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Original Research Article

Antibacterial Activity of *Commiphora molmol* Extracts on Some Bacterial Species in Iraq

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Abstract: This study aimed to evaluate the inhibitory effect of *Commiphora molmol* (Myrrh) extracts on the growth of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii* from Iraqi hospital isolates. The chloroform, methanol and water extracts of *C. molmol* were tested against the five clinical microbial strains. Gel diffusion method, minimum inhibitory concentration (MIC) values were used in this study. Also Phytochemical analysis of most biological active extract was determined by general phytochemical screening and GCMS, The findings indicated that *C. molmol* methanol and chloroform extracts had growth inhibitory effect against tested bacteria. Methanolic extract of *C. molmol* exhibited the highest inhibitory effect on all tested microorganisms, MIC of methanolic extract was 3.12mg/mL for each of *E.coli, K.pneumoniae* and *P.aeruginosa*, 12.5mg/mL for *A.baumannii* and 6.25mg/mL for *S.aureus*. Phytochemical screening showed the presence of terpenoids, tannins, alkaloids, saponins and flavonoids. GC MS investigation of *C. molmol* methanol extract showed the different peaks of the different constitutes with highest intensity that recorded β-elemene (classified as sesquiterpenes) with retention time 10.82 and other sesquiterpenes constitutes showing that the resin is rich in sesqiuterpenes that possess the antibacterial activity.
Keywords: *Commiphora molmol*, Antibacterial Activity, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*.

INTRODUCTION

Commiphora molmol (C. molmol) (Myrrh) is an oleo-gum resin, obtained from the stem of various species of genus *Commiphora* of family Burseraceae, which grow in north-east Africa and Arabia. *C. molmol* consists of water-soluble gum, alcohol-soluble resins and volatile oil. The gum contains polysaccharides and proteins, while the volatile oil is composed of steroids,sterols and terpenes. Myrrh's characteristic odor is derived from furanosesquiterpenes [1].

The medicinal values of the resinous exudates of the genus *Commiphora* have been gradually recognized by humankind **[2]**, they are used in indigenous medicines for the treatment of wound, pain, arthritis, fractures, obesity, parasitic infection and gastrointestinal diseases [3].

The plant-derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness. So, a return to natural substances is an absolute need of our time [4,5].

The aim of this study was to evaluate the antibacterial activity of *C. molmol* chloroform, methanol and water extracts against microbial strains related to many human infections caused by tested microorganisms, since these organisms have now gained more importance due to increased concerns about safety in food and better quality of life.

MATERIALS AND METHODS

Plant materials and extraction

The oleo-gum-resin of *C. molmol* (Myrrh) was collected from local herbal apothecary in Baghdad and authenticated by department of botany / MOH. Plant material was cut into smaller pieces and washed with distilled water, dried in incubator at 37°C and then grinded into fine powder.[6]

Preparation of the extracts

The amount of (500 g) dried powder of *C. molmol* oleo-gum-resin was extracted using successive solvent extraction (SSE) [7, 8].

The extraction process was performed using the following solvents in increasing order of polarity; chloroform, methanol and distilled water, respectively, by soaking 50g in each of the ten conical flasks with 200ml of the first solvent (chloroform) with continuous shaking by using the shaker water bath for eight hours at 40°C [9].

The crude extracts were filtered under vacuum using filter paper Whatman number 1 filter (20 cm), and then filtrate was concentrated with rotary evaporator at 45°C. Each time before employing the solvent of higher polarity the residue was dried and extracted by the same procedure with the two other solvents (Methanol and then Water).

The concentrated extracts were then transferred into pre-weighed beakers, dried under a stream of cold air, and weighed to determine the yield. The resulting extract was then stored in closed glass amber vessels at +4 °C until tested [10].

Microbial strains

The bacterial strains were isolated from human, and belong to the microbiological laboratory collection of the Clinical Laboratory Sciences department in College of Pharmacy/ Al-Mustansiriya University / Baghdad / Iraq.

Antibacterial tests

The chloroform, methanol and water extracts of *C. molmol* were tested for antimicrobial effect against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

The growth inhibitory effect was determined by the agar well diffusion method [11], active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth, they were then incubated at 37°C for 24 hours. The cultures were diluted with broth to achieve an optical density corresponding to 2.0×10^{-6} colony forming units per milliliter (CFU/ml).

