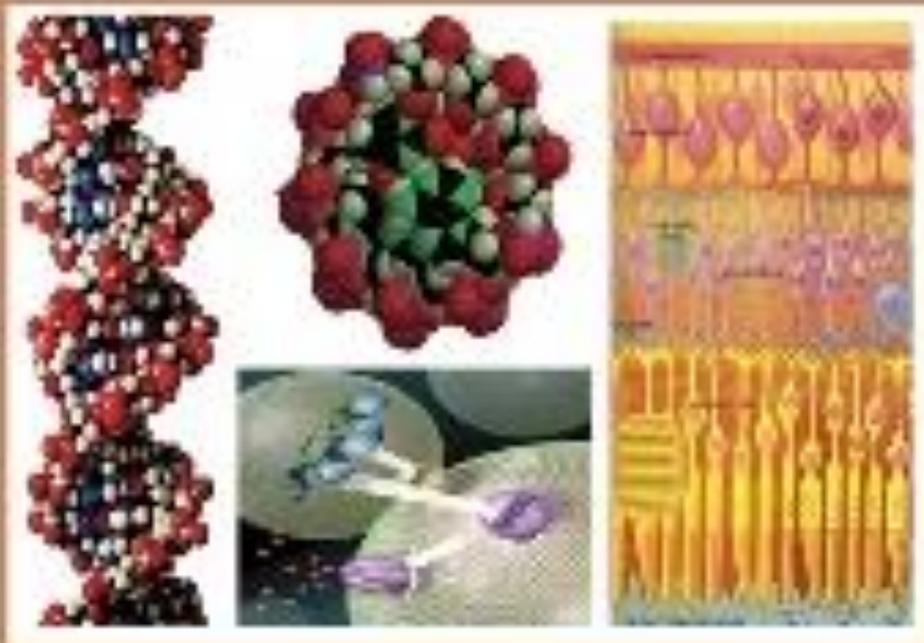




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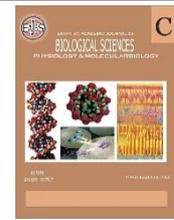
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A laboratory Evaluation of Antibacterial Activity of *Commiphora myrrha* Gum Resins from Kingdom of Saudi Arabia 2023

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ABSTRACT

Background: Today therapeutic plants are regarded as an essential part of human life, the study of antimicrobial activities of plants involves the care of the possible practice of these ordinary crops **Objective:** The main goal of the present work is to assess the antibacterial activity of *Commiphora myrrah* on pathogenic bacteria.**Methods:** The experimental study was conducted between April and May 2023. Fresh *Commiphora myrrah* gums used in my work were collected from Aqiq town, Saudi Arabia. *Commiphora myrrah* gums were effectively extracted by using cool and hot water for 48 hrs. as traditional uses. Five strains of bacteria were used. Minimum Inhibitory Concentration was determined by using the agar well diffusion method as the method reported by Saadabi *et al.* (2012). We used three drops of overnight cultured bacteria. Each extract; hot water and cool water was verified in contradiction of the five strains of the organism using the agar diffusion method (Groove and Randall, 1955) and measured the zones of inhibition.

Results: In this study resin *Commiphora myrrah* was selected to estimate their antimicrobial activities against five isolated bacteria (one strain Gram-positive and 4 strains Gram-negative). Our findings similarly exposed that *C. myrrah* hot extract gives advanced antimicrobial activities on tested isolate strains. Despite, there were similar efficiency of *C. myrrah* and the used antibiotics on almost all tested microorganisms, sometimes *C. myrrah* has more efficiency than some antibiotics against some tested bacterial strains. Cool extracts of *C. myrrah* have less efficiency against all tested strains.

