Impact of *Commiphora myrrha* on bacteria (*Streptococcus mutans* and *Lactobacillus* species) related to dental caries

Reem Izzeldien[1*], Sondos Abdulaziz[2], Ayat Ahmed[3], Zwaher Abu Elbashar[4], Omer Ibrahim[5], Mounkaila Noma[6]

1. Research Methodology and Biostatistics, University of Medical Sciences and Technology, Khartoum, Sudan. E-mail: Reeme_19@hotmail.com .

2. Department of Microbiology, Faculty of Medical Laboratory Sciences, University of Medical Sciences and Technology, Khartoum, Sudan. E-mail: Sondosabdlalaziz076@gmail.com .

3. Department of pharmacognosy, Faculty of Pharmacy, University of Medical Sciences and Technology, Khartoum, Sudan. E-mail: ayatwarag@yahoo.com .

4. Department of Diary Science and Technology, Sudan University of Science and Technology, Khartoum ,Sudan. E-mail: Zawahirabuelbashar@gmail.com .

5. Department of Diary Science and Technology, Sudan University of Science and Technology, Khartoum ,Sudan. E-mail: Omerhi30@yahoo.com .

6. Research Methodology and Biostatistics, University of Medical Sciences and Technology, Khartoum, Sudan. E-mail: mounkailanoma@gmail.com.

***Corresponding author**

E-mail: reeme_19@hotmail.com

Abstract

Dental caries is a chronic contagious disease caused by the interaction of oral microorganisms, diet and host factors over time. *Streptococcus mutans* is considered as the main bacteria involvs in dental decay, while the level of *Lactobacillus spp*. is directly related to the presence or onset of caries. *Commiphora myrrh* is an ancient plant which extracts are used as antiseptic and anti-inflammatory for mouth and throat due to it is antimicrobial activity. This study assessed the impact of *Commiphora myrrha* on two bacteria *Streptococcus mutans* and *Lactobacillus spp*. involved in dental caries. Three samples of *Streptococcus mutans* bacteria were collected randomly from patients with dental caries in Khartoum dental teaching hospital, while *Lactobacillus spp*. were obtained from fermented milk. 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 biochemical analysis of *Commiphora myrrha* oil was carried out using Gas Chromatography/Mass Spectrophotometric 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) with disc diffusion method regardless the two methods this inhibition zones were recorded at 100 mg/ml, with Minimum Bactericidal Concentration (MBC) at 3.125 mg/ml. While *Lactobacillus spp.* bacteria sensitive to three concentrations (100,50 and 25 mg/ml) and resistance to concentration 12.5 mg/ml, it is MBC found to be 25 mg/ml. In conclusion, this research revealed that Myrrh oil is effective on both *S.mutans* and *Lactobacillus spp*. Hence, Myrryh oil is a potential antibacterial product of interest in dental caries.

Key words: Commiphora myrrha, Dental carie, Lactobacillus spp, Streptococcus mutans.

Author summary

Dental caries is a chronic contagious disease caused by the interaction of oral microorganisms, diet and host factors over time. This study assessed the impact of *Commiphora myrrha* on two bacteria *Streptococcus mutans* and *Lactobacillus spp*. involved in dental caries. The research foud that Myrrh oil is effective on both *S.mutans* and *Lactobacillus spp*. Hence, Myrryh oil is a potential antibacterial product of interest in dental caries.

Introduction

Dental caries is a chronic contagious disease caused by the interaction of oral microorganism in dental plaque, diet and host factors (including teeth and saliva) over time, this result in localized destructions of hard tissues of teeth. Various factors includ social, environmental, genetic, biochemical, and immunologic factorsWHO declares that deprived oral health and it is related diseases may have dreadful effect on common health as well as eminence of life. Dental caries is a common and major public health oral disease which hampers the attainment and protection of oral health in different age groups [1,2].

Diet type play an important role in caries formation, as fermentable sugar and carbohydrates interact with the acidogenic bacteria in the mouth leading to acid formation which result in tooth decalcification and destruction of hard parts of the tooth [3,4]. *Streptococcus mutans*, one of the acidogenic bacteria, is the main bacteria responsible of dental decay, as indicated by epidimilogical studies which revealed that 74% to 100% of the bacteria associated with dental decay was *Streptococcus mutans*; *Lactobacillus* is another acidogenic bacteria which level had a direct relation with the presence or onset of caries [5].

Streptococcus mutan is a Gram-positive lactic acid bacterium which produces energy by glycolysis and metabolizes large amount of carbohydrates which is its characteristic cariogencity signature. *S.mutans* lives naturally in dental plaque with several other microorganisms; but the ability of *S.mutans* bacteria to adapt in environmental change is the key reason explaining that the bacteria is a major etiological agent of dental caries. Furthermore, it has the ability to synthesize large amount of extracellular polymer of glucan from sucrose.

Strains of *mutans* bacteria are classified based on the composition of cell-surface rhamose-glucose polysaccharide in four serological groups (c, e, f, k); it was reported 75 % of the bacteria on dental plaque are from group c [6].

Lactobacillus spp. Is an opportunistic Gram- positive bacilli bacteria that needs specific environmental requirement to sustain. This includes access to fermentable carbohydrate, anaerobic niches and low Ph environment as mostly found in dental lesion, stomach and vagina. This last is considered as one of the ways of transmitting the bacteria from mother to the child during birth. Another source of *lactobacilli* transmission is contaminated food and infected human [7].

Some plants are used since the Bible time for wound dressing, women health and beauty care. *Commiphora myrrha* is one of this ancient plant used as antiseptic and anti-inflammatory for mouth and throat. It is from genus *Commiphora* and *Burseraceae* family, which are found in southern Arabia, Somalia, Kenya and some Asian countries.

Myrrh composed of alcohol-soluble resin, water-soluble gum which contain polysaccharides and proteins, and volatile oil that composed of steroids, sterols and terpenes [9]. Myrrh volatile oil is extracted by distillation method (hydro-distillation). Furano-type compounds has been reported as major constituents; other components were also reported furanodiene, 19.7%, furanoeudesma-1,3-diene, 34.0%; and lindestrene, 12.0% as the major constituents of Ethiopian species [10].

Herbal extract is used in dental care as tooth cleansing, anti-inflammatory, antimicrobial agent, and analgesic. WHO stated that 80% of the world depend on herbal for their primary health care because of the safety and the low cost. *Commiphora myrrha* is used in dentistry for malodour and as anti-inflammatory in periodontitis as it promotes healing [11]. Twenty-three types of 100% natural essential oils were tested to assess their antimicrobial effects on oral strain using disk diffusion method in Korea [12]; The findings indicated that seventeen oils were effective on *S.mutans*, with myrrh, basil, and carrot seed which had a high antimicrobial activity. Of those 17 oils, myrrh had the highest antimicrobial activity (18.34 mm±0.26). Eighteen oils were found effective against *Porphyromonas gingivalis*; the high antimicrobial activity was obtained from tea tree, carrot seed, and cinnamons. Sixteen oils had high antimicrobial activity on *Lactobacillus rhamnosus*, these oils were from carrot seed and peppermint cinnamon.

