant sources were searched for the treatment of oral infections of bacterial cause, and for this reason the current study selected five plants of *Dracaena cinnabari*, *Saussurea lappa*, *Boswellia carteril*, *Commiphora myrrha* and *Salvia officinalis* which are known for their medical applications to evaluate their antimicrobial 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 (*Boswellia carteril*, *Commiphora myrrha* and *Saussurea lappa*) were collected from a local market in Sana'a-Yemen. The *Salvia officinalis* was collected from Ibb Governorate in Nov-2020. Methanol extract of *Dracaena cinnabari* was obtained from dr. Nahed Al-Baoqai, Department of Pharmacology and Therapeutics, Faculty of Medicine and Health Sciences, Sana'a University, Yemen. All collected plants were dried and ground and stored in airtight bottles.

Preparation of ethanolic 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 (135.25g *Saussurea lappa*, 146.16g *Boswellia carteril*, 168g *Commiphora myrrha* and 366.54g *Salvia officinalis*) with methanol by using maceration method. The extracts was 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 was be stored in bottles for further studies.

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

Bacterial sample

The collection of samples was conducted in the Department of Dentistry and Oral Health in Republican Teaching Hospital Authority, Sana'a, Yemen. A sixty five active dental caries samples were collected from patients attending the operative clinics of Republican Teaching hospital Authority and

transported to the laboratory immediately after collection using Thioglycollate broth and processed on same day. The sample was vortexed (15 sec) and diluted 1:1000 in isotonic saline solution prior to inoculation. One loop (1/1000th ml of sample) was inoculated on the 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 studied and identified.

Antibacterial assay

Disc diffusion assay

Anti-bacterial activity of the methanol extracts of five extracts against *S.mutans* was been evaluated using the disc diffusion method^{33,34}. Chlorohexidine 0.1% was 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 ($1-2 \times 10^8$ 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 by 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 < 10 mm³³.

Broth micro dilution assay MIC AND MBC

The quantitative assay of anti-bacterial activity of plants extracts was performed according to the reference method recommended by NCCLS³⁴. Sterility condition was maintained throughout experimental. Figure 3.1 summarizes the microdilution 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 *Dracaena 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. Tube 1 was filled with 30 μ l of stock solution of the test material (500mg/ml) with 1470 μ L nutrient broth to obtain a concentration of (10mg/ml). About 750 μ L of nutrient broth was dispensed in to the tube 2 to 6. Then 750 μ L of the solution in tube 1 was transferred to tube 2. This was repeated sequentially for the solutions in tube 2 to 6 in order to obtain intermediate concentrations ranging between 10 to 0.3125 mg/ml.

Aliquots (100 μ L) of each resultant solution then transferred into 96-well micro titer plate. Each well later filled with 100 μ L of bacterial suspension ($1-2 \times 10^8$ 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 microtiter was incubated for 24hr at 35°C. Four controls was used for the broth microdilution assay including:

- Negative control: 100 μ L of 2% MeOH in nutrient broth was mixed with 100 μ L of bacterial suspension in the well giving the concentration of the solvents not exceeding 1% as recommended by NCCLS³⁴.
- Growth control: 100 μ l of bacterial suspension was mixed with 100 μ L of nutrient broth.
- Positive control: 0.1% chlorhexidine was used to determine the sensitivity of tested bacteria.
- Sterility control (purity control): 200 μ L of nutrient broth alone was used to confirm the sterility of the broth.

Minimal inhibition concentration (MIC) of the tested material was determined visually as recommended by NCCLS³⁴. 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 $1.00 \leq MIC \leq 4.9$ mg/ml and weak if $MIC \geq 5.00$ mg/ml.

Antibacterial combination assay(Checkerboard assay)

In vitro antibacterial combination assay was performed to investigate the combined effect of tested plants with chlorohexidine against *S.mutans*. by using the checkerboard technique, as described, as described by Davidson and Parish³⁵. The assay involved multiple dilution 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 dilution 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 *Dracaena cinnabari* (1.25-0.02 mg/ml), *Commiphora myrrha* (2.5-0.04mg/ml), *Saussurea lappa* (0.625- 0.01mg/ml), *Salvia officinalis* (0.625-0.01mg/ml) and chlorohexidine (0.1-0.002 mg/ml) were dispensed in to the wells vertically down the 96-well microtitre plate and 50 μ l aliquots of each plants with the same previous concentration was dispensed horizontally. A 100 μ l suspension ($1-5$ CFU/ml) of *S. mutans* was added into each well. The final concentration in the reaction mixture ranged from (0.3-0.005 mg/ml) for *Dracaena cinnabari*, (0.625-0.01mg/ml) for *Commiphora myrrha*, (0.16-0.0025mg/ml) for *Saussurea lappa*, (0.16-0.0025mg/ml) for *Salvia officinalis* and (0.025-0.0005mg/ml) for chlorohexidine. 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 4.1. In general, the percentage yields of the *Boswellia carteril* extract was the highest among the five extracts, and the *Commiphora 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. Cowan³⁶ found that MeOH has a high efficiency in extracting most of non-polar and polar phytochemicals from plant materials.