After agar solidification, Mueller Hinton agar plates were swabbed with a suspension of each bacterial species using sterile cotton swab. The medium was punched with six millimeters diameter wells and filled with 100 µl of the test sample and allowed to diffuse at room temperature for 20 minutes. The final concentration of each extracts was 50 mg/ml. Gentamicin (Oxoid, England) was used as positive control at a concentration of 0.2 mg/ml [**12].**

The plates were incubated aerobically at 37°C for 24 hours and inhibition zone formed around the wells were measured in millimeters (mm) using a ruler as inhibition zone diameter (IZD). All tests were done in triplicate and the growth inhibitory effect of plant extracts was recorded.

The Minimum Inhibitory Concentration (MIC) of extracts was determined by broth microdilution technique as described by National Committee for Clinical Laboratory Standards (NCCLS, 2000). Extracts were serially diluted with Mueller Hinton broth to give a final concentration ranging from 25 mg/mL to 0.39 mg/mL. Each tested strain of bacteria was run in duplicate. Tests were incubated aerobically at 37°C for 24 hours.

The MIC was considered as the minimum concentration of the dilutions that inhibited the growth of the test microorganism.

Phytochemical analysis

Phytochemical screening of most biological active extract of *C. molmol* was determined as following: [13, 14].

- Test for terpenes: One gram of extract of *C. molmol* was dissolved in chloroform and filtered, 1 ml of the clear filtrate was mixed with 5 ml of glacial acetic acid in a test tube, the solution was then treated with small volume of concentrated sulfuric acid and mixed properly. The appearance of red color indicates the presence of terpens.
- Test for saponins: Two grams of *C. molmol* was boiled in 20 ml of distilled water using a water bath then filtered, ten milliliters of the filtrate was mixed with 5 ml of distilled water in a test tube and shaken vigorously. Characteristic persistent froth at least 1cm in hight indicates the presence of saponins.
- Test for tannins: About 0.5gm of *C. molmol* extract was boiled in 20ml of distilled water in a test tube and filtered, few drops of 0.1% ferric chloride was added. A brownish-green or blue-black coloration indicates the presence of tannins.
- Test for flavonoids: Five milliliters of dilute ammonia solution was added to the aqueous filtrate of most active extract of *C. molmol*. The aooerance of yellow precipitate indicates the presence of flavonoids.
- Test for alkaloids: Few drops of freshly prepared Mayer's reagent were added to 5ml of the *C. molmol* extract sample. The appearance of white precipitate indicates the presence of alkaloids.

Gas chromatography-mass spectrometry (GC-MS)

It is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC-MS analysis was performed on GC-MS QP-2010. InertCap 1MS capillary column was used (30 m x 0.25 mm x 0.25 µm film thickness) with helium as carrier gas at a flow rate of 1.6 ml/min. The source was operated in positive ionisation mode (electron impact energy: 70eV) and the detection was performed in full-

scan mode. The inlet and the transfer line temperatures were both maintained at 250°C while the ion source was kept at 200°C. Samples were injected in splitless mode (10:1) and separated using a temperature gradient program as follows: 100°C for 1 min, to 200°C at 12°C/min and then maintained at 200°C for 5 mins; then to 300°C at 5°C/min and maintained at 300 C for further 5 mins. GC-MS spectra were evaluated by Postrun software and searched in the National Institute of Standards and Technology (NIST) MS Search V2.0 browsers[15].

Statistical analysis

Data analysis results were expressed as means ± standard deviation, and differences between means were analyzed statistically using an analysis of variance (ANOVA) according to Turkey's test through SPSS 16.0 software package in Microsoft Windows 7.0 operating system. Differences are considered significant when P≤0.05.

RESULTS

The extraction procedure yielded a highest percentage of 232gm (46.4%) of water extract and the lowest yield was for the methanol extract 25.5gm (5.1%) while the chloroform extract was 42gm (8.4%).

The inhibitory effect of the chloroform, methanol and water extracts of *C. molmol* olego-gum resin was evaluated. The results of inhibitory zone diameter of the plant extracts against the five bacterial species are presented in figure-1. The methanol extract of *C. molmol* olego-gum resin, exhibited the highest inhibitory effect against all five bacterial species in compared to both chloroform and water extracts (*P≤0.05*), also there was a noticed inhibitory effect of chloroform extract in compared to water extract that showed no inhibitory effect, while gentamicin as positive control kept the highest values in terms of inhibitory antibacterial effect.