The antibacterial efficacy of Chlorohexidine Gluconate (0.2%) and Saudi Myrrh (1g % w/v aqueous colloidal solution) mouthwashes were compared on oral microbial flora and other microorganisms on a sample of female participants. The nine microorganisms tested were *Escherichia coli* 25922, *Salmonella* 25566, *Klebsiella pneumonia* 13883, *Pseudomonas* 27853, *Proteus*, *Staphylococcus aureus* 25923, *Streptococcus pneumonia*, *Streptococcus mutans*, and *Candida albicans*. The results of the test revealed that Myrrh produced antimicrobial activity against *S.aureus*, *S.mutans* and *Candida albicans* and other microorganisms that comparable to Chlorohexidine gluconate [13]. The pharmaceutical formulations of mouthwashes, containing extracted Yemeni Myrrh as a single active constituentindicated that the hydro-alcohol extracted by ethanol (phosphate buffer pH 7 (85:15)) had a antimicrobial activity. Of 10 pharmaceutical formulations of Myrrh tinctures prepared and tested, two formulations, containing 9.5% (M9) and 10.5 % w/v (M10) of sodium lauryl sulphate had satisfactory results. In addition, the antimicrobial activity of the formulation M9 was higher to those of two commercial mouthwashes and one oral antifungal suspension [14]. Myrrh has also an antimicrobial activity; It is used for treatment of oral ulcers, gingivitis, sinusitis, glomerulonephritis, brucellosis and a variety of skin disorders [15]. Myrrh has a known anti-inflammatory effect proved by it is ability to supress interleukin 31(IL-31) mRNA and IL-31 protein expressions, production of IL-31cytokine and inhibits histamine production.. Our research aimed to assess the clinical impact of *Commiphora Myrrha* on *Streptococcus mutans*, and *Lactobacillus spp*. bacteria as casual agents of dental caries. and contribute in providing scientific-based evidence of the antibacterial effect of Myrrh.

Results

Yield percentage of *C.myrrha*

C.myrrha volatile oil yield was found 2 ml from 250 g of raw material using hydro-distillation method.

Identification of *S.mutans*

In *Mitis Slaivarius* agar colonies growth detected.

Microscopic examination revealed Gram-positive cocci in chains. That produced α-haemolysis in Muller Hinton agar supplemented with blood and resistant to Optochin disks "Fig 1"

Figure 1: Bacteria with α-haemolysis resistant to optochin disk in Muller Hinton agar supplemented with blood.

All 3 isolates fermented the four types of sugars (Glucose, Sucrose, Lactose and Mannitol). the fermentation was detected by the turning of the colour of phenol from red to yellow "Fig 2". Esculin and V.P tests were positive. the bacteria were catalase negative. As a final result all three isolated bacteria were identified as *Streptococcus mutans* bacteria.

Figure 2: Sugar fermentation tests (Glucose, Sucrose, Lactose, Mannitol)

Methods used in testing oil

Six bacteria were tested in using two methods. Disc diffusion method represented 66.7% (6/9) of the method and the remaining 33,3% (3/9) was Well method as revealed by "Table 1"

Table 1: Types of bacteria (*Streptococcus mutans* and *Lactobacillus*) and testing methods used

	Method of testing		
Bacteria	**Disc diffusion method**	**Well method**	**Total**
Streptococcus mutans bacteria 1	1	1	2
Streptococcus mutans bacteria 2	1	1	2
Streptococcus mutans bacteria 3	1	1	2
Lactobacillus bacteria 1	1	0	1
Lactobacillus bacteria 2	1	0	1
Lactobacillus bacteria 3	1	0	1
Total	**6**	**3**	**9**
%	66.7	33.3	

Inhibition zone of *S,mutans* was measured across four concentrations of oil using two methods as revealed by "Table 2" and "Fig 3"

In both disc diffusion method and well method the highest inhibition zones were recorded in 100 mg/ml concentration with respectively (14.0 mm) and (18.7 mm ± 0.6)

whereas *Lactobacillus ssp*. higher inhibition zone was obtained at 100 mg/ml concentration of *C.myrrha* oil of 10.00 mm ± 2.

Table 2: Inhibition zones of *S.mutans* and *Lactobacillus ssp*. across the four concentrations

	Myrrh volatile oil concentrations (mg/ml)			
Variables	**100**	**50**	**25**	**12.50**
Inhibition zone for *Streptococcus mutans* bacteria using disc diffusion method (n=3)				
Mean	14.0	12.7	11.7	10.7
Std. Deviation	0.0	0.6	0.6	0.6
Minimum	14.0	12.0	11.0	10.0
Maximum	14.0	13.0	12.0	11.0
Inhibition zone for *Streptococcus mutans* bacteria using well method(n=3)				
Mean	18.7	17.7	15.7	14.0
Std. Deviation	0.6	0.6	0.6	1.7
Minimum	18.0	17.0	15.0	12.0
Maximum	19.0	18.0	16.0	15.0
Inhibition zone for *Lactobacillus ssp*. using disc diffusion method (n=3)				
Mean	10.0	8.3	6.7	0.0
Std. Deviation	2.0	1.5	1.2	0.0
Minimum	8.0	7.0	6.0	0.0
Maximum	12.0	10.0	8.0	0.0

Figure 3 (A,B): Antibacterial activity of *C.myrrha* oil

(A) Inhibition zone against *S.mutans* in MH supplemented with blood using disc and well diffusion methods
(B) Inhibition zone against *Lactobacillus spp.* in MH using disc diffusion method.

Results of antibiotics

Antibiotics were tested against *S.mutans* and *Lactobacillus ssp*. As revealed by "Table 3" and "Fig 4"

Using sensitivity Discs *S.mutans* and *Lactobacillus ssp*. were tested with Ampicillin, *S.mutans* had inhibition zones of 17.7 mm ± 2.5 and *Lactobacillus ssp*. inhibition zone for Ampicillin was nil .

View the fact that *Lactobacillus ssp*. was resistant to Ampicillin it was tested with two other antibiotics namely Vancomycin and Ciprofloxacin.

Vancomycin had inhibition zones of 23.7 mm ± .2 followed by Ciprofloxacin with inhibition zone of 18.3 mm ±1.5.

Figure 4: Sensitivity of Antibiotics

(A) Ampicillin inhibition zone against *S.mutans* in MH supplemented with blood (B) *Lactobacillus spp.* resistant to Ampicillin in Nutrient Agar media. (C) Vancomycin and Ciprofloxacin inhibition zones against *Lactobacillus spp.* in Nutrient Agar media.

Table 3: Antibacterial activity of antibiotics against bacterial strains

	Inhibition zones measured in mm			
	Mean	**Std.**	**Min**	**Max**
Bacteria/Antibiotics				
Streptococcus mutans (n=3)				
Ampicillin	17.7	2.5	15.0	20.0
Lactobacillus ssp. (n=3)				
Ampicillin	0.0	0.0	0.0	0.0
Vancomycin	23.7	1.2	23.0	25.0
Ciprofloxacin	18.3	1.5	17.0	20.0

Determination of Minimum Bactericidal Concentration (MBC)

With the three concentrations of *C.myrrha* oil at 12.5, 6.25 and 3.125 mg/ml no growth was recorded across the three isolates. however, growth of each of three isolates was observed at *C.myrrha* oil concentration 1.56 mg/ml “Table 4”

In overall concentration of *C.myrrha* oil 3.125 mg/ml was considered as the (MBC).

Lactobacillus ssp. Was not tested with this procedure viewing that MBC was considered 25mg/ml as it was the last concentration that showed the activity against these isolates.

Table 4: Growth of bacteria with four concentrations, (N.G: no growth; G: growth of bacteria).