Disc diffusion assay

The antibacterial activity of MeOH extracts from *Dracaena cinnabari*, *Boswellia carteril*, *Salvia officinalis*, *Commiphora myrrha* and *Saussurea lappa* was carried out using the disc diffusion method. All of MeOH extracts used in this study showing strong antibacterial activity against *mutans streptococci* with varying sizes of zone of inhibition figure 4.2. Also, The readings for the positive and negative controls were obtained. The results obtained are shown in figure 4.1. *Commiphora myrrha* had the highest inhibition diameter (36.6 ± 5.1 mm) followed by *Saussurea lappa* (35.6 ± 3.6 mm), the lowest inhibition zone diameter was with *Salvia officinalis* (21 ± 1.5 mm). No zone indicative of the lack of growth around the methanol which was used as negative control was observed 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 *Dracaena cinnabari*, *Saussurea lappa*, *Boswellia carteril*, *Commiphora myrrha* and *Salvia 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 bacterial pathogens in the mouth. Flavonoids also have anti-viral activity and play a vital role in the general health of a person.

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 Rattanakit³⁷ in which they investigated the inhibitory effect against *S. mutans in vitro* of the 95% ethanol extracts from five herbs, *Psidium guajava* L., *Momordica cochinchinensis* Spreng, *Glycyrrhiza glabra* L., *Syzygium aromaticum* L. and *Piper retrofractum* Vahl, 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 (Cinnamon, Turmeric, Ginger, Clove and Black seed) 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.

Commiphora myrrha and *Boswellia carterii* in our present study showed strong result in the disc diffusion assay against *S. mutans*, the inhibitory zones were 36 mm and 25 mm in diameter respectively, this result is higher than the results of previous studies that assessed the impact 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 *Boswellia frereana* and *Boswellia 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 ± 1mm) against *S. mutans*. In study by Izzeldien, *et al.*⁴¹ disk and well diffusion methods were used to test the effect of four concentration (100, 50, 25 and 12.5 mg/ml) of Myrrh volatile oil, extracted by hydro-distillation technique, the finding revealed that the four concentrations of oil were effective on *Streptococcus mutans* with the largest inhibition zone (18.7± 0.6 mm) through the well diffusion method and inhibition zone of (14.00 mm).

Salvia officinalis in our current study showed strong result against *S. mutant* with inhibitory zone (21±1.5 mm), this result were in agreement with the result of previous studies that tested antimicrobial activity of *Salvia officinalis* against *S. mutans* and is not in agreement with others. For example, Krumin *et al.*⁴² tested the impact 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 antimicrobial activity of ten medicine plants including *Salvia officinalis* against *S. mutans* and showed very weak antimicrobial activity of *Salvia officinalis* against tested bacteria with inhibitory zone (0.6 mm).

Saussurea lappa also in this study showed great strong result against *S. mutans* with inhibition zone (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 *Dracaena cinnabari* collected from Soqatra Island, Yemen, has antimicrobial activity our study is the first -to our knowledge- to investigate antimicrobial activity of *Dracaena cinnabari* against *S. mutans*. *Dracaena 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 *Dracaena cinnabari* on different types of bacteria, for example, Ansari, *et al.*⁴⁵ investigated the antimicrobial activity of the of *Dracaena cinnabari* resin on both antibiotic multi-resistant human pathogens and on poly-microbial culture, the results of this study showed that ethanolic extract of *Dracaena cinnabari* resin has a considerable antimicrobial 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 *Commiphora myrrha*, *Boswellia carterii*, *Salvia officinalis* and *Dracaena cinnabari* have more antibacterial properties than other strain in other countries.