INTRODUCTION

The therapeutic plant has had an extended trial implementation in the past, for example, *Commiphora myrrah*, which is usually used in some areas of Arabia, Somalia, Kenya and the northeastern state of Africa. It has remained used conventionally for treating mouth ulcers, aches wounds, stomach disorders, microbial infections, inflammatory diseases and fractures. It is used disinfection agent and as medicine. Phytochemical research reveals that *C. myrrah* gum contains terpenoids (sesquiterpenoids monoterpenoids, and volatile/essential oil), diterpenoids, triterpenoids, and steroids. Its important oil has been requested in aromatherapy, cosmetics and perfumery. The study has publicized that it employs several biotic actions for instance anti-microbial, anti-parasitic, anti-inflammatory, analgesic, neuroprotective, antioxidant, anti-diabetic, anti-cancer, and recently, used against respiratory infections such as COVID-19. Through the progress in drug expansion, expectantly, its annoying phytochemical components can be discovered for medication progress as a pesticide owed to its great antiparasitic activity. Also, its interactions with drugs can be fully elucidated (Batiha *et al.*, 2023).

Commiphora myrrah is a common herbal amongst the several species that belong to the family Burseraceae. Its home is the Arabian Peninsula and some African nations. It has been used since ancient times in the treatment of many diseases, where it was used as an astringent, anti-parasite, antiseptic, and anti-cough, for spasm and menstruation (Wanner *et al.*, 2010, Singh and Singh, 2011).

Commiphora genus is a member of the Burseraceae family, which comprises more than 200 species. It cultivates in hot dry places like Africa, India, and Arabia (Gadir and Ahmed, 2014). It produces numerous viable balsms; myrrah gum resin is considered one (Başer *et al.*, 2003). The gums' colors are yellow to red and continually are shielded with a grimy powder that has a brighter color. However, exact myrrah is formed by cutting *Commiphora myrrah* (Hosseinkhani *et al.*, 2017).

History of *Commiphora myrrah*:

Commiphora myrrah from *Commiphora myrrah* tree, which perform bud classified into the family Burseraceae (Alyafei, 2020).

Traditional Usages:

The excessively studied and more frequently handled and used types of *Commiphora* are *C. molmol*, *C. myrrah*, *C. mukul*, and *Commiphora opobalsamum*. These species of resins have revealed a display of pharmacological activity in managing wounds, mouth ulcers, aches, fractures, stomach disorders, microbial infections, and inflammatory disease. In *Unani* treatment, gums are applied as stomachic, astringent, anthelmintic, carminative, emmenagogue, expectorant and antiseptic.

They are also implemented in amalgamation with other medications as protective drugs against widespread diseases and used ectopically in gouty and tender joints and for dealing with injuries (Akbar, 2020).

Antimicrobial Activity:

A study performed in Saudi Arabia published on 14 February 2023 showed that *C. myrrah* possesses antibacterial potential

activities, probably due to comprising high volumes of many active ingredients example polyphenolic components and flavonoids including alkaloids (Mansouri *et al.*, 2023).

A study done by Rasha A. Mansouri *et al* 2023 reported that *C. myrrah* exhibited promising inhibition of bacterial growth, and found that there is a clear zone of inhibition against *E. coli* (ATCC 10536) followed by *S. epidermidis* (ATCC 12228), *B. subtilis* (ATCC 11774) (27 mm), *S. pyogenes* (ATCC 12344), *K. pneumoniae* (ATCC 10031), *P. aeruginosa* (ATCC 27853), *P. fluorescens* (ATCC 13525), and *S. typhimurium* (ATCC 13311), respectively (Mansouri *et al.*, 2023).

Experiential indications have exposed that myrrah extracts give good care to the virus feature of which these extracts possess an antimicrobial and antiviral effect on many viruses (Khalil *et al.*, 2020). In a specific study, fungicidal, bactericidal and antiviral actions of myrrah crucial oil extracts advised their possibility of delaying the growth of bacteria and viruses (Brochot *et al.*, 2017). Moreover, in research, essential oils from myrrah showed antiviral activities against two viruses: herpes simplex virus type 1 (HSV-1) and influenza virus type A (H1N1). Myrrah was noticed that act on free viral particles' direct inactivation and disrupt the virion envelope structures whose major role is in host cell virus invasion.

Commiphora Africana and *Commiphora myrrah* oils possess antibacterial and antifungal and are rich in compounds that play a significant part in therapy. *Commiphora Africana* oil displays pure and high antiviral activity towards NDV extra study can principal to control this hostile virus, low LC50 values of the two oils elucidate their cytotoxicity and anti-tumour belongings, and the core chemical complexes in the two oils originate as sesquiterpenes and sesquiterpene lactones the antitumor possessions of sesquiterpene lactones have involved good attention, more study is desired for these oils and their mechanisms can lead to fascinating pharmaceuticals normal products (Gadir and Ahmed, 2014).