Streptococcus mutans bacteria	12.5mg/ml	6.25mg/ml	3.125mg/ml	1.56mg/ml
Bacteria1	N.G	N.G	N.G	G
Bacteria 2	N.G	N.G	N.G	G
Bacteria 3	N.G	N.G	N.G	G

Gas Chromatography Mass Spectrometry

The results of GC/MS revealed 48 compounds after comparing retention index and mass fragmentation patents of the oil component with those available in the library, the National Institute of Standards and Technology (NIST). The highest percentage compounds were; Benzofuran 29.13% with retention time (R.T) 14.922 followed by Cyclohexane 19.88% with R.T 12.986 and 1,3-Diphenyle-1,2-butanediol 15,17% with R.T 17.134 “Tables 5a and 5b”

Table 5a: Component of oil as analysed by GC-MS

SN	Name	Retention Time	Formula	Molecular weight	Retention index	Area%
1	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	4.125	$C_{10}H_{16}$	136	902	0.02
2	.alpha.-Pinene	4.244	$C_{10}H_{16}$	136	948	0.05
3	Camphene	4.495	$C_{10}H_{16}$	136	943	0.08
4	.beta.-Pinene	4.973	$C_{10}H_{16}$	136	943	0.02
5	D-Limonene	5.916	$C_{10}H_{16}$	136	1018	0.02
6	Bicyclo[2.2.1]heptane, 2-methoxy-1,7,7-trimethyl-	7.546	$C_{11}H_{20}O$	168	1087	0.02
7	(+)-2-Bornanone	8.237	$C_{10}H_{16}O$	152	1121	0.01
8	1,5-Cyclooctadiene, 3,4-dimethyl-	10.247	$C_{10}H_{16}$	136	1046	0.03
9	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	10.97	$C_{12}H_{20}O_2$	196	1277	0.11
10	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-	11.929	$C_{15}H_{24}$	204	1377	4.56
11	.alpha.-Cubebene	12.154	$C_{15}H_{24}$	204	1344	0.24
12	.gamma.-Muurolene	12.42	$C_{15}H_{24}$	204	1435	0.04
13	.alpha.-ylangene	12.587	$C_{15}H_{24}$	204	1221	0.09
14	Copaene	12.67	$C_{15}H_{24}$	204	1221	0.37
15	(-)-.beta.-Bourbonene	12.851	$C_{15}H_{24}$	204	1339	1.78
16	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-	12.986	$C_{15}H_{24}$	204	1398	19.88
17	Caryophyllene	13.5	$C_{15}H_{24}$	204	1494	1.76
18	.gamma.-Elemene	13.571	$C_{15}H_{24}$	204	1431	0.14
19	.beta.-copaene	13.671	$C_{15}H_{24}$	204	1216	0.25
20	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethylidene)-, (E,E)-	13.721	$C_{15}H_{24}$	204	1603	6.76
21	.beta.-ylangene	13.944	$C_{15}H_{24}$	204	1216	0.23
22	Humulene	14.122	$C_{15}H_{24}$	204	1579	0.58
23	Alloaromadendrene	14.253	$C_{15}H_{24}$	204	1386	0.15
24	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [S-(E,E)]-	14.353	$C_{15}H_{24}$	204	1515	0.05
25	4,5-di-epi-aristolochene	14.401	$C_{15}H_{24}$	204	1474	0.09
26	Guaia-1(10),11-diene	14.508	$C_{15}H_{24}$	204	1490	1
27	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,	14.611	$C_{15}H_{24}$	204	1339	1.43
28	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	14.715	$C_{15}H_{24}$	204	1469	2.48
29	Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans-	14.922	$C_{15}H_{20}O$	216	1532	29.13

Table 5b: Component of oil as analysed by GC-MS

SN	Name	Retention Time	Formula	Molecular weight	Retention index	Area%
30	1,4-Methano-1H-indene, octahydro-4-methyl-8-methylene-7-(1-methylethyl)-, [1S-(1.alpha.,3a.beta.,4.alpha.,7.alpha.,7a.beta.)]-	15.057	$C_{15}H_{24}$	204	1339	0.27
31	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-	15.187	$C_{15}H_{24}$	204	1435	0.57
32	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	15.324	$C_{15}H_{24}$	204	1469	0.57
33	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.alpha.)]-	15.56	$C_{15}H_{24}$	204	1474	0.54
34	Selina-3,7(11)-diene	15.67	$C_{15}H_{24}$	204	1507	0.5
35	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,3a.beta.,4.alpha.,7.beta.)]-	15.947	$C_{15}H_{24}$	204	1461	1.31
36	Carbamic acid, p-tolyl ester	16.318	$C_9H_{11}NO_2$	165	1372	0.37
37	Caryophyllene oxide	16.415	$C_{15}H_{24}O$	220	1507	0.11
38	Cyclohexanone, 5-ethenyl-5-methyl-4-(1-methylethenyl)-2-(1-methylethylidene)-, cis-	16.741	$C_{15}H_{22}O$	218	1602	0.13
39	cis-Z-.alpha.-Bisabolene epoxide	16.855	$C_{15}H_{24}O$	220	1531	0.07
40	1,3-Diphenyl-1,2-butanediol	17.134	$C_{16}H1_8O_2$	242	2025	15.17
41	4,4'-Dimethyl-2,2'-dimethylenebicyclohexyl-3,3'-diene	17.244	$C_{16}H_{22}$	214	1618	5.88
42	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	17.835	$C_{15}H_{26}O$	222	1522	0.62
43	Spiro[2.4]heptane, 1,2,4,5-tetramethyl-6-methylene-	18.095	$C_{12}H_{22}$	164	1065	0.44
44	Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl-	18.294	$C_{15}H_{24}$	204	1518	0.37
45	Bicyclo[3.1.1]hept-2-ene-2-ethanol, 6,6-dimethyl-, (1R)-	18.56	$C_{11}H_{18}O$	166	1290	0.83
46	Androsta-3,5-dien-7-one	19.902	$C_{19}H_{26}O$	270	1933	0.4
47	Acetic acid, 6-(1-hydroxymethyl-vinyl)-4,8a-dimethyl-3-oxo-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester	20.871	$C_{17}H_{24}O_4$	292	2244	0.27
48	.alpha.-Bulnesene	21.362	$C_{15}H_{24}$	204	1490	0.21

Discussion

Natural plant has been used more commonly as medications due to it is availability and safety and as the resistance to antibiotics drugs is successively increase, and as *C.myrrha* has antibacterial and anti-inflammatory agents it has been used in oral health to treat periodontitis and malodour [11,12].

S.mutans and *Lactobacillus ssp*. bacteria responsible in formation and progression of dental caries ,were tested with volatile oil of *C.myrrha* with different methods as antibacterial [5]. In our study *C.myrrha* oil was effective at all concentrations levels tested for *S.mutans* bacteria with MBC 3.125mg/ml while *Lactobacillus ssp*. did not show activity at concentration 12.5 mg/ml.

A Study [12] using standard organisms and disc diffusion method revealed results of oil activity against *S.mutans* and *Lactobacillus rhamnosus* in line with ours with respectively 18.34 mm±0.26 and 11.72 mm±0.85. Higher results were recorded in a comparative study testing two different formula of Myrrh mouth wash (containing 65ml of Myrrh extract or each 100 ml) with another ingredients, on *Streptococcus mutans* isolates. Their findings for the two formula were 32.367 mm ± 0.262 and 22.367 mm ± 0.102 [14].

In our study, Ampicillin antibiotic inhibition zone of 17.7 mm ±2.5 was comparable to the one of myrrh oil against *S.mutans* using same disc method. This indicates that with antibiotic resistance increasing rapidly Myrrh can be used as alternative and effective antibacterial agent under condition that further investigations are conducted. Furthermore, *Lactobacillus ssp.* was completely resistant to Ampicillin while Myrrh was effective at the three concentrations of respectively 100,50, and 25 mg/ml. Vancomycin was more effective than Ciprofloxacin with inhibition zones of respectively 23.7 mm ±1.2 and 18.3 mm ±1.5.

