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Impact of *Commiphora myrrha* on Bacteria (*Streptococcus mutans* and *Lactobacillus spp.*) Related to Dental Caries

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Abstract: *Commiphora myrrha*, is an ancient plant which exhibits an antiseptic and anti-inflammatory effect for mouth and it has been traditionally used for its antimicrobial properties in oral health care. This study assessed the impact of *C. 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*. isolates were obtained from fermented milk. Disc and well diffusion methods were used to test the effect of four concentrations (100, 50, 25 and 12.5 mg/ml) of Myrrh volatile oil, extracted by hydro-distillation technique. Ampicillin, Vancomycin and Ciprofloxacin were used as control positive. The phytochemical analysis of *C. myrrha* oil was carried out using Gas Chromatography Mass Spectroscopy technique. The finding of this study revealed that the four concentrations of essential 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 with disc diffusion method, regardless the two methods; the inhibition zones were recorded at 100 mg/ml, with Minimum Bactericidal Concentration (MBC) at 3.125 mg/ml. On the other hand, *Lactobacillus spp*. bacteria was found to be sensitive to three concentrations of oil (100, 50 and 25 mg/ml) and resistance to lowest concentration (12.5 mg/ml), the MBC found to be 25 mg/ml. The results of GC-MS revealed the presence of 48 compounds, the highest percentage were; Benzofuran (29.13%) followed by Cyclohexane (19.88%) and 1,3-Diphenyle-1,2-butanediol (15.17%) respectively. Myrrh oil is effective on both *S. mutans* and *Lactobacillus spp*. Hence, it is a potential antibacterial product of interest in dental caries.

Keywords: Myrrh, Essential Oil, Cavities, *Streptococcus mutans*, *Lactobacillus spp.*

1. Introduction

Dental caries is a common and major public health oral disease which hampers the attainment and protection of oral health in different age groups, it is a chronic 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 destruction of hard tissues of teeth. WHO declare that deprived oral health and it is related diseases may have dreadful effect on common health as well as eminence of life [1, 2]. Dental caries disease can lead to deadly consequences ranging from simple abscess to chronic facial space infections. “Oral health condition have an impact on the quality of life of the affected, whereas treatment is costly for individual, communities and countries” as declared by regional Director of WHO in Africa when launching oral health strategy 2016-2025. 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 epidemiological studies which revealed that 74% to 100% of the bacteria associated with dental decay were *Streptococcus mutans* [5], it produces energy by glycolysis and metabolizes large amount of carbohydrates which is its characteristic cariogenicity 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 [6]. *Lactobacillus* is other bacteria which had a direct relation with the presence or of caries, it is an opportunistic Gram-positive bacilli 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. Another source of *Lactobacilli* transmission is contaminated food and infected human [7]. Some plants are used since the ancient time for wound dressing, women health and beauty care. *Commiphora myrrha* Belongs to family Burseraceae, it is used as antiseptic and anti-inflammatory for mouth and throat. It is commonly known as myrrh, it has been traditionally used for its antimicrobial properties in oral health care [8]. The plant distributed in southern Arabia, Somalia, Kenya and some Asian countries. Myrrh contains polysaccharides, proteins, and volatile oil that composed of sterols and terpenes [9]. Furano-type compounds has been reported as major constituents; other components were also reported furanodiene, furanoeudesma-1,3-diene, and lindestrene as the major constituents of Ethiopian species [10]. According to WHO reports, 80% of the population in the world depends on herbal medications for their primary health care. Myrrh is used in dentistry for malodour and as anti-inflammatory in periodontitis as it promotes healing [11]. A previous studies indicated that myrrh oil was effective on *S. mutans* [12, 13]. Myrrh has also an antimicrobial activity; It is used for treatment of oral ulcers, gingivitis, sinusitis, brucellosis and a variety of skin disorders [14, 15]. Despite that limited research were conducted on the impact of myrrh on bacteria related to dental caries, the present study aimed to assess the effect of *Commiphora myrrha* on *Streptococcus mutans* and *Lactobacillus spp.* as causes of dental caries and contribute in providing scientific-based evidence of the antibacterial effect and to determine the chemical constituents in myrrh essential oil.