All the test bacteria were susceptible to methanolic extract of *C. molmol* with inhibitory zone diameters ranged from 6.17±0.28mm in *A.baumannii*, 9.67±0.57mm in *S.aureus*, 12.17±0.28mm in *K.pneumoniae*, 12.67±0.57mm in *E.coli* and 12.67±0.28mm in *P.aeruginosa*. And because the methanolic extract exhibited the highest inhibitory effect, it was chosen for further testing.

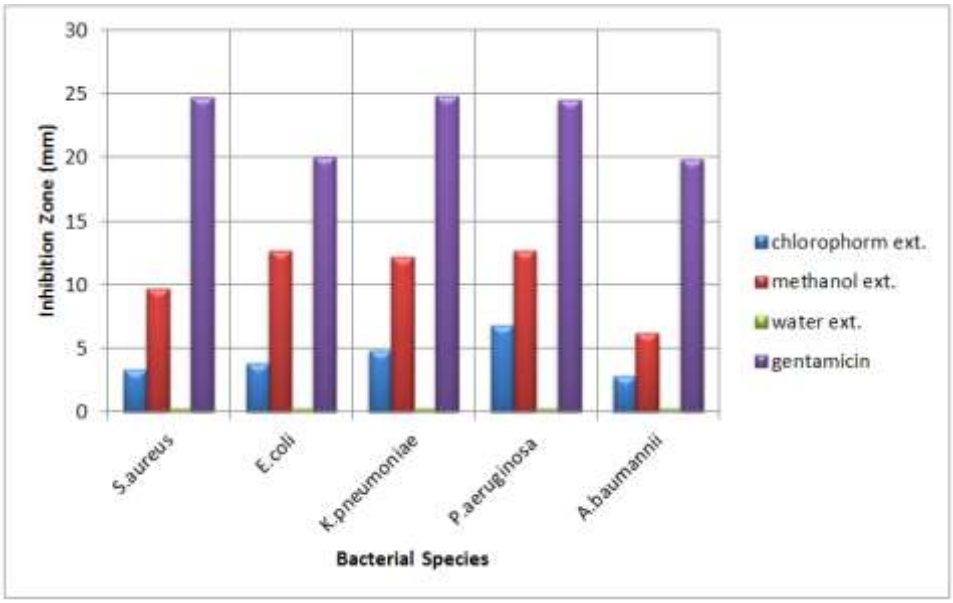

Fig-1: Inhibition zone in diameter (mm) around the discs impregnated with each of the three extract (100µg/disc), gentamicin is the positive control. (Values are average of triplicate)

The minimum inhibitory concentration (MIC) of methanolic extract ranged from 3.12mg/mL for each of *E.coli*, *K.pneumoniae* and *P.aeruginosa*, to 12.5mg/mL for *A.baumannii* and 6.25mg/mL for *S.aureus*. (Table 1). Antibacterial effect of *C. molmol* methanol extract was significantly higher on *E.coli*, *P.aeruginosa* and *K.pneumoniae* in compared to *A.baumannii and S.aureus*.

Table-1: Minimum inhibitory concentration (MIC) of methanol extract of *C. molmol* against bacterial isolates.

Minimum Inhibitory Concentrations (MIC) in mg/ml					
***C. molmol* Methanol extract**	**Bacterial isolates**				
	S. aureus	***E. coli***	***K. pneumoniae***	***P. aeruginosa***	***A. baumannii***
	6.25	**3.12**	**3.12**	**3.12**	**12.5**

Phytochemical screening

The methanol extract of oleo-gum resins of *C. molmol* was phytochemically screened and the results are shown in figure (2). The methanol extract showed the presence of terpenoids, tannins in high levels concentration, also alkaloids, saponins and flavonoids. These phytoconstitutes detected in *C. molmol* may be responsible for the antibacterial activity of the plant.

