Antibacterial activity of Commiphora molmol in wound infections

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Abstract

The isolates of bacteria from patients' infected wound from Hail General Hospital and King Khalid Hospital (Hail city, KSA) showed Gram positive as 37.5% while Gram negative as 62.5%. The Gram positive isolates were *Staphylococcus aureus* (31.25%) and *Bacillus subtilis* (6.25%) while Gram negative isolates were *Escherichia coli* (12.5%), *Klebsiella pneumoniae* (12.5%), *Pseudomonas aeruginosa* (12.5%), *Serratia marcescens* (12.5%) and *Enterobacter cloacae* (12.5%).

The sensitivity of bacteria isolates tested against six different antibiotics: Streptozocin (STN), Piperacillin (PRL), Ceftazidime (CAZ), Ciprofloxacin (CIP), Imipenem (IMI), and Aztreonam (ATM) antibiotics. All these antibiotics showed sensitivity against *Bacillus subtilis*; *Staphylococcus aureus* found resistant to all except IMI; *E. coli* sensitive to all except PRL; while *Enterobacter clocae* was sensitive to all except STN and CIP.

The inhibitory effect of diethyl ether extract from *Commiphora molmol* (Myrrh) on *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae* was obvious but no inhibitory effect shown on *Pseudomonas aeruginosa* and *Serratia marcescens.*

The hexane extract from *Commiphora molmol* (Myrrh) showed minor inhibitory effect against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae.*

The water extract from *Commiphora molmol* (Myrrh) showed no inhibitory effect against all bacterial islaots.

The antimicrobial properties of *C. molmol* diethyl ether, hxane and water extracts against clinical isolates of Gram positive and Gram negative bacteria could be the first report. Based on the results diethyl ether extract tested can be considered as effective anti-bacterial natural product while the results for water extract and hexane extract showed no efficacy.

Keywords: Bacterial isolates, Antimicrobial resistance pattern.

Introduction

Myrrh resins are normally collected after the bark of Commiphora molmol species excretion, it consists of about (2-8%) volatile oil, (23-40%) resin and (40-60%) gum. The main secondary metabolites which were identified in literature are cadinene, elmol, eugenol, cuminaldehyde, commipherol and commipherin.

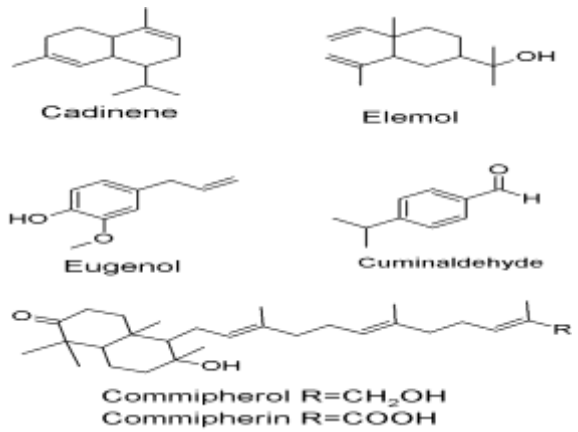

Fig. 1: **The main compounds obtained from Commiphora genera draw by using ACD Chemsketch**

Myrrh is a natural gum or resin extracted from a number of small, thorny tree species of the genus Commiphora.(1) Myrrh resin has been used throughout history as a perfume, incense, and medicine. Gum (resin) is a yellowish harvest of Commiphora myrrha(2) and the commonly used name is Commiphora molmol(3) which is now considered a synonym of Commiphora myrrha.(4) Commiphora myrrha is native to parts of Saudi Arabia, Oman, Yemen, Somalia, Eritrea and eastern Ethiopia. The oleo gum resins of a number of other Commiphora species are also used as perfumes, medicines (such as aromatic wound dressings), and incense ingredients. These myrrh-like resins are known as opopanax, balsam, bdellium, guggul bisabol, and Indian myrrh. In pharmacy, myrrh is used as an antiseptic in mouthwashes, gargles, and toothpastes.(6) Myrrh is currently used in some linaments and healing salves that may be applied to abrasions and other minor skin ailments. Myrrh has also been recommended as an analgesic for toothaches (as common ingredient of tooth powders) and can be used in linament for bruises, aches, and sprains.(7) Myrrh gum is used for indigestion, ulcers, colds, cough, asthma, lung congestion, arthritis pain, and cancer.(8) Research data indicated that the extract killed all of the cancer cells in laboratory dishes.(9) Myrrh is combined with such herbs as Panax notoginseng, Safflower