Sesquiterpenes and furanotype, are major constituents of *C.myrrha*, have antimicrobial activity leading them to have therapeutic effect [10,14,16]. The highest percentage compound in our result is Benzofuran 29.13%, which have antimicrobial activity, this results in line with a study which revealed that benzofuran constitutes 26.63% of *C.myrrha* oil analysed by GC-MS technique [16], and higher than another study which indicated that Curzerene (Benzofuran) was 11.9% [9].

Caryophyllene $C_{15}H_{24}$ considered as anti-tumor, antibacterial, anti-inflammatory represents 1.76% of constituents higher than a study which revealed 0.29% [16].

Other important components of myrrh oil in our study, measured through R.T 12.986 and R.T 17.134. ,were Cyclohexane (19.88 %) and 1,3-Diphenyle-1,2-butanediol (15.17%).

Myrrh oil was effective on both *S.mutans* and *Lactobacillus ssp*. with a higher activity on *S.mutans.* The two bacteria are involved in dental caries; hence Myrrh oil is a potential antibacterial product which needs to be recognized as such by further multi-centre studies as it can be affordable at low cost for oral health to control caries as a public health challenge.

Materials and methods

A quasi experimental study was implemented from August 2019 to February 2020.

the swaps were obtained from outpatients of Khartoum dental teaching hospital. Isolates of lactobacillus bacteria were received from Sudan University of Science and Technology. The Laboratory of microbiology department of the Faculty of Medical Laboratory Sciences of the University of Medical Sciences and Technology was used to performed the laboratory tests.

Commiphora myrrha plant

Plant sample was collected from local shop in Omdurman traditional market. Taxonomical authentication was made by Sudanese taxonomist at herbarium of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTRI), (Dr. Yahya Sulieman Mohamed taxonomist). the botanical name was identified : *Commiphora Myrrha (Nees)Eng* .and Vernacular name of the plant known as Murr EL Hegazy; it belongs to the Family of Burseraceae “Fig 5”

Figure 5: Myrrh plant (oleogum resin part)

Preparation and extraction of Myrrh oil. The volatile oil extracted by hydro-distillation technique [9] this was done in Environmental and Natural Resources and Desertification Research Institute (ENDRI), Khartoum.

The sample was cleaned and crushed into small pieces, about 250 grams weighted and introduced into 1,5 litres of distilled water into glass flask. The content of flask boiled for 6 h till distillation of the volatile oil, the volatile oil transferred into calendrical bottle by pipette. Sodium sulfate added to the bottle to absorbed the water as displayed by “Fig 6”

The oil yield was calculated by volume and transferred into another glass bottle ready for analysis.

Figure 6: Myrrh resin volatile oil separated from water by sodium sulfate

Bacterial strains

Lactobacillus spp. bacteria were obtained from Department of Diary Science and Technology of the Faculty of Animal Production, Sudan University of Science and Technology. The bacteria were isolated from fermented milk and the department availed to us three isolates.

Streptococcus mutans isolated from dental caries.Samples were collected randomly in Khartoum Dental Teaching Hospital from patients with dental caries, sterile cotton swabs were used to take the swabs from inside carious teeth, the swabs were transported in Amies transport medium till delivery to the laboratory.

Identification of *S.mutans* bacteria

The caries swabs were inoculated on sterile Mitis Salivaries Agar plates. The plates incubated anaerobically at 37□ overnight, microscopic identification made then subculture in Muller Hinton Agar supplemented in blood plates, for further identification biochemical test applied [17].

Microscopical examination

Gram stain method used prior the microscopical examination ,the bacteria specimen were fixed in slide using heat then kept to dry for 1 minute, crystal violet stain applied for 1 minute then the slide washed with water for 2 seconds, iodine solution was applied for 1 minute and washed for 2 sec, for decolorization the slide flood with alcohol for 10 seconds and washed with water then safranin stain for 1 min then washed with gentle indirect tap water. The slides were put in absorbent paper to be dried.

The results of the staining procedure were observed under oil immersion (100x) using a bright light microscope.

Biochemical tests for identification of isolates

Antibiotic sensitivity test. Optochin discs were used to test sensitivity of isolates [17].

Sugar fermentation tests. Sugar fermentation tests and Esculin hydrolysis are commonly tests used for identification and confirmation of bacteria [18].

Phenol red indicator preparation. About 0.25 gram of phenol red powder added to 10 ml of methanol and 10 ml distilled water and mixed.

Sucrose. About 1 gram of sucrose sugar was dissolved in 100 ml of peptone water, then 1 ml of phenol red was added to sugar. The bacteria were put to test tube from the media and incubated for 24 h at 37 □ then the colour changed were observed.

Glucose. About 1 gram of Glucose sugar was dissolved in 100 ml of peptone water, then 1 ml of phenol red was added to sugar. The bacteria were put to test tube from the media and incubated for 24 h at 37 □ then the colour changed was observed.

Mannitol. About 1 gram of Mannitol sugar was dissolved in 100 ml of peptone water, then 1 ml of phenol red was added to sugar. The bacteria were put to test tube from the media and incubated for 24 h at 37 □ then the colour changed was observed.

Lactose. About 1 gram of Lactose sugar was dissolved in 100 ml of peptone water, then 1 ml of phenol red was added to sugar. The bacteria were put to test tube from the media and incubated for 24 h at 37 □ then the colour changed was observed.

If colour changed to yellow = positive results

If colour remain red = negative results

Esculin test. The surface of aesculin agar bacteria inoculated using sterile loop and incubated at 37 °C for 24 h development of black colour indicates positive result.

Voges Proskauer (VP)test. Bacteria inoculated in Methyl red-Voges-Proskauer broth and incubated for 24 h at 37°C, after that 1 ml of 40% KOH and 3 ml of 5% solution of α-naphthol were added. A positive reaction indicated by development of eosin-pink colour.

Catalase test. Test used to differentiate bacteria produce enzyme catalase such as *Staphylococci* from non-catalase producing such as *Streptococci*.

In sterile glass slide drops of hydrogen peroxide H_2O_2 added, using sterile wooden stick several colonies were removed and immersed in, then immediate bubble production observed.

Activity of the Myrrh oil

Activity of Myrrh against isolated pathogens was tested by using four different concentrations 100, 50, 25, 12.5 mg/ml prepared by serial dilution method from stock of 0.1 gm/1 ml.

Two different methods were used to detect antibacterial activity: (i) the disc diffusion method and (ii) the well diffusion method.

Disc diffusion method

The antibacterial activity of plant extract was determined using the disc diffusion method which was described elsewhere [18].

Wattman filter paper No.1 was cut into small round discs (6 mm) and sterilized in autoclave at 121□ for 15 min.

Pure bacterial culture was suspended in sterile normal saline according to standard procedures, then swabbed uniformly with sterile cotton swab in the surface of the agar plates. After dryness of the plate the disc of the four concentration were placed on the surface of the agar and gently pressed with sterilized forceps.

Different antibiotics (Ampicillin 10 mcg, vancomycin 30 mcg, ciprofloxacin 5 mcg) were used as control positive against tested bacteria, while DMSO was used as control negative. All the plates incubated anaerobically in at 37□ for 24 h and the zone of inhibition (mm) was measured.

Well diffusion method

The second antibacterial activity of plant extract was determined using Well diffusion method described in the literature [19].

After swapping the bacteria uniformly in the surface of the agar plate a hole with diameter of (6 mm) was made using sterile tip, different well introduced with 20 µl of different oil concentration. The plate incubated anaerobically at 37 □ for 24 h. the zone around each well observed and recorded in millimetre.