2. Materials and Methods

2.1. Study Design

An experimental *in vitro* study was conducted at the Department of Microbiology, Faculty of Medical Laboratory Sciences, University of Medical Sciences and Technology.

2.2. Preparation and Extraction of Commiphora myrrha Oil

Plant sample (oleogum resin part) obtained from local shop in Omdurman market. The specimen was deposited in the herbarium of Medicinal and Aromatic plants and Traditional Medicine Research Institute (MAPTRI), Khartoum, Sudan. The sample was cleaned, air dried and ground to fine powder. About 250 gram of the powder was extracted by Clevenger [9], this experiment was done in Environmental and Natural Resources and Desertification Research Institute (ENDRI), Khartoum, Sudan. The yield percentage of obtained oil was calculated.

2.3. Isolation and Identification of Bacterial Strains

2.3.1. Lactobacillus spp

The tested bacteria were obtained from Department of Dairy Science and Technology, Faculty of Animal Production, Sudan University of Science and Technology. Three isolated bacteria were isolated from traditional fermented milk and identified using standard procedures.

2.3.2. Streptococcus mutans

Samples were collected randomly from patients with dental caries in Khartoum Dental Teaching Hospital, sterile cotton swabs were used to take the samples from inside carious teeth.

2.3.3. Identification of S. mutans Bacteria

The caries swabs were inoculated on sterile Mitis Salivarius Agar plates. The plates incubated anaerobically at 37°C overnight, microscopic identification was made, then subcultured in Mueller Hinton agar in blood plates. Finally; biochemical tests were applied for further identification [16].

(i). Microscopical Examination

Dried and fixed smear were prepared from culture media. Gram stain were applied crystal violet stain for one minute, washed with tape water and decolorized by alcohol for few seconds, washed immediately with tape water and covered with safranin for 2 minutes then washed again, allowed to dry and examined microscopically using oil immersion lens (X100).

(ii). Biochemical Tests

a. Antibiotic sensitivity test

Optochin discs were used to test the sensitivity of isolates [16].

b. Sugar fermentation tests

Sugar fermentation tests with Phenol red indicator and Esculin hydrolysis are commonly tests used for identification and confirmation of bacteria [17].

Sucrose test

One gram of sucrose sugar was dissolved in 100 ml of peptone water; 1 ml of phenol red was added to sugar. The bacteria were put to test tube from the media and incubated for 24 hour at 37°C then the colour changed was observed.

Glucose test

One gram of Glucose was dissolved in 100 ml of peptone water, then one ml of phenol red was added to sugar. The bacteria were put to test tube and incubated for 24 hour at 37°C, the colour change was observed.

Mannitol test

One gram of Mannitol sugar was dissolved in 100 ml of peptone water, then 1 ml of phenol red was added. The bacteria were put to media and incubated for 24 hour at 37°C, the colour change was observed.

Lactose test

One gram of Lactose sugar was dissolved in 100 ml of peptone water, then one ml of phenol red was added. The bacteria were put to test tube from the media and incubated for 24 hour at 37°C, the colour change was observed. If the colour to yellow, the result considered positive while if the colour remain red, the result considered negative.

c. Esculin test

The surface of esculin agar bacteria inoculated using sterile loop and incubated at 37°C for 24 hour, development of black colour indicates positive result.

d. Voges Proskauer (VP) test

The bacteria were inoculated in Methyl red-Voges-Proskauer broth and incubated for 24 h at 37°C, after that, one ml of 40% KOH and 3 ml of 5% solution of α-naphthol were added. A positive reaction indicated by appearing of pink colour.

e. Catalase test

Test used to differentiate bacteria produce enzyme catalase such as *Staphylococci*, from non-catalase producing such as *Streptococci*. In sterile glass slide, few drops of hydrogen peroxide (H_2O_2) were added, using sterile wooden stick several colonies were removed and immersed in, then immediate bubble production observed.