(Carthamus tinctorius) petals, Angelica sinensis, Cinnamon, and Salvia miltiorrhiza, usually in alcohol, and used both internally and externally.[10]

Microbial wound contaminants are likely to originate from environment (exogenous microorganisms in the air or introduced by traumatic injury), surrounding skin (normal skin microflora) and endogenous sources involving mucous membranes.[11] The potential wound pathogen are: Gram positive cocci (*Streptococcus pyogenes*; *Enterococcus faecalis*; and *Staphylococcus aureus* "MRSA as the most causative agent associated with wounds"), Gram negative aerobic rods (*Pseudomonas aeruginosa*), Gram negative facultative rods (*Enterobacter* species; *Escherichia coli*; *Klebsiella* species; and *Proteus* species), Anaerobes (*Bacteroides* and *Clostridium*).[12,13] Of 71 clinical bacterial isolates at Khartoum (Sudan) nine genera including Gram-positive bacteria (42.25%) and Gram-negative bacteria (57.75). The genera included Gram positive *Staphylococcus* species, *Staphylococcus delephini, Streptococci* species, and *Corynebacterium* species while genera included Gram negative isolates were *E. coli Serratia marcescens*, *Serratia plymuthica*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Proteus mirabilis*, *Vibrio* species, *Aeromonas* species and *Shewanella putrefaciens.*[14]

The aim of this study is to evaluate the antibacterial activity of *C. molmol* water extract, hexane extract and diethyl ether extract against clinical bacteria isolates obtained from human infected wounds.

Occurrence of myrrh: Different species of myrrh have been found in different countries as shown in the Table.

Species	Country
C. molmol Engler	Ethiopia, Somalia, Arabia
C. mukul Engler	Somalia, India
C. guidotti Chiov	Somalia
C. abyssinica Engler	China, East Africa, Ethiopia
C. incisa Chiov.	India, East Africa, Ethiopia
C. pyracanthoides Engler	East Africa
C. rostrate Diels	Arabia
C. gileadensis Engler	Djibouti, Ethiopia, Kenya, Sudan Somalia,
C. wightii Engler	Pakistan, India
C. guillauminiperr Engler	Sudan, Kenia
C. erythraea(Var.)	Engler India, Somalia
C. opobalsamum Engler	Near Cairo "at mataria"

Constituents: Myrrh contains a 2-8% volatile oil (myrrhol), 23-40% resin (myrrhin), 40-60% gum, and a bitter principle 10-25%.

Volatile oils: The volatile oil from Commiphoramolmol is thick. It has a pale yellow color which upon exposure to the vapour of bromine or fumes from nitric acid give a violet color. The constituents of essential oil in two kinds of myrrh were analyzed by GC/MS and identified with their percent contents.

The components from Commiphoramolmol were detected chromatographically with a simple colorimetric determination method using vanillin-sulfuric acid reagent to form a stable violet colored reaction product with a maximum absorption at 518 nm. It contains cadinene, elemol, eugenol, cuminaldehyde, numerous furanosesquiterpenes.

Resins: The chemistry of myrrh resin is uncompletely elucidated. It is generally classified into a bigger ether soluble fraction and a smaller insoluble fraction. The ether soluble fraction consists of a-, b- and g-commiphoric acid, esters of a resin acid, commiphorinic acid, and two phenolic resins, a- and b-heerabomyrrhol. The ether insoluble fraction contains a- and b-heerabomyrrholic acids. It shows a fluorescent spot on the TLC due to the probable formation of dehydroabietic acid. From the gum-resin of Commiphoramukul long chain aliphatic tetrols 25 were isolated and were found to be a mixture of homologues of octadecan-1,2,3,4-tetrol (50%), nonadecan-1,2,3,4-tetrol (7%) and eicosan-1,2,3,4-tetrol (40%)

Gums: The crude gum from the alcohol insoluble matter of Commiphora molmol contains 18% protein and 64% carbohydrates as galactose, arabinose and glucuronic acid. The gum is associated with an oxidase enzyme. It is apparently allied to Acacia gum and contains an oxidase enzyme whose activity was destroyed at 100˚C. About 50 of the gum was established by Wiendle and Franz. The gum of Commiphoramukul was found to be a highly branched polysaccharide 51 containing (16), (15) and (13) linkages. Its structure was deduced from the methylation of Commiphoramukul gum with Me2SO4/ NaOH and subsequent treatment with Purdie reagent followed by hydrolysis with methanolic HCl, and saponification with Ba(OH)2.