Determination of minimum bactericidal concentration (MBC)

The lowest concentration of each antimicrobial agent that inhibits the growth of the microorganisms being tested is known as minimum inhibitory concentration (MIC) and is detected by lack of turbidity matching with a negative control. Furthermore, the minimum bactericidal concentration (MBC) is defined as the lowest concentration of an agent killing the majority of bacterial inoculums [20].

To determine the MBC of *C.myrrha* oil four concentrations were prepared by serial dilution starting by stoke oil concentration (12.5 mg/ml).

Each tube seeded with bacteria prepared according to Mcfarland 0.5 turbidity standard and incubated in 37□ for 24 h **"**Fig **7**A"

After 24 h, each tube swabbed in plate of Muller Hinton agar suspended in blood using sterile cotton swab and incubated anaerobically for 24 h in 37□, results were recorded to observe the growth of bacterial colonies "Fig 7B"

Figure 7: determination of MBC (A) serial dilution of myrrh oil in four tubes seeded with the bacteria and (B) four concentrations swabbed in Muller Hinton agar supplemented with blood media after 24 h.

Gas Chromatography Mass Spectrometry (GC/MS)

The qualitative and quantitative analysis of the oil sample was carried by using GC/MS technique model (GC/MS-QP2010-Ultra) from Japan, Simadzu company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25mm). the sample was injected by using split mode, instrument operating in EI mode at 70 eV. Helium as the carrier gas passed with flow rate 1.69 ml/min, the temperature program was started from 50□ with rate 7□/min to 180□ then the rate change to 10□/min reaching 280□ as final temperature degree , the injection port temperature was 300□, the ion source temperature was 200□ and the interface temperature was 250□ .

The sample was analysed by using scan mode in the range of m/z 40-500 charges to ratio and the total run times was 28 minutes.

Identification of component for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST), results were recorded.

Acknowledgments

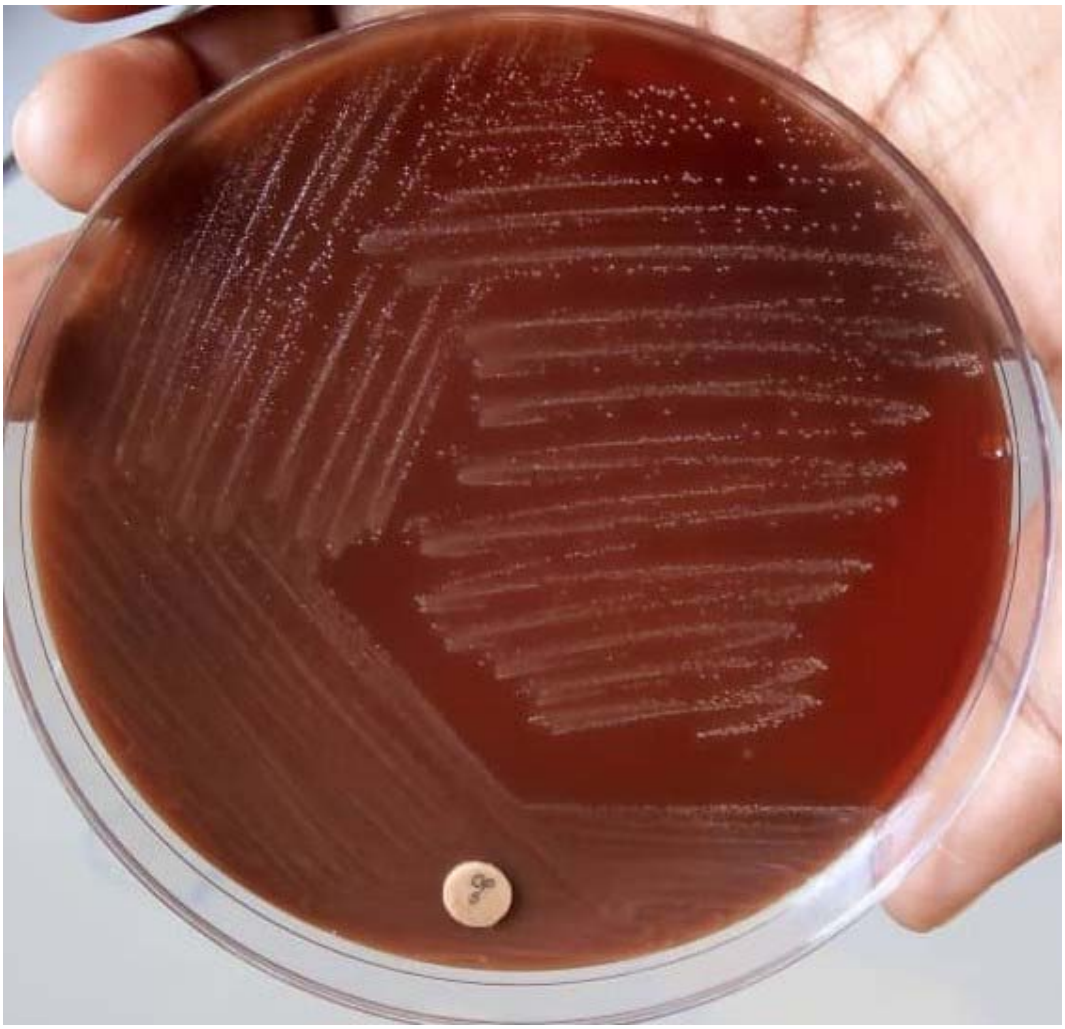
Special thanks to Afraa M.Alhaj, Department of Microbiology, Faculty of Medical Laboratory Sciences, University of Medical Sciences and Technology . for facilitating laboratory experiments.

Special thanks to the faculty of Medical Laboratory Sciences, University of Medical Sciences and Technology in which I process this study, for their support.