2.4. Antimicrobial Activity of Myrrh Oil

Two different methods were used to evaluate the antibacterial activity of Myrrh oil against dental caries pathogens; disc diffusion method and well diffusion method.

2.4.1. Disc Diffusion Method

The antibacterial activity of oil in different concentrations (100, 50, 25 and 12,5 mg/ml) was determined using the disc diffusion method [17]. Twenty ml of prepared Mueller Hinton agar were distributed into sterile Petri-dishes. About 0.1 ml of the isolates and standardized bacterial stock suspension 10^8CFU mL-1 was streaked on Mueller Hinton agar medium plates using sterile cotton swab. Standardized sterilized filter paper discs 6 mm (What man NO1) diameter was soaked in the prepared samples, then it were placed on the surface of the tested bacteria plates. The plates incubated anaerobically at 37°C for 24 hour and the zone of inhibition (mm) was measured. Ampicillin, Vancomycin and Ciprofloxacin were used as control positive.

2.4.2. Well Diffusion Method

The second antibacterial activity method of myrrh oil was determined using Well diffusion method [18]. Bacterial culture has been adjusted to Mueller Hinton agar plate similar to disc diffusion method. The wells were made and impregnated with oil samples; after 24 hours, the diameter of the inhibition zone was measured.

2.4.3. 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 (MBC) and is detected by lack of turbidity matching with a negative control [19]. To determine the MBC of *C. myrrha* oil, four concentrations (100, 50, 25 and 12.5 mg/ml) of oil were prepared by serial dilution, each tube seeded with bacteria prepared according to Mcfarland 0.5 turbidity standard and incubated in 37°C. After 24 hour, each tube swabbed in plate of Mueller Hinton agar suspended in blood using sterile cotton swab and incubated anaerobically for 24 hour in 37°C, results were recorded by observing the growth of bacterial colonies.

2.5. Determination of Chemical Constituents Using Gas Chromatography Mass Spectroscopy (GC-MS) Technique

The qualitative and quantitative analysis of the sample was carried out by using GC-MS technique (GC-MS-QP2010-Ultra) from japans Simadzu Company, with capillary column (Rtx-5ms-30 m×0.25 mm×0.25 µm). The sample was injected by using split mode, Helium as the carrier gas passed with flow rate 1.61 ml/min. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patterns with those available in the library from the National Institute of Standards and Technology (NIST).

3. Results

3.1. Yield percentage of C. myrrha

C. myrrha hydro-distilled essential oil yield was found to be 0.8%.

3.2. Identification of S. mutans

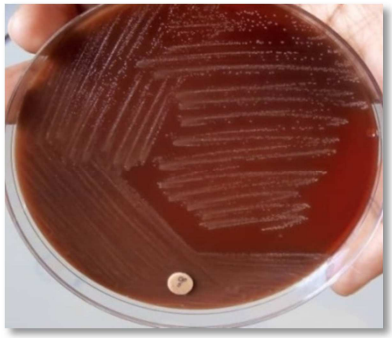

Figure 1. *Bacteria with α-hemolysis resistant to optochin disc in Mueller Hinton agar with blood.*

Microscopic examination revealed Gram-positive cocci in chains. That produced α-haemolysis in Mueller Hinton agar with blood and resistant to Optochin discs (Figure 1).

All three 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 (Figure 2).

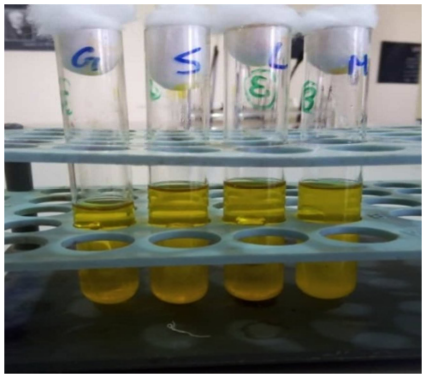

Figure 2. *Sugar fermentation tests (Glucose, Sucrose, Lactose and Mannitol).*

Esculin and VP tests were positive. The bacteria were catalase negative. As a final result all three isolated bacteria were identified as *Streptococcus mutans* bacteria.