Myrrah has several active therapeutic uses and has been implemented to treat tumors, disorders of the gall bladder, fever, dysmenorrhea, and skin infections (El Ashry *et al.*, 2003, Massoud *et al.*, 2001). Various earlier research has established the presence of numerous phytochemicals that possess the cited biological activities as steroids, terpenoids, flavonoids, carbohydrates, lignans, and others (Shen *et al.*, 2007).

Objective: The main goal of the present work is to assess the antibacterial activity of *Commiphora myrrah* on pathogenic bacteria.

MATERIALS AND METHODS

Preparation of the Extracts:

The experimental study was conducted between April and May 2023. Fresh *Commiphora myrrah* gums used in my work were collected from Aqiq town, AL Baha region, Saudi Arabia. The principles for selecting the top and perfect form of oleo-gum resin of *C. myrrha* involved central characteristics like its color, odor, transparency, and period of storage. The gum must not be kept for further than 3 months and should be translucent with a golden to brownish-yellow color (Mansouri *et al.*, 2023).

A10 grams of the crudely powdered Gum of *Commiphora myrrah* herbal were consecutively extracted with cool and hot water for 48 hrs.

The water extracts were extracted by using distilled water to solve 10 g of crudely powdered resins in a pointed bottle and left at a dark place to immerse for 48 hours. The remainder was then filtered and the ending size was adjusted to 10 mL with the addition of distilled water and the resolution used directly (Saadabi, 2006) ⁽¹⁶⁾.

Bacterial Strains:

Five bacterial strains namely *Streptococcus agalactiae*, *Escherichia coli*, *Citrobacter koseri*, *Klebsiella pneumoniae spp pneumoniae* and *Pseudomonas aeruginosa* were used. The bacteria strain identification and antibiotic susceptibility were done in South Qunfuzah General Hospital Laboratory, Qunfozah City, Makkah region, Saudi Arabia by using the Vitek2

machine system.

Determination of MIC:

MIC was assessed by using the Mueller-Hinton agar well diffusion method based on the method applied by Saadabi *et al.* (2012). In this method prepared solutions of different concentrations from *Commiphora myrrah* resins including 100, 50, 25, 12.5 and 6.25 mg mL.

3 droplets of 24 hrs bacterial growth i.e., *Streptococcus agalactiae*, *Escherichia coli*, *Citrobacter koseri*, *Klebsiella pneumoniae spp pneumoniae* and *Pseudomonas aeruginosa* have been cultivated into the dilutions and incubated at 37°C for overnight on sterile molten Mueller-Hinton agar ⁽¹⁶⁾.

Antibacterial Susceptibility:

Each extract; hot water and cool water was examined and evaluated their activities against the five species of bacteria using the cup plate agar diffusion method (Grove and Randall, 1955) ⁽¹⁷⁾ and measured the diameter of inhibition zones.

RESULTS

As an overall regulation, extractions of medicinal herbal were regarded as effective against both bacteria and fungi when it provided an inhibition zone more than 6 mm in diameter (16,17).

Table 1 provided the medium of the growth inhibition zones diameters of *Commiphora myrrah* cool and hot water extracts, the result was interpreted as susceptible, intermediate and resistant. Based on the results the existing extract resulting in 6 mm or great growth inhibition zone is measured to be effective and those read under 6 mm are active less. As presented in Table (1) hot aqueous extracts of the resin of *C. myrrah* appeared active against all tested organisms, whereas cool extract produced inactive results for all tested organisms.