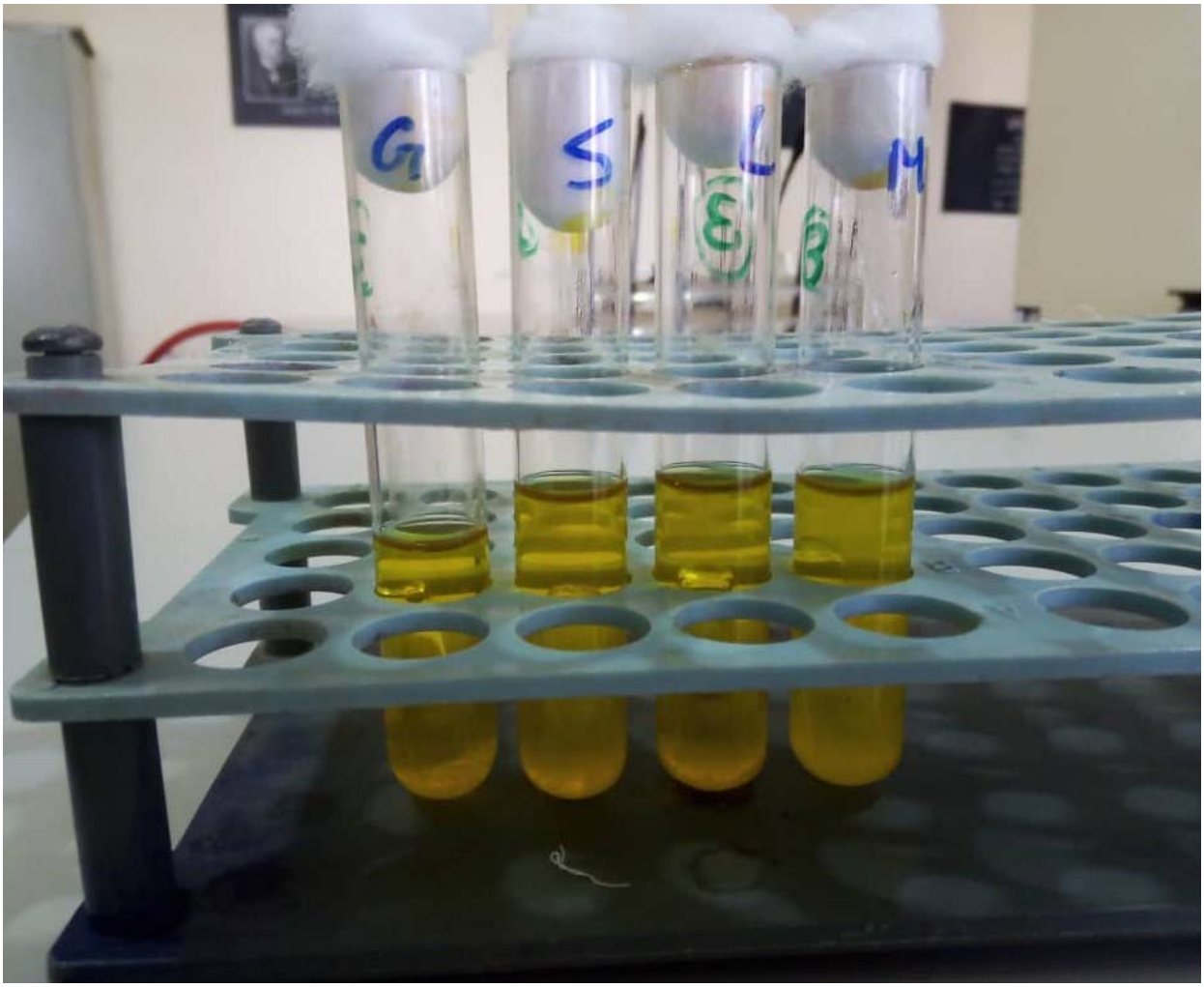
Our greatest thanks to Khartoum Dental Teaching hospital and Sudan University of Sciences and Technology, Department of Diary Science and Technology for facilitating access to the study materials.

References

1. Poul Erik Petersen et al. The global burden of oral diseases and risks to oral health. Bulletin of the World Health Organization, September 2005, 83 (9), p:661-669.
2. Yu OY, Zhao IS, Mei ML, Lo EC-M, Chu C-H. Dental Biofilm and Laboratory Microbial Culture Models for Cariology Research. *Dentistry Journal*. 2017; 5(2):21.
3. Boucher GO. Dental Caries. California medicine. 1951 Feb;74(2):128..
4. Tafere Y, Chanie S, Dessie T, Gedamu H. Assessment of prevalence of dental caries and the associated factors among patients attending dental clinic in Debre Tabor general hospital: a hospital-based cross-sectional study. BMC oral health. 2018 Dec;18(1):119.
5. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiological reviews. 1986 Dec;50(4):353.
6. Lemos JA, Palmer SR, Zeng L, et al. The Biology of Streptococcus mutans. Microbiol Spectr. 2019;7(1):10.
7. Caufield PW, Schön CN, Saraithong P, Li Y, Argimón S. Oral lactobacilli and dental caries: a model for niche adaptation in humans. Journal of dental research. 2015 Sep;94(9_suppl):110S-8S.
8. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environmental health perspectives. 2001 Mar;109(suppl 1):69-75.
9. Hanuš LO, Řezanka T, Dembitsky VM, Moussaieff A. Myrrh-commiphora chemistry. Biomedical papers. 2005 Jul 1;149(1):3-28.
10. De Rapper S, Van Vuuren SF, Kamatou GP, Viljoen AM, Dagne E. The additive and synergistic antimicrobial effects of select frankincense and myrrh oils–a combination from the pharaonic pharmacopoeia. Letters in applied microbiology. 2012 Apr;54(4):352-8.
11. Kumar, Gunjan et al. “Emerging trends of herbal care in dentistry.” Journal of clinical and diagnostic research : JCDR vol. 7,8 (2013): 1827-9. doi:10.7860/JCDR/2013/6339.3282
12. Park C., Yoon H. Antimicrobial Activity of Essential Oil against Oral Strain. Int J Clin Prev Dent 2018;14(4):216-221. https://doi.org/10.15236/ijcpd.2018.14.4.216.
13. Sambawa Z. M. et al. Comparison of Antibacterial Efficacy Chlorohexidine Gluconate and Saudi Myrrh Mouthwashes in the Oral Cavity. Oriental Journal of Chemistry, 2016, Vol. 32, No. (5): P. 2605-2610.
14. Almekhlafi S,Thabit AA, Alwossabi AM, Awadth N, Thabet AA, Algaadari Z. Antimicrobial activity of Yemeni myrrh mouthwash. J Chem Pharm Res. 2014;6(5):1006-13.
15. Shin JY, Che DN, Cho BO, Kang HJ, Kim J, Jang SI. Commiphora myrrha inhibits itch-associated histamine and IL-31 production in stimulated mast cells. Experimental and therapeutic medicine. 2019 Sep 1;18(3):1914-20.
16. Gadir Suad , Ahmed Ibtisam. Commiphora myrrha and commiphora Africana essential oils. Journal of Chemical and Pharmaceutical Research. 2014. 6. 151-156.
17. Al-Jumaily E, AL-Seubehawy HM, Al-Toraihy FA. Isolation and Identification of Streptococcus mutans (H5) produced glucosyltransferase and cell-associated glucosyltransferase isolated from dental caries. Int. J. Curr. Microbiol. App. Sci. 2014;3(6):850-64.
18. John Gerald Collee.,Mackie and Mccartney Practical Medical Microbiology. Elsevier ;1996.
19. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of pharmaceutical analysis. 2016 Apr 1;6(2):71-9.
20. Ahrari F, Eslami N, Rajabi O, Ghazvini K, Barati S. The antimicrobial sensitivity of Streptococcus mutans and Streptococcus sangius to colloidal solutions of different nanoparticles applied as mouthwashes. Dental research journal. 2015 Jan;12(1):44.