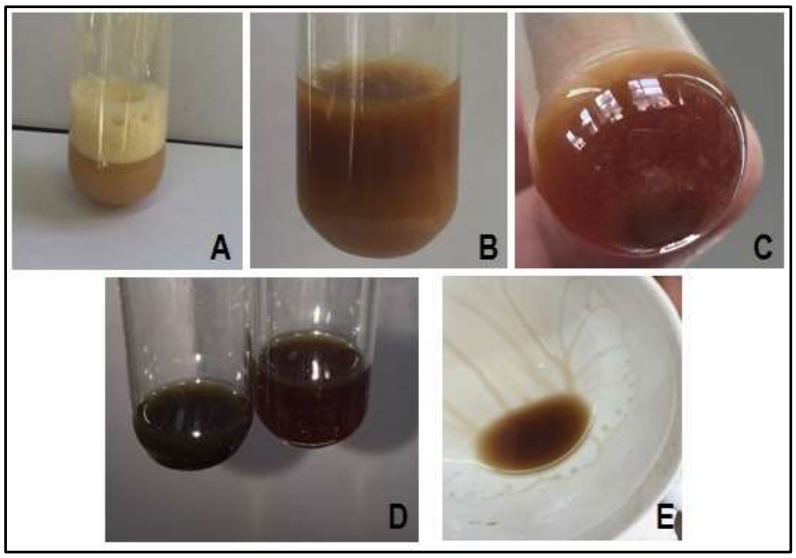

Fig-2: General phytochemical screening, where A, B, C, D and E represent the test results for saponins, flavonoids, alkaloids, tannins and terpines respectively.

Gas Chromatography Mass Spectrometry (GC-MS) investigation of *Commiphora molmol* (Myrrh) methanol extract

The data of the results obtained through GC-MS analysis of the methanol extract of *C. molmol* (Myrrh) is presented in table (x) and chromatogram showing the different peaks of the different constitutes of the methanol extract with highest intensity in peak number twenty two that recorded β-elemene with retention time 10.82 (Figure X). And other sesquiterpenes constitutes: δ-Elemene (Line #15, 2.15%), Copaene (Line #17, 0.17%), δ-Cadinol (line #25, 0.15%), and other sesquiterpenes in Line # 18, 20, 21, 27, 33 & 40. (Figure 3, Table 2)

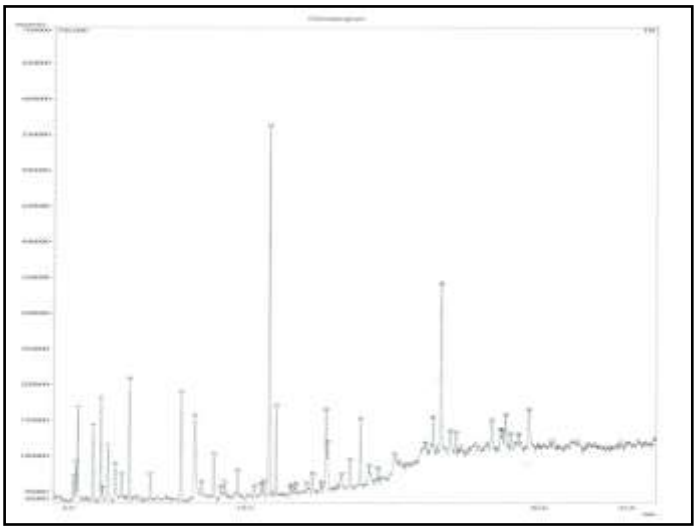

Fig-3: GC-MS chromatogram showing the different peaks of the different constitutes of the methanol extract of *C. molmol*

Table-2: List of selected chemical fractions identified by GC-MS in the methanol extract of *C. molmol.*

Peak	R.Time	Area	Area %	Height	Height%	Name
15	8.94	165310	2.15	58434	2.09	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)- $$ p-Menth-3-ene, 2-isopropenyl-1-vinyl-, (1S,2R)-(-)- $$ .delta.-Elemene $$ 3-Isopropenyl-1-isopropyl
17	9.31	54454	0.71	18742	0.67	Copaene $$ Tricyclo[4.4.0.02,7]dec-3-ene, 1,3-dimethyl-8-(1-methylethyl)-, stereoisomer $$ Tricyclo[4.4.0.02,7]dec-3-ene, 8-isopropyl-1,3-dimethyl-, (1R,2S,6S,7S,8S)-(-)- $$ .alpha.-
18	9.75	94287	1.22	31712	1.13	Cyclobuta[1,2:3,4]dicyclopentene, decahydro-3a-methyl-6-methylene-1-(1-methylethyl)-, [1S-(1.alpha.,3a.alpha.,3b.beta.,6a.beta.,6b.alpha.)]- $$ Cyclobuta[1,2:3,4]dicyclopentene, 1,2,3,3a
20	10.56	21699	0.28	12160	0.43	Tricyclo[2.2.1.0(2,6)]heptane, 1,7-dimethyl-7-(4-methyl-3-pentenyl)-, (-)- $$ .alpha.-Santalene $$ (-)-.alpha.-Santalene $$ Santalen $$ Santalene $$
21	10.64	34810	0.45	16102	0.58	1H-Benzocycloheptene, 2,4a,5,6,7,8,9,9a-octahydro-3,5,5-trimethyl-9-methylene- $$ 3,5,5-Trimethyl-9-methylene-2,4a,5,6,7,8,9,9a-octahydro-1H-benzo[a]cycloheptene # $$
22	10.82	1305318	16.96	515241	18.43	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]- $$ Cyclohexane, 2,4-diisopropenyl-1-methyl-1-vinyl-, (1S,2R,4R)- (-)- $$ .beta.-Elemene, (-)-
25	11.62	11835	0.15	6430	0.23	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1.alpha.,4.beta.,4a.beta.,8a.beta.)]- $$ 1.beta.-Cadin-4-en-10-ol $$ .delta.-Cadinol, (-)- $$ (-)-.delta.-Cadinol
27	12.12	65454	0.85	11224	0.40	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene
33	13.58	86730	1.13	33768	1.21	beta.-Panasinsene
40	16.69	656605	8.53	231852	8.29	(-)-solongifolol, acetate