The wider inhibition zone measured is (11 mm) for *Pseudomonas aeruginosa* after that *Klebsiella pneumoniae spp pneumoniae* (10 mm), *E. coli* (9mm) and (8mm) in the case of *Citrobacter koseri* and *Streptococcus agalactiae* in the hot extract. The smallest inhibition zones were found in cool extracts, (3mm) for *Citrobacter koseri*,

followed by (4mm) in *Streptococcus agalactiae*, and (5mm) in case of *Escherichia coli*, and (5mm) in case of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae spp pneumoniae*, *Staphylococcus aureus* and

Table 1: An antimicrobial activity of *Commiphora myrrah* extract

Extract type	Isolated strains /MDIZ mm				
	<i>Strep. agalactiae</i> ¹	<i>E. coli</i> ²	<i>C. koseri</i> ³	<i>K.p.p</i> ⁴	<i>P.aeruginosa</i> ⁵
Cool aqueous	4	4	3	4	5
Hot aqueous	8	9	8	10	11

Key: 1=*Streptococcus agalactiae*, 2= *Escherichia coli*, 3=*Citrobacter koseri*, 4=*Klebsiella pneumoniae spp pneumoniae*, 5=*Pseudomonas aeruginosa*

MDIZ: Mean diameter inhibition zone. Extracts concentration of 0.1 mL/cup (100 mg/ mL).

Table 2, revealed the minimum inhibitory concentration for cool and hot aqueous extract of *C. myrrha* showed several grades of activeness against the test bacteria, it was 12.5mg per ml and 6.25mg/ml respectively for *Streptococcus agalactiae* and *Pseudomonas aeruginosa*, 100 mg/ml and 50mg/ml for *Escherichia coli*, 50mg/ml and 25 mg/ml for *citrobacter koseri* and 25mg/ml and 12.5mg/ml for *Klebsiella pneumoniae spp pneumoniae*.

The Minimum Inhibitory Concentration (MIC) weight of water extracts of gum resins of *C. myrrah* exposed that the peak MIC values were found in hot extracts in *E. coli* (50mg/ml) while low grads of MIC

values for the similar extracts was(6.25mg/ml) for *Streptococcus agalactiae* and *Pseudomonas aeruginosa*. The maximum MIC values were gained in cool extracts for *Escherichia coli* (100mg/ml), followed by *Citrobacter koseri* (50mg/ml) and *Klebsiella pneumoniae spp pneumoniae* (25mg/ml). on the other hand, the lowermost MIC value for the equivalent extracts was (12.5mg/ml) for *Streptococcus agalactiae* and *Pseudomonas aeruginosa*.

It was obviously observed that the hot extract of *C. myrrah* gum of the plant *Commiphora myrrah*, has a wide range of activeness against all tested bacteria.

Table 2: Minimum inhibitory concentration of *C. myrrah* extracts.

Extract type (fragment used = resin)	MIC of isolated bacteria - mg/ml				
	<i>Strep. agalactiae</i> ¹	<i>E. coli</i> ²	<i>C. koseri</i> ³	<i>K.p.p</i> ⁴	<i>P.aeruginosa</i> ⁵
Cool aqueous	12.5	100	50	25	12.5
Hot aqueous	6.25	50	25	12.5	6.25

Key: 1=*Streptococcus agalactiae*, 2= *Escherichia coli*, 3=*Citrobacter koseri*, 4=*Klebsiella pneumoniae spp pneumoniae*, 5=*Pseudomonas aeruginosa*.

Table 3 presents the antibiotic efficiency of the used chemotherapeutic agents on the isolated bacteria. Once the present results of this study were compared to

antibiotics findings; it can be determined that the extract of *Commiphora myrrah* was similar to or more sufficient than the used antibiotics.

Table 3: Antibacterial activity of used antibiotics against isolated strains.

Antibiotics	MIC of isolated microorganisms mg/ml				
	<i>Strep. agalactiae</i> ¹	<i>E. coli</i> ²	<i>C. koseri</i> ³	<i>K.p.p</i> ⁴	<i>P.aeruginosa</i> ⁵
Benzylpenicillin	0.12				
Ampicillin	0.25	32R		32R	
Levofloxacin	1				8R
Moxifloxacin	0.25				
Clidamycin	0.25 R				
Quinupristin/dalfopristin	0.25				
Linezolid	2				
Vancomycin	0.5				
Tetracycline	16 R				
Tigecycline	0.12	0.5		0.5	8R
Nitrofurantoin	16	16	16	16	
Amoxicillin/clavulanic acid		16I	32R	2	
Piperacillin/tazobactam		4	4	4	4
Cefotaxime			16I		
Cefalotin		64R		2	
Cefoxitin		4		4	
Ceftazidime		4	64R	1	4
Ceftriaxone		64R			
Cefepime		2	1	1	1
Imipenem		0.25	1	0.25	16R
Meropenem		0.25	0.25	0.25	0.25I
Amikacin		8I	2	2	2
Gentamicin		16R	1	1	1
Ciprofloxacin		4R		0.25	4R
Trimethoprim/sulfamethoxazole		20	20	20	
Ertapenem			0.5		
Norfloxacin			1R		
Fosfomycin			16		
Ticarillin/clavulanic Acid					32
Tobramycin					1
Colistin					0.5