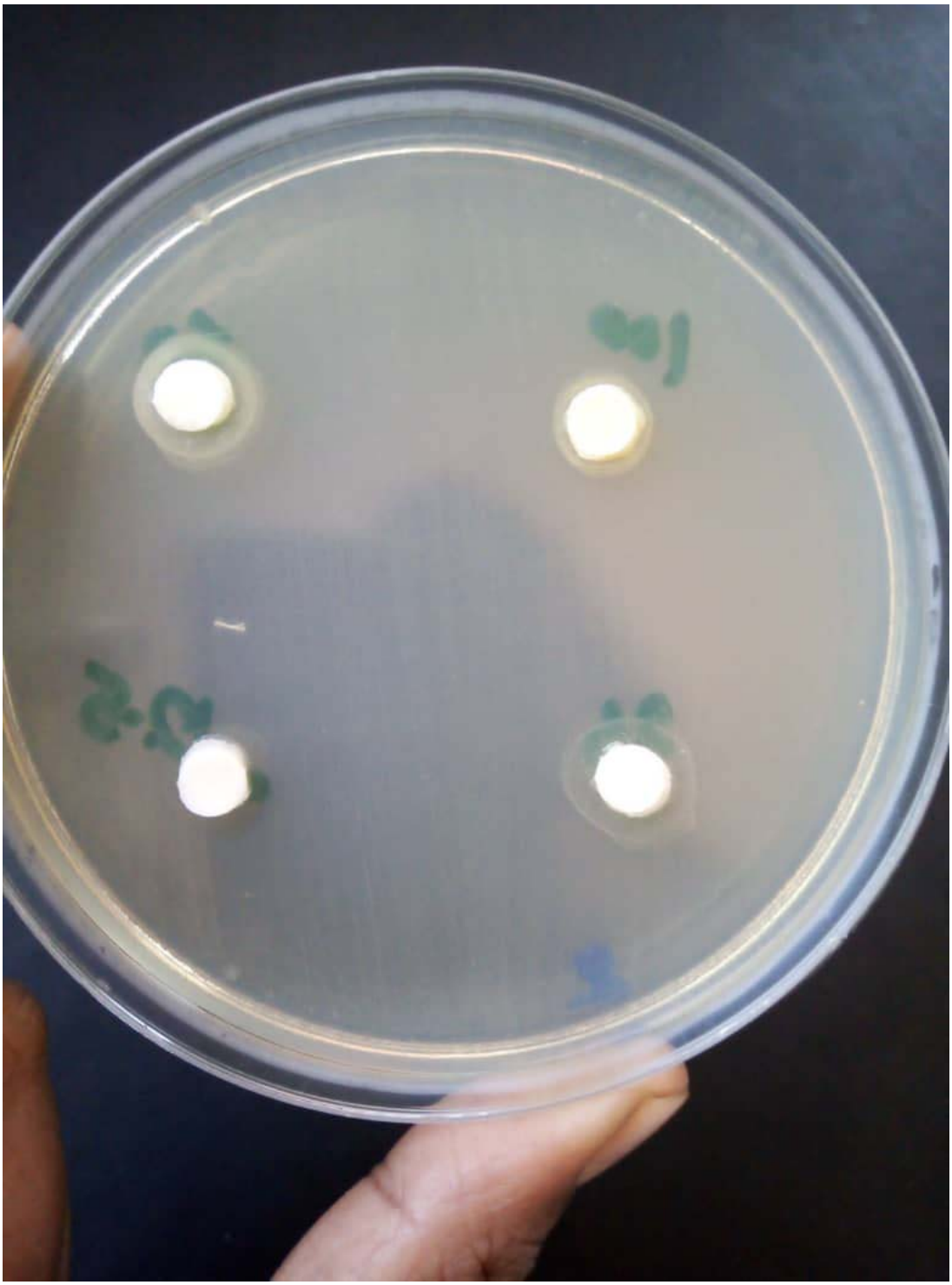


S1 Figure 1: Bacteria with α-haemolysis resistant to optochin disk in Muller Hinton agar supplemented with blood.

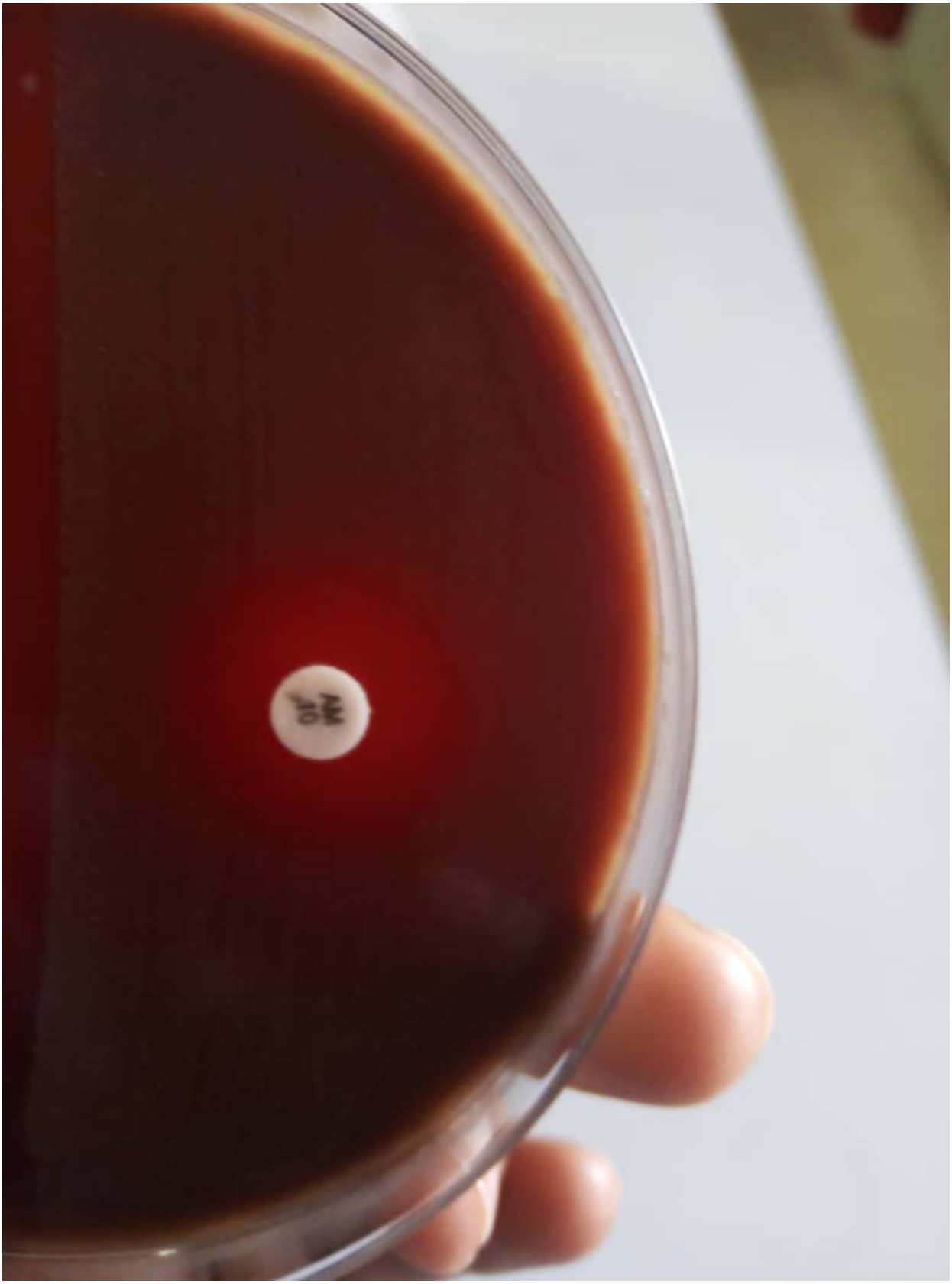


S2 Figure 2: Sugar fermentation tests (Glucose, Sucrose, Lactose, Mannitol)

S3 Figure 3 (A): Antibacterial activity of *C.myrrha* oil. Inhibition zone against *S.mutans* in MH supplemented with blood using disc and well diffusion methods