Broth micro-dilution assay

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 4.2. The results of microdilution assay were consistent with the disc diffusion results confirming the antibacterial activity of used plants except for *Boswellia 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 *Boswellia sacra* did not show inhibition for *S. mutans* at the lowest concentration (5 mg/ml) used in their study while it exhibit anti-microbial 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 *Boswellia sacra* in our 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.

Antibacterial combination

The checkerboard technique was performed to investigate antibacterial combination of chlorhexidine with *Dracaena cinnabari*, *Salvia officinalis*, *Commiphora myrrha* and *Saussurea lappa* against *streptococcus mutans*. The combination of chlorhexidine with *Dracaena cinnabari*, *Commiphora myrrha* and *Saussurea lappa* showed synergistic effect as the FICI was less than 1. The combination of chlorhexidine with *Salvia officinalis* did not show any inhibition of bacteria. Table 4.3 and figure 4.3 show the MICs and FICI of the chlorhexidine and *Dracaena cinnabari*, *Commiphora myrrha* and *Saussurea lappa* combination against *streptococcus 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 (cinnamon, tea-tree, manuka, *Leptospermum morrisonii*, arnica, eucalyptus, grapefruit, the essential oil mouthrinse Cool Mint Listerine and two of its components alone and in combination with chlorhexidine gluconate against planktonic and biofilm cultures of *S. mutans* and *Lactobacillus* 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 cinnamon, manuka, *L. morrisonii*, thymol, and Listerine. In other 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 investigated and concluded that; the use of sub-MIC of chlorhexidine digluconate with sub-MIC of *L. japonica* and *R. officinalis* extracts resulted in synergistic inhibitory effects of these antibacterial agents except for chlorhexidine digluconate and *L. japonica* combination.

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 anticariogenic agents such as Toothpastes and mouthwash. The study also successfully evaluated the antibacterial combination of *Dracaena cinnabari*, *Commiphora myrrha*, and *Saussurea 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.

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TABLE 4.1: The percentage yield of the methanol extracts of plants

Plants	Yield %
<i>Saussurea lappa</i>	25.3
<i>Boswellia carteril</i>	44.7
<i>Commiphora myrrha</i>	6.4
<i>Salvia officinalis</i>	10.9
<i>Dracaena cinnabari</i>	unknown

