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Full Length Research Paper

Antibacterial activity of *Cymbopogon citratus* and *Rosmarinus officinalis* essential oil against carbapenems resistant *Klebsiella pneumonia* strains (as nosocomial pathogen) isolated from intensive care units of two hospitals

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The present study shows the antibacterial activity of *Cymbopogon citratus* and *Rosmarinus officinalis* essential oils. The essential oil from the dried leaves was extracted by Hydrodistillation. the disc diffusion test was performed as antibacterial tests. Monoterpene hydrocarbons were The dominant compounds in *Cymbopogon citratus* oil, camphor□□-pinene, □-terpineol and 1,8-cineole were the major compounds in Rosmarinus officinalis oil. *Cymbopogon citratus* oil revealed a good antimicrobial activity against carbapenems resistant *Klebsiella pneumonia* strains while no *Rosmarinus officinalis* activity was acheived . The findings suggest possibility of application of Cymbopogon citratus oil in treatment of the nosocomial infections caused by this microorganism.

Keywords: *Cymbopogon citrates, Rosmarinus officinalis*, essential oil, antibacterial activity, *Klebsiella Peumoniae*, nosocomial infections

INTRODUCTION

The emergence of antimicrobial resistance, despite the availability of newer antibiotics, has become an increasing problem throughout the world, particularly in pathogens causing nosocomial infections (Syed *et al.,* 2013) and the occurrence of this resistance in the intensive care unit (ICU) is an emerging global concern (Vlek *et al.,* 2012). Because of the spread of antibiotic resistance and the growing demand on antimicrobials for preventing bacterial infections, there is an increasing interest in medicinal plants as an alternative to antibiotics (Mimoune *et al.,* 2013). The essential oils and their components are gaining increasing interest because of their relatively safe status and their potential use. The main advantage in the use of such natural agents is that they do not present the phenomenon of drug-resistance, commonly encountered with the long-term use of antibiotics (Abdellatif *et al.,* 2014).

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Essential oils are a rich source of biologically active compounds.these compounds had specific as well as general antimicrobial activity and antibiotic potential. An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable (Shaik et al., 2014)

MATERIALS AND METHODS

1. Plant Material

The leaves of *Cymbopogon citratus* and *Rosmarinus officinalis* plants were collected from fields of agricultural research center at Giza, this species was identified by medicinal plants department, horticulture institute, agricultural research center. The specimens were dried (drying in the shade, away from humidity, at room temperature for 7 days).

1) Extraction of essential oil

Hydrodistillation of the plant material was performed in a Clevenger-type apparatus for 4h . The *Cymbopogon citrates* and *Rosmarinus officinalis* oil obtained were amber yellow, clear, liquid at room temperature and its odor was intensive lemony, herbal. After its isolation, the essential oil was collected and stored in steeled glass vials in refrigerator at 4°.

Antibacterial activity

In vitro antibacterial activity of *Cymbopogon citratus* and *Rosmarinus officinalis* essential oil was evaluated by disc diffusion method.

Microorganisms under study

Fourteen strains of carbapenems resistant *Klebsiella peumoniae* were isolated from blood, sputum, wounds and urine samples from trauma and chest intensive care units of Assiut university hospital and trauma intensive care unit of Sohag university hospital . Stock cultures were grown in Mueller-Hinton broth at 37° for 18-24h before the test.

Disc diffusion method

The bacterial suspension was adjusted to a density of bacterial cells of 1.0×10^8 UFC/mL (or 0.5 McFarland turbidity units). It spread on the Mueller Hinton Agar (MHA) plates by sterile swab. Sterile filter paper discs Whatman (