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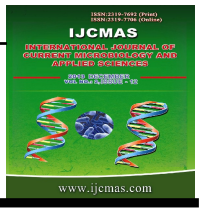

Original Research Article

Antimicrobial activity of essential oil and methanol extract from *Commiphora molmol* (Engl.) resin

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A B S T R A C T

Keywords

Antibacterial activity; *Commiphora molmol*; Essential oil; *Staphylococcus aureus.*

The aim of this work was to investigate the antibacterial properties of essential oil (Eo) and methanol extract from *C. molmol* resin against clinical microbial strains. The agar dilution method was used for assessment of bacterial growth inhibition at various concentrations. The results of our experiment showed that the oil from *C. molmol* has strong activity against clinical *S. aureus* isolates— including multidrug resistant strains. To the best of our knowledge, this is the first report of their antimicrobial properties of *C. molmol* resin oil against clinical isolates of *S. aureus*. Based on the results, the Eo and methanol extract tested can be considered as effective anti-staphylococcal natural products.

Introduction

The genus *Commiphora* (Burseraceae) with more than 150 plant species, is distributed in the tropical and subtropical regions, especially occurring in northeastern Africa, southern Arabia and India (Langenheim, 2003). The plants of *Commiphora* species are characterized as small trees or shrubs with spinescent branches, pale-gray bark and reddish-brown resinous exudates. The resinous exudates of the genus *Commiphora* are commonly used as perfume, incense, or embalming ointment, and their medicinal values have been gradually recognized by humankind (Langenheim, 2003). They are used in indigenous medicines for the treatment of wound, pain, arthritis, fractures, obesity, parasitic infection and gastrointestinal diseases (Abdul-Ghani *et al*., 2009). Diverse secondary metabolites including terpenoids, steroids, flavonoids, sugars, lignans, etc. have been discovered in this genus (Hanuš *et al*., 2005). Antiproliferative, anti-inflammatory, antimicrobial, hepatoprotective and cardiovascular properties of the purified metabolites and the crude extracts have been investigated (El Ashry *et al*., 2003).
The distribution of fifty-one constituents and medical uses of myrrh was reviewed

by El Ashry *et al* (2003). A review covering the chemical aspects of *Commiphora* species has appeared (Hanuš *et al*., 2005). The resin of *C.molmol* mainly used in Egypt as an antiparasitic agent, its medical use has been summarized recently (Abdul-Ghani *et al*., 2009 and Tonkal and Morsy, 2008). Plant resins with antimicrobial potential have been summarized, the resin of *Commiphora* species were included (Termentzi *et al*., 2011). Different from the writing objectives of above literatures, our review presents a comprehensive and up-to-date report on traditional uses, phytochemical aspects, pharmacological functions and toxicity of this genus. Besides, we focus on the pharmacological data reported since the year of 2000, to provide a probable scope of future research concerning this genus.

Not only the resin of this genus displays antimicrobial potential (Termentzi *et al*., 2011), but also the leaf, stem and bark are active against microorganism. Paraskeva *et al*. (2008) have studied antimicrobial potential of the leaf and stem extracts of ten *Commiphora* against four bacteria and two yeasts. Inhibitory effects of *C. swynnertonii* on bacteria and fungus have been investigated by Bakari *et al*. (2011). Mansumbinoic acid (**30**) possessed potent antibacterial activity against a multidrug-resistant strain *Staphylococcus aureus* with a MIC value of 4 μg/mL (Rahman *et al*., 2008).

The aim of this study was to evaluate the antibacterial activity of *C. molmol* essential oil and methanol extract from resin against microbial strains related to many human infections such as food spoilage, food safety and persistent hospital infection, 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

The resin powder of bark and stem of the plant by the process incision of *C. molmol* collected from supermarket in Al jouf Region, KSA. 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 sharing sunlight until water droplets got completely evaporated. Peel and plant were then kept in hot air oven for two days so that it could get dried. 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.

Preparation of the methanol extract

Resin *C. molmol* (50 g) was extracted with methanol (200 ml X 3 times) at room temperature. The methanol extract was combined and evaporated by a vacuum rotary evaporator at 45^{o}C to the dried powdered form (yield 2. 6%, w/w). The resulting extract was then lyophilized and kept in the dark at +4 °C until tested (Selim 2011).