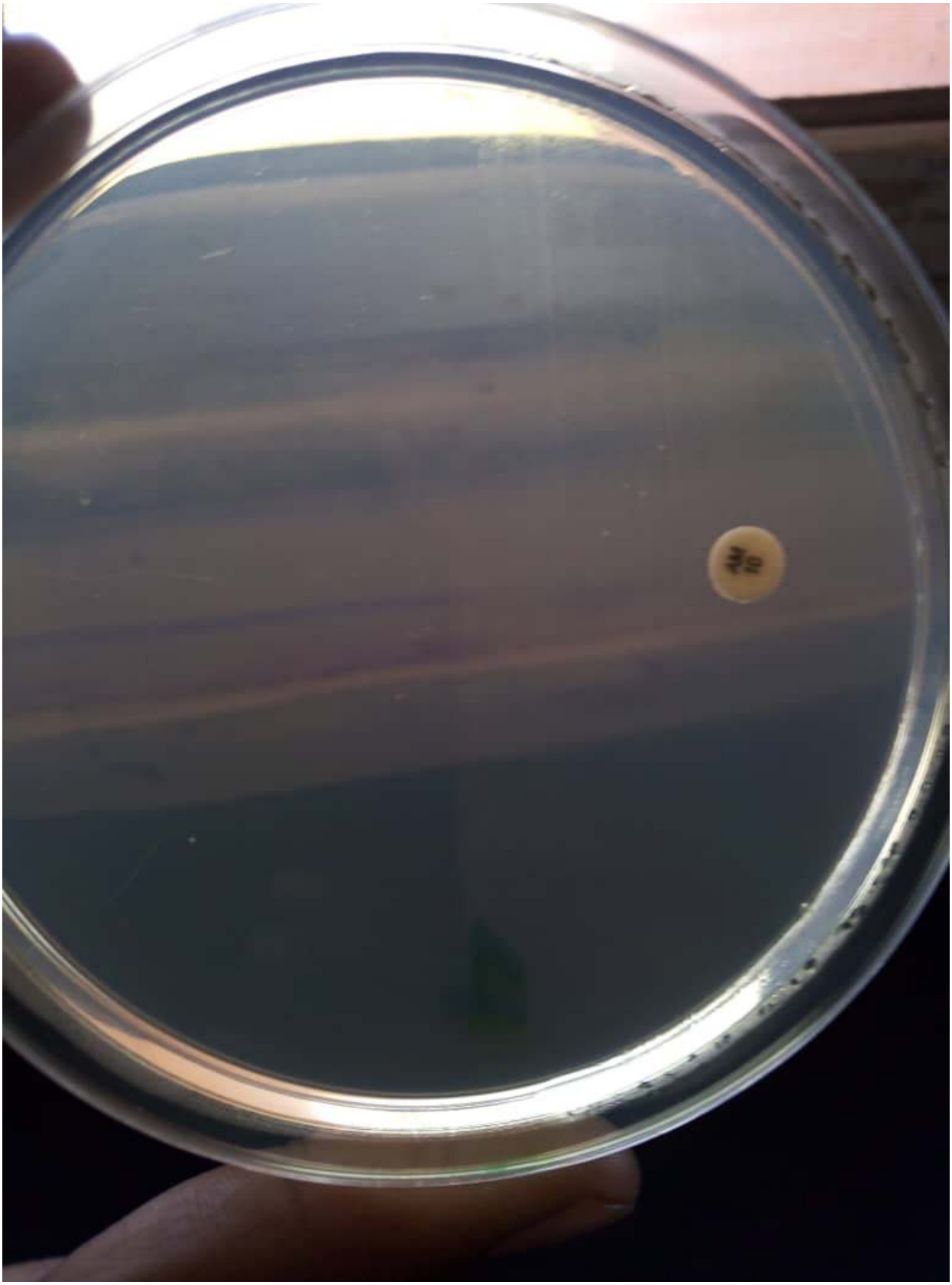


S3 Figure 3 (B): Antibacterial activity of *C.myrrha* oil. Inhibition zone against *Lactobacillus spp.* in MH using disc diffusion method.



S4 Figure 4 (A): Sensitivity of Antibiotics

(A) Ampicillin inhibition zone against *S.mutans* in MH supplemented with blood .

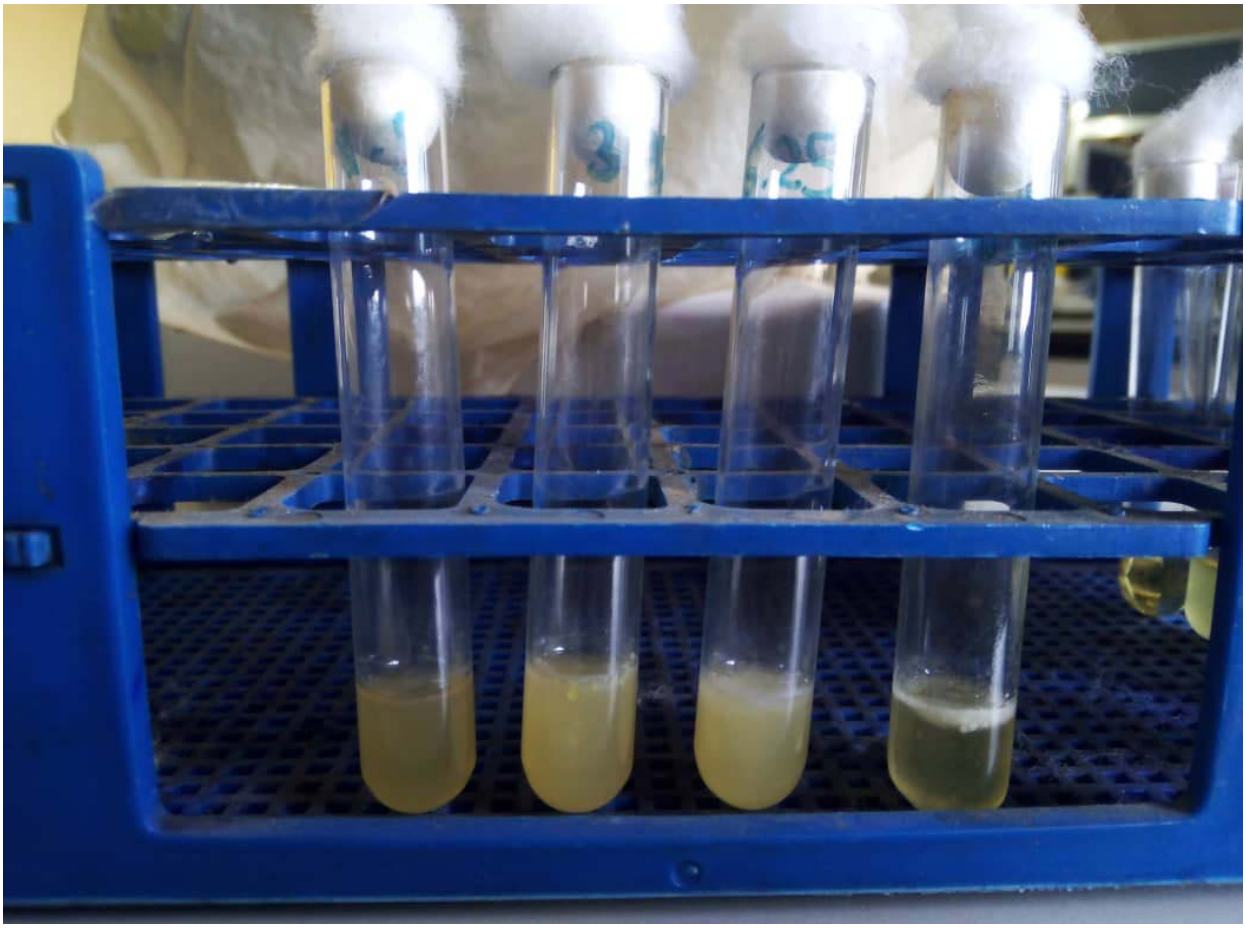


S4 Figure 4 (B): ***Lactobacillus spp.*** **resistant to Ampicillin in Nutrient Agar media.**

S4 Figure 4 (C): Vancomycin and Ciprofloxacin inhibition zones against *Lactobacillus spp.* in Nutrient Agar media.

S5 Figure 5: Myrrh plant (oleogum resin part)

S6 Figure 6: Myrrh resin volatile oil separated from water by sodium sulfate.



S7 Figure 7 (A): determination of MBC serial dilution of myrrh oil in four tubes seeded with the bacteria .

S7 Figure 7 (B): four concentrations swabbed in Muller Hinton agar supplemented with blood media after 24 h.