Materials and Method

Myrrh resins was collected from Hail tradition shop, Hail city, KSA and it was identified by local folk practitioner, the material was checking physically to select the bright yellow parts, then it was crushed and grinded it to powder form in order to make solution.

Stock solution: Myrrh stock solution was prepared by dissolving about 7.77g of powdered myrrh in a 100ml of distilled water. The solution were placed on hotplate at about 45 °C with continuous steering for about 30 min until it show cloudy solution with mucilage touch. The solution the cooled and stored in refrigerator for further use.

Fractionation: Aqueous solution representative polar extract thus, for providing another fractions liquid-liquid extraction method were applied. Though, Two different organic solvents were used for fractionate the aqueous extract particularly (n-hexane and Diethyl

ether) which representative non-polar and semi-polar organic solvents respectively.

In a separatory funnel about 10 ml of stock solution were mixed with 10 ml of ether n-hexane or Diethyl ether to form two immiscible phases, the funnel were rotated gently to avoid emulation occurring. Then, the organic phase was separated from the aqueous phase and collected in a special container.

Plant Materials: The plant (*Commiphora myrrha*) was collected from "Perfumery Center at Hail city in KSA". The plant material was then cut into smaller pieces and then first washed with tap water followed by washing with distilled water. It was than dried under sunlight until water droplets completely evaporated. Peel and plant were then kept in hot air oven for two days to dry. Dried resin was then taken for grinding by the help of mixer grinder. The coarse powder of plant sample was then used throughout the study.

Microbial strains: The clinical isolates were isolated from infected patients' wounds. The isolated bacteria come from the "General Hail Hospital" and "King Khalid Hospital" at Hail city (KSA). The bacteria were isolated by Microbiology laboratory at University of Hail Research Centre (MDXPTU) using automated (MicroScan Walk-Away Microbiology Systems in the Identification and Susceptibility Testing of Bacteria) device and the bacterial isolates were used during the study to test Myrrha antibacterial activity.

Extracts:

Three ways were used to get the extracts of *Commiphora myrrha*:

1. Water extract (polar extract).
2. Hexane extract (non-polar extract).
3. Diethyl ether extract (semi-polar extract).

Water extractions (Polar extract): For water extract we used 10 grams of *Commiphora myrrha* and mixed with 100 ml of water in a beaker under the evaporation temperature mixing about 30 minutes. There was impurities after mixed where the total of impurities (2.23) and removed for the solution that make the total concentration of water extraction 7.77 g/ 100ml.

Hexane extract (Non polar extract): The hexane extract was made from water extract by addition of the hexane to the water extract which cannot mixed to the water due to the solution characteristics differences (the polar and non-polar characteristics). After gentle mixing the components that cannot dissolve in water can dissolve in hexane (non-polar components). The concentration of hexane extract is 0.020g/ 10ml.

Diethyl ether extract (Semi polar extract): The useful of this extract is the components they do not dissolved in polar extract and non-polar extract they will be dissolved in the diethyl ether extract. The used concentration of diethyl ether extract is 1.4g/ 100ml.

Method of testing:

Disk diffusion method: Because of convenience, efficiency and cost, the disk diffusion method is probably the most widely used method for determining antimicrobial resistance. Mueller-Hinton agar usually used where it is firstly evenly seeded throughout the plate with the isolate of interest that has been diluted at a standard concentration (approximately 1 to 2 x 10^8 colony forming units per ml). Commercially prepared disks, each of which are pre-impregnated with a standard concentration of a particular antibiotic, are then evenly dispensed and lightly pressed onto the agar surface. The test antibiotic immediately begins to diffuse outward from the disks, creating a gradient of antibiotic concentration in the agar, such that the highest concentration is found close to the disk with decreasing concentrations further away from the disk. After an overnight incubation, the bacterial growth around each disc is observed. If the test isolate is susceptible to a particular antibiotic, a clear area of "no growth" will be observed around that particular disk.