Preparation of essential oil

Eo was obtained using the Clevenger hydrodistillation method. The plant material (about 300 g), was cut into small pieces, and placed in a flask (4 l) together with doubly distilled water (1.5 l). The mixture was boiled for 3 h, the collected Eo was dried with anhydrous sodium sulphate and kept at –18 °C until use.

Microbial strains

The clinical isolates were isolated from human and belong to the microbiological laboratory collection of the department of microbiology from Al Jouf University, Saudi Arabia.

Drop-diffusion assay

The drop diffusion assay was performed according to the modified Kirby-Bauer disc diffusion method (Diab *et al*., 2002; 2004; Selim *et al*., 2013). One ml of each test organism liquid culture was individually suspended in 3 ml of a 0.9% NaCl solution. The Eo and methanol extract were dissolved in 10% dimethylsulfoxide (DMSO) to a final concentration of 500 µg/ml as stock solution and sterilized by filtration through 0.45 µm Millipore filters. Antimicrobial tests were then carried out using 100 µl of suspension containing 10^8 cfu/ml of bacteria spread on nutrient agar media. The drop with 100 and 250 µg of the essential oil and methanol extract, and then placed onto inoculated agar. Negative controls were prepared using the same solvent employed to dissolve the extract. The inoculated plates were incubated at 37°C for 24 h for clinical bacterial strains and 48 h for yeast isolates. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms (Selim *et al*., 2012).

Minimal inhibitory concentration (MIC)

The EO and methanol extract were tested for antibacterial activity using the macrobroth dilution method in broth media Mueller-Hinton (Difco). In these experiments, 0.4 ml of a suspension containing 1 x 10^6 CFU/ml was added to 3.6 ml of susceptibility test broth containing serial twofold dilutions of the EO and methanol extract in glass test tubes (13 by 100 mm) fitted with loose plastic non screw caps. All tubes were incubated in air at 37°C for 24 hr before being read. The MIC was considered the lowest concentration of the sample that prevented visible growth. Minimum bactericidal concentrations (MBCs) were determined by subculturing, 10 ml from each negative tube and from the positive growth control. MBCs were defined as the lowest concentration yielding negative subcultures or only one colony. All samples were examined in duplicate in three separate experiments (Al-Ruwaili *et al*., 2012a; 2012b; Selim 2012).

Results and Discussion

The antimicrobial activity of the *C. molmol* Eo and methanol extract from resin were screened against nine organisms by disc diffission method. The Methanol, negative control showed no inhibiting effect for all three species. The results of the antimicrobial activity of the Eo and methanol extract are presented in Table 1. At the 100 µg/disc concentration, the methanol extract of *C. molmol* had a very broad spectrum of activity against *Staphylococcus aureus* (Fig. 1). The essential oil of *C. molmol* was found to be more effective than standard antibiotic against *B. cereus*, *E. coli* and *K. pneumoniae* with a zone diameter of 16, 13, 8and 12 mm, respectively. The essential oil of *C. molmol* was found to have a low activity against tested microorganisms than methanol extract. No activity found against yeast.

MICs for all bacterial tests are reported in Table 2. The EO inhibited *S. aureus* at a concentration of 100 µg/ml. In contrast to

Table.1 Antimicrobial activity of the essential oil and methanol extract from of *C. molmol* by diffusion technique on solid media.