3.3. Antimicrobial Activity

Disc and well diffusion methods were used to test the effect of four concentrations (100, 50, 25 and 12.5 mg/ml) of Myrrh oil against Six bacteria strains. 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 spp.) and testing methods used.*

Bacteria	Method of testing		Total
	Disc diffusion method	Well method	
Streptococcus mutans bacteria 1	1	1	2
Streptococcus mutans bacteria 2	1	1	2
Streptococcus mutans bacteria 3	1	1	2
Lactobacillus spp. bacteria 1	1	0	1
Lactobacillus spp. bacteria 2	1	0	1
Lactobacillus spp. bacteria 3	1	0	1
Total	6	3	9
%	66.7	33.3	100

The Inhibition zone of *S. mutans* and *Lactobacillus spp.* was measured across four concentrations of oil as revealed by Table 2.

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

Variables	Myrrh volatile oil concentrations (mg/ml)			
	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 diffusion 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 spp.* 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

In both disc diffusion method and well diffusion method, the highest inhibition zones against *S. mutans* were recorded in 100 mg/ml (14.0 mm and 18.7 mm ± 0.6) respectively. Whereas the highest inhibition zone against *Lactobacillus spp.* was found to be 10.00 mm ± 2.00 at 100 mg/ml.

3.4. Sensitivity Test of Antibiotics

Three antibiotics were tested against *S. mutans* and *Lactobacillus ssp*. Results showed in Table 3. The inhibition zone of Ampicillin using Disc diffusion method against *S. mutans* was found to be 17.7 mm ± 2.5, while *Lactobacillus ssp*. was resistant to Ampicillin. Vancomycin showed inhibition zone of 23.7 mm ± 0.2 followed by Ciprofloxacin with inhibition zone of 18.3 mm ±1.5 against *Lactobacillus ssp*.

Table 3. *Antibacterial activity of antibiotics against bacterial strains.*

Bacteria/Antibiotics	Inhibition zones measured in mm			
	Mean	Std.	Minimum	Maximum
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

3.5. Determination of Minimum Bactericidal Concentration (MBC)

The three concentrations of *C. myrrha* oil at 12.5, 6.25 and 3.125 mg/ml did not show any growth across the three isolates of *S. mutans*. However, growth of three isolates was observed at in *C. myrrha* oil at concentration 1.56 mg/ml (Table 4). The concentration 3.125 mg/ml of oil was considered as the MBC.

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

Streptococcus mutans	12.5mg/ml	6.25mg/ml	3.125mg/ml	1.56mg/ml
Bacteria 1	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

3.6. Gas Chromatography Mass Spectroscopy Analysis

The results of GC-MS of Myrrh oil revealed the presence

of 48 compounds after comparing retention index and mass fragmentation patents of the oil components with those available in the library of 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 respectively. The results showed in Table 5.

Table 5. *Components of C. myrrha oil as analyzed by GC-MS.*

NO.	Name of compound	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)	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
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