DISCUSSION

The methanol extract of *C. molmol* olego-gum resin, exhibited the highest inhibitory effect against the bacterial species in compared to the other extracts, this result resembled previous studies stating that the methanol extract exhibited the highest antibacterial activity [16,17], Methanolic extract dominated in inhibiting the growth of the pathogenic bacteria under study, but were less potent when compared to those of gentamicin used as positive controls, similar results were found in a previous study using kanamycin as positive control [18].

According to Salvat *et al.*, plant extracts with MIC less than/or around 0.5 mg/ml (500µg/ml) indicate good antibacterial activity [19]. Based on this, it is concluded that methanol extracts of *C. molmol* exhibited good antibacterial activity against *E.coli*, *K.pneumoniae* , *P.aeruginosa* and *S.aureus*. A previous study confirmed that the methanol extract of *C. molmol* had very good antibacterial activity with lower MIC values [16], High MIC values may indicate that active compounds in the extracts may be present in low concentrations could be due to the method of extraction.

The methanol extract of *C .molmol* oleo-gum resin was phytochemically screened and the results showed the presence of terpenoids, tannins, alkaloids, saponins and flavonoids, which may be responsible for the antibacterial activity of the extract, current investigation revealed that studied herbal extracts possess potential antibacterial activity against entire tested organisms and the methanol extract was found to have shown the strongest and broadest spectrum.

These results are in agreement with a recent study where phytochemical screening of *C. molmol* oleo-gum resins (myrrh) showed the presence of Isoprenoids (Terpenoids), sterols, steroids and tannins in high levels concentration [20].

In the present study the Gas Chromatography Mass Spectrometry (GC-MS) investigation of *C. molmol* (myrrh) methanol extract showed the different peaks of the different constitutes with highest intensity in line twenty two that recorded β-elemene (classified as sesquiterpenes) with retention time 10.82. And other sesquiterpenes constitutes; δ-Elemene (Line #15,

2.15%), Copaene (Line #17, 0.17%), δ-Cadinol (line #25, 0.15%), sesquiterpenes are a class of terpenes that consist of three isoprene units and have the formula $C_{15}H_{24}$, result are in line with the fractions of phytochemicals documented previously for *C. molmol* by GC-MS shows that the resin is rich in sesqiuterpenes that possess anti-inflammatory and antitumor activity Antibacterial and antifungal activity in myrrh [21].

As a herbal medication, *C. molmol* is used as an effective antimicrobial agent, for sore throats, and gingivitis the sesquiterpene rich fractions of *C. molmol* have antibacterial and antifungal activities against Gram positive and Gram negative bacteria and *Candida albicans* at a minimum inhibitory concentration of 0.18–2.8 μg/mL [22].

However, further investigations regarding the chemical constituents of the oleo-gum resins of *C. molmol* (myrrh) plant are required.

CONCLUSION

In conclusion, oleo-gum resin methanol extract from *C. molmol* (myrrh) has shown some degree of antibacterial activity. The phytochemical analyses of methanol extract contain some active principles: terpenoids, tannins, alkaloids, saponins and flavonoids, GC-MS analyses showed the presence of sesquiterpenes as major constituents of the oleo-gum resins of the plant. These results confirm the antibacterial activity of gum resin and support the traditional use of the myrrh in therapy of bacterial infections.

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