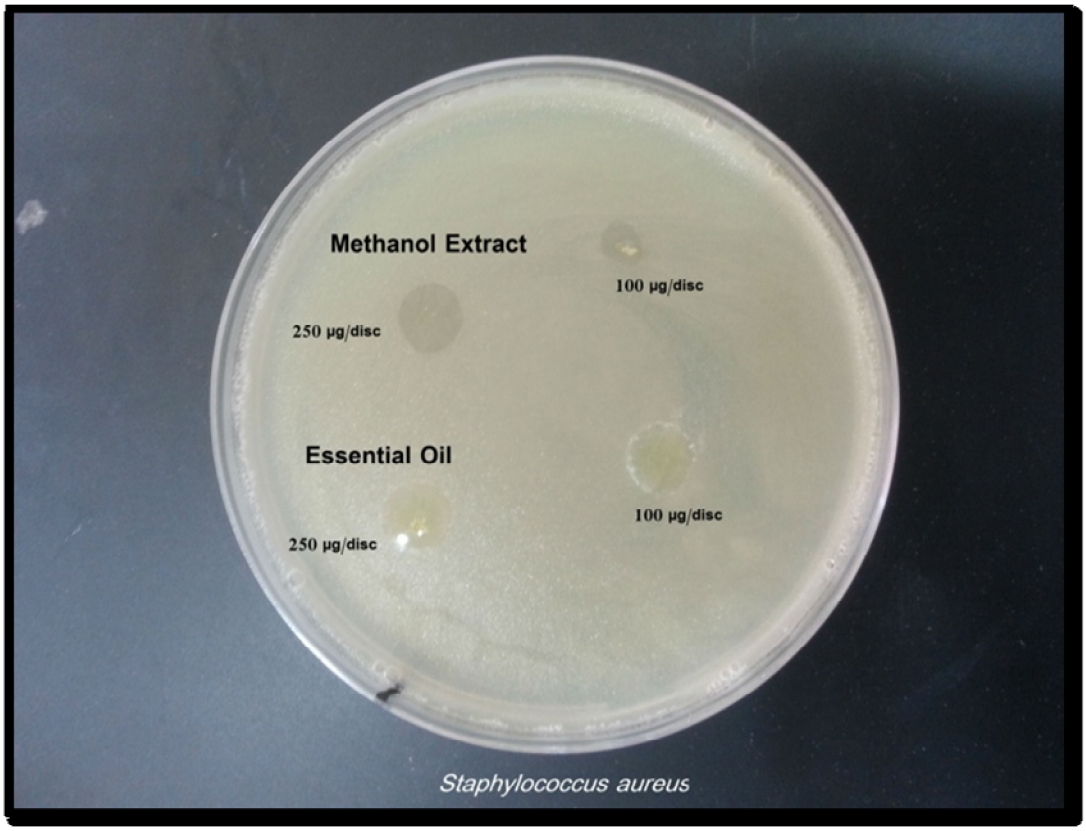
Microorganism	**Essential oil**	**Methanol extract**
Gram Positive Bacteria		
Bacillus cereus	7	13
Bacillus subtilis	7	11
Enterococcus feacalis	-	-
Serratia marcescens	-	-
Staphylococcus aureus	8	18
Gram Negative Bacteria		
Aeromonas hydrophila	-	-
Escherichia coli	6	8
Klebsiella pneumoniae	9	12
Proteus vulgaris	-	-
Pseudomonas aeruginosa	-	-
Salmonella indica	-	-
Yeast		
Candida albicans	-	-
Saccharomyces cerevisiae	-	-

* Inhibition zone in diameter (mm) around the discs impregnated with essential oil and methanol extract (100 µg/disc); (–) no antimicrobial activity. Values are average of triplicate.

Table.2 Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (µg/ml) of the essential oil and methanol extract from of *C. molmol*

Microorganism	**Essential oil**		**Methanol extract**	
	MIC	**MBC**	**MIC**	**MBC**
Bacillus cereus	250	250	100	150
Bacillus subtilis	250	250	100	150
Staphylococcus aureus	100	250	50	50
Escherichia coli	250	250	100	250
Klebsiella pneumoniae	50	100	100	250

Figure.1 Showed antimicrobial activity of the essential oil and methanol extract of *C. molmol* by diffusion technique on *Staphylococcus aureus*.



the relatively low MIC of methanol extract for *S. aureus* were inhibited by methanol extract with MICs 50. The minimum concentration of antimicrobial necessary to kill an organism, MBC, should be equal to or greater than the MIC for that microbe. In this study six bacterial strains presented MBCs which were within one twofold dilution of the MIC obtained for these organisms. The MICs for *B. cereus*, *E. coli* and *K. pneumoniae* ranged from 50 to 250 µg/ml.

The findings of this study are in agreement with El-Ashry *et al*., (2003) who showed that there is a different *Commiphora* species have a considerable antimicrobial activity against some gram positive and gram negative bacteria. Recently, it was found that *C. molmol* has antibacterial activities against some strains of *S. aureus*, *Salmonella enterica* and *Klebsiella pneumoniae* (Rahman *et al*., 2008). Indeed, little is known about the sensitivity of *S. aureus* stains to plant extracts as they were poorly documented in literature. However, medicinal plant extracts might be a promising source of new antibacterial agents, particularly against MRSA. The results obtained with the *C. molmol* Eo and methanol extract suggest that these species have antimicrobial activity against gram positive and gram negative bacteria. These results suggest that *C. molmol* Eo and methanol extract could be used to treat infections and used for traditional medicine in the future. Further investigations are necessary to identify the components of these essential oils of *C. molmol*.

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