Key: 1=*Streptococcus agalactiae*, 3=*Citrobacter koseri*, 4=*Klebsiella pneumoniae spp pneumoniae*, 5=*Pseudomonas aeruginosa*.

R: Resistance, I: Intermediate.

DISCUSSION

Hot and cool water extracts of *C. myrrha* resin were examined for their antimicrobial possibility against five isolated microorganisms; one is Gram-positive bacteria *Streptococcus agalactiae* and four are Gram-negative bacteria (*Escherichia coli*, *Citrobacter koseri*, *Klebsiella pneumoniae spp pneumoniae* and *Pseudomonas aeruginosa*). The cool aqueous extract of *C. myrrha* was branded by lower activity when compared to that of hot extracts.

It is clear from Table 1 that the gum hot aqueous extracts displayed higher activity against *Pseudomonas aeruginosa* followed

by *Klebsiella pneumoniae spp pneumoniae*, *Escherichia coli* and *Citrobacter koseri* and *Streptococcus agalactiae*. This finding agreed in points with the study used a similar extract against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* prepared by Abd-Ulgadir K S *et al.* (Abd-Ulgadir, 2017), Al-Daihan *et al.* (Al-Daihan *et al.*, 2013)19 in Saudi Arabia, and Rahman *et al* (Rahman *et al.*, 2008).

Moreover, the present study findings agree with the findings of research done in Saudi Arabia prepared by Rasha A. Mansouri *et al* 2023, which showed that *C. myrrha* extract gives a clear zone of inhibition

against *E. coli* (ATCC 10536) followed by *S. epidermidis* (ATCC 12228), *B. subtilis* (ATCC 11774) (27 mm), *S. pyogenes* (ATCC 12344), *K. pneumoniae* (ATCC 10031), *P. aeruginosa* (ATCC 27853), *P. fluorescens* (ATCC 13525), and *S. typhimurium* (ATCC 13311), respectively (Mansouri *et al.*, 2023).

On the other hand, the cool aqueous extract revealed low activities against all tested organisms. The comparison of present MIC findings presented in Tables 2 and 3 established that the gum hot extract of *C. myrrah* exposed high activity (MIC= 6.25mg/ml) against *Streptococcus agalactiae*, and *Pseudomonas aeruginosa*, which is almost similar to the activity of used antibiotics and more than activity of Clindamycin and Tetracycline for streptococcus and Levofloxacin, Tigecycline, Imipenem, Ciprofloxacin which showed no activity against *p.aerogenosa* and Meropenem which showed intermediate activity (Tables 2&3).

It also inhibits *Klebsiella pneumoniae* spp *pneumoniae* (MIC= 12.5mg/ml), which is almost similar to the activity of used antibiotics and more than the activity of Ampicillin which showed no activity.

In addition, the same extract showed activity against *C. koseri* at 25mg/ml which is similar to the activity of used antibiotics and more than the activity of Amoxicillin/clavulanic acid, Ceftazidime, Norfloxacin which showed no activity and Cefotaxime which produced intermediate activity.

Although the same extract provides activity against *E. coli* at high concentrations (50mg/ml) it is similar to the activity of used antibiotics at different concentrations, and more than the activity of Ampicillin, Cefalotin and Ceftriaxone, Gentamicin, Ciprofloxacin Which produced no activity, and Amoxicillin/clavulanic acid, Amikacin Which gives intermediate effect.

Recommendation:

The current research recognized gum resins of *Commiphora myrrah* as a possible source of antimicrobial, meanwhile, it

exhibited high activity against many pathogenic bacteria, therefore, Additional studies are also needed to isolate and describe the active component of *Commiphora myrrah*.

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