Prepared filter paper discs were used and impregnated with by the three prepared concentrations of the extract from Myrrh (water extract, hexane extract and diethyl ether extract) were used to test against the clinical isolates.

Results

The isolates of wound infection bacteria were identified as shown in Table 1. About 32 isolates were identified. Gram positive represented 37.5% and Gram negative represented 62.5% of the clinical isolates. The percentages of these isolates were shown in the Table 1.

Table 1: Bacteria isolated from clinically infected wounds from patients admitted to Hail General Hospital and King Khalid Hospital at Hail City (KSA)

Samples	No of isolates	Percentages
Gram Positive:		
Bacillus subtilis	2	6.25
Staphylococcus aureus	10	31.25
Gram Negative:		
Escherichia coli	4	12.5
Klebsiella pneumonie	4	12.5
Pseudmnas aureginosa	4	12.5
Serratia marcescens	4	12.5
Enterobacter cloace	4	12.5

Table 2: Sensitivity of the isolates to different tested antibiotics

Antibiotic	C. cloace	Escherichia coli	Staphylococcus aureus	Bacillus subtilis
STN	R	S	R	S
PRL	S	R	R	S
CAZ	S	S	R	S
CIP	R	S	R	S
IMI	S	S	S	S
ATM	S	S	R	S

R= Resistant. S= Sensitive

The results of inhibition of C. molmol extracts (water "polar"; hexane "non-polar" and diethyl ether "semi-polar" by disk diffusion method were shown in Table 3. Inhibition zone in diameter (mm) around the discs impregnated with water (7.7 gm/10 ml), hexane (0.02 gm/ 10 ml) and diethyl ether (1.4 gm/10 ml) extracts.

It is noticed that Pseudomonas aeruginosa and Serratia marcescens were the resistant isolates to Myrrh. Also it is observed that water extract of Myrrh was found to enhance the growth of isolates around the discs impregnated with water extract of Myrrh.

Table 3: Anti-bacterial effect of different Myrrh extracts against clinical bacterial isolates

	Water extract (polar extract)	Hexane extract (nonpolar extract)	Diethyl ether extract (semi polar extract)
Gram Positive Bacteria			
Bacillus subtilis	resistance	6mm	9mm
Staphylococcus aureus	resistance	5mm	13mm
Gram Negative Bacteria			
Escherichia coli	resistance	4mm	6 mm
Klebsiella pneumoniae	resistance	6mm	10mm
Pseudomonas aeruginosa	resistance	resistance	resistance
E.cloacae	resistance	resistance	3
Serratia marcescens	resistance	resistance	resistance

Discussion

The bacterial isolates are same as those previously isolated genera during previous studies.[19,20,21,22] These isolates were tested against six different antibiotics: (STN), Piperacillin (PRL), Ceftazidime (CAZ), Ciprofloxacin (CIP), Imipenem (IMI), and Aztreonam (ATM) and the sensitivity to these antibiotics were showed varying degrees of sensitivity. Same isolates were tested against the three extracts of Myrrh and they showed good inhibition zones only for diethyl ether extract of Myrrh. The diethyl ether extract is the best of inhibition zone activity as antibacterial with zone of inhibition reached 13 mm in diameter.

Hexane extract showed inhibitory action against the bacteria isolates but it is poor compared to diethyl ether extract where the diameter of inhibition reached only 6 mm maximum.

The water extract have no activity against bacteria isolates from both Gram positive and Gram negative. In some cases the water extract observed to show increase the growth of bacteria around the discs.

Pseudomonas aeruginosa was known as a resistant microorganism to most of antibiotics[23] here also it is found to be resistant to Myrrh with all extracts.

Serratia marcescens was found also to show same resistance to Myrrh for all extracts like Pseudomonas aeruginosa.

Conclusion and Recommendations

1. The Camphor myrrh can be used as antibacterial especially diethyl ether extract where its action is very obvious especially to Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and E.cloacae.
2. Hexane extract second compared to diethyl ether extract as antibacterial where it is not very effective especially to Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia.
3. Water extract has no visual effect as antibacterial to all clinical isolates. Although, it is observed that the water extract of Myrrh was found to enhance the growth of isolates around the water extract impregnated discs.

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