4. Discussion

Medicinal plants have been used for centuries as a natural remedy for various ailments including dental infections caused by microorganisms; several studies revealed that Plants can have various effects on bacteria related to dental caries and many researches demonstrated the effects of medicinal plants on dental pathogens such as *Streptococcus mutans*, *Staphylococcus aureus*, *Candida albicans* and *Porphyromonas gingivalis*, it has been used more commonly as medications due to it is availability and safety and as the resistance to antibiotics drugs is successively increase. Some previous studies demonstrated that *C. myrrha* 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 *C. myrrha* oil using two different methods [5]. In this study *C. myrrha* oil was effective at all concentrations against *S. mutans* bacteria with MBC 3.125mg/ml while *Lactobacillus ssp*. MBC was considered to be 25mg/ml. The highest activity of oil against *S. mutans* and *Lactobacillus spp.* using disc diffusion method were found to be 18.34 mm±0.26 and 11.72 mm±0.85 respectively. In a study by Almekhlafi et al., higher results were recorded in a comparative study testing two different formula of Myrrh mouth wash (containing 65 ml of Myrrh extract for each 100ml) with another ingredient against *Streptococcus mutans* isolates. Their findings for the two formulas were 32.367 mm ± 0.262 and 22.367 mm ± 0.102 respectively [14]. Furthermore, *Lactobacillus ssp.* was completely resistant to Ampicillin while Myrrh was effective at the three concentrations of 100, 50, and 25 mg/ml respectively. Another Previous study on the antibacterial effect of the resin of *Commiphora myrrha* was evaluated against three dental pathogens, namely *Streptococcus mutans*, *Streptococcus sobrinus* and *Porphyromonas gingivalis* showed that the resin extract of *C. myrrha* significantly inhibited the growth of all three pathogens, with minimum inhibitory concentrations (MICs) ranging from 0.025 to 1 mg/ml. The extract also exhibited bactericidal activity against *S. mutans* and *S. sobrinus* at concentrations of 0.5 and 1 mg/ml respectively [11]. The finding of this study was complying with literature review. According to the results, Myrrh can be used as alternative and effective antibacterial agent.

Sesquiterpenes and furanotype are major constituents of *C. myrrha*, have antimicrobial activity leading to have therapeutic effect [10, 14, 20]. The highest percentage compound in the present study is Benzofuran, which have antimicrobial activity, this result was comparable with a previous study which revealed that benzofuran constitutes 26.63% of *C. myrrha* oil analyzed by GC-MS technique [20], and differ from another study which indicated that Curzerene (Benzofuran) was 11.9% [9]. The differences may be due to plant origin or method used for extraction. Caryophyllene considered as anti-tumor, antibacterial and anti-inflammatory represents 1.76% of constituents was found higher than a study which revealed 0.29% [20]. Other important components of myrrh oil in the present study were Cyclohexane (19.88%) and 1,3-Diphenyle-1,2-butanediol (15.17%). Further studies revealed that the antimicrobial effect of *C. myrrha* was due to the presence of various phytochemicals such as; terpenes, flavonoids, and phenolics. The high concentration of terpenoids, especially sesquiterpenes, in *C. myrrha* has been shown to be responsible for its antimicrobial activity [20]. Overall, the results suggest that *Commiphora myrrha* has a significant impact against dental pathogens, it considered as potential natural antibacterial agent and can be used in the development of oral health care products.

5. Conclusion

The results support some of the traditional uses of Myrrh for help prevent the dental caries, it offers a natural alternative to conventional antibiotics and anti-fungal agents with minimal side effects. Further research is needed to identify the active components and to fully understand the mechanisms of action and optimal use of this medicinal plant in the prevention and treatment of dental infections.

Data Availability

The data generated and analyzed during this study are included in this manuscript.

Disclosure

An earlier version of this manuscript has been presented as a preprint copy according to the following link: Impact of Commiphora myrrha on bacteria (Streptococcus mutans and Lactobacillus species) related to dental caries | bioRxiv

Conflict of Interests

The authors declared that they have no conflicts of Interests.

Authors' Contributions

Reem Izzeldien initiates the idea, performed sample collection, performed the laboratory work, performed analysis, and wrote the original draft. Sondos Ibn Ouf participated in Laboratory work and was responsible for conceptualization and investigation of the study. Ayat A. Alrasheid participated in Co-Supervision, experimental process, responsible for conceptualization and investigation of the study and revising the manuscript. Zawahir Abu Elbasha was responsible for collection and identification of samples used in the study. Mounkaila Noma was responsible for conceptualization, investigation, data analysis and supervision. All authors have read and approved the final manuscript.

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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.

[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] 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.

[17] John Gerald Collee., Mackie and Mccartney Practical Medical Microbiology. Elsevier; 1996.

[18] 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.

[19] 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.

[20] Gadir Suad, Ahmed Ibtisam. Commiphora myrrha and commiphora Africana essential oils. Journal of Chemical and Pharmaceutical Research. 2014. 6. 151-156.