Yield based on dry weight plant

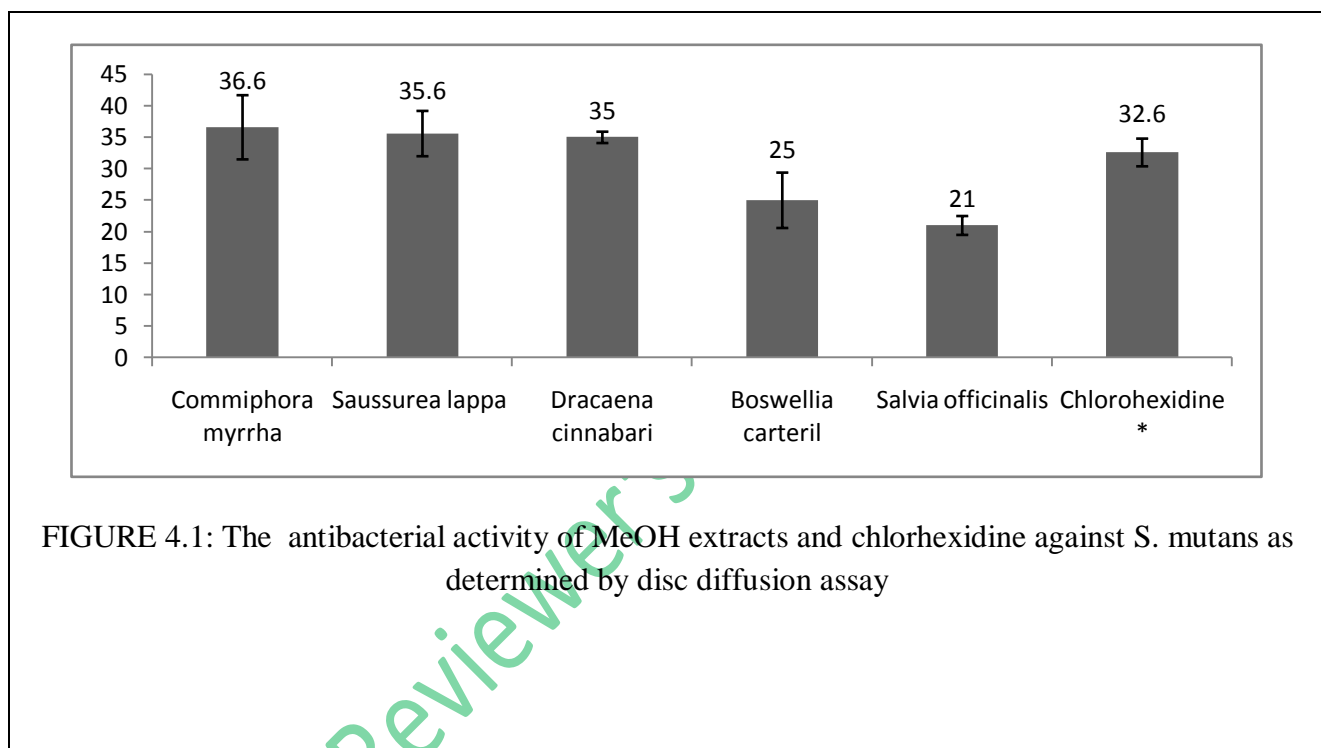


FIGURE 4.1: The antibacterial activity of MeOH extracts and chlorhexidine against *S. mutans* as determined by disc diffusion assay



FIGURE 4.2: Plates showing the inhibitory zone of MeOH extracts of plants

Cin = *Dracaena cinnabari*, -ve = negative control)MeOH(, chx = chlorhexidine, chlo = Chloramphenicol. myr = *Commiphora myrrha*, cos = *Saussurea lappa*, Sag = *Salvia officinalis*, fra = *Boswellia carteril*, against streptococcus mutans

TABLE 4.2: The minimum inhibitory concentration (MICs)(mg/ml) of methanol extracts against *S. mutans* as determined by broth microdilution assay.

Plant	MIC	MBC
<i>Dracaena cinnabari</i>	(0.3125)	(0.625)
<i>Commiphora myrrha</i>	(0.625)	(0.625)
<i>Salvia officinalis</i>	(0.156)	> (0.156)
<i>Saussurea lappa</i>	(0.156)	> (0.156)
<i>Boswellia carteril</i>	> 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 4.3 : Combination between chlorohexidine and *Darceana cinabari*, *Commiphora myrrha* and *Saussurea lappa*.

Plants	effect	FICI	Plants MIC (mg/ml)		Chlorohexidine MIC(mg/ml)	
			alone	combined	MIC alone	MIC combined
<i>Dracaena cinnabari</i>	Synergistic	0.131	0.313	0.002	0.02	0.002
<i>Commiphora myrrha</i>	Synergistic	0.43	0.63	0.006	0.08	0.006
<i>Saussurea lappa</i>	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

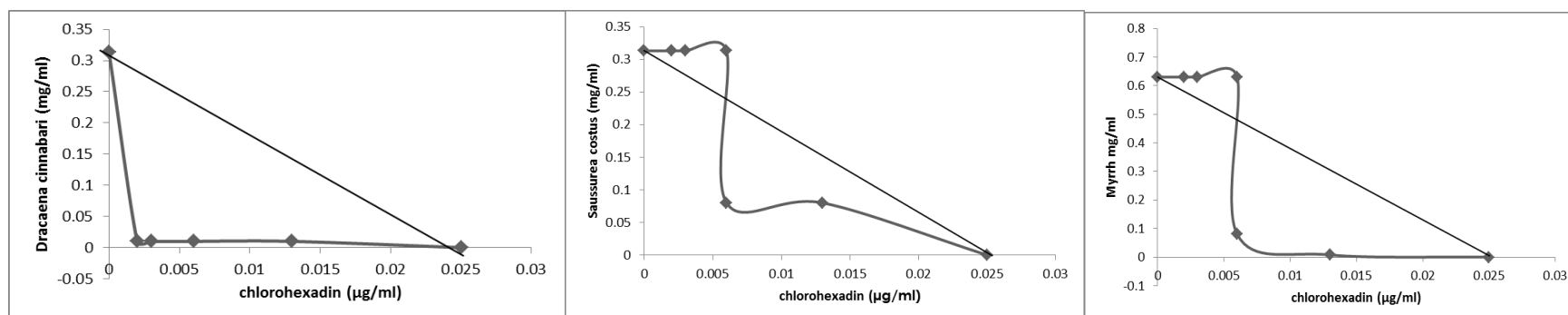


FIGURE 4.3: Isobologram showing the synergistic effect of the combining the chlorohexidine and *Darceana cinnabari*, *Saussurea lappa* and *Commiphora myrrha* against *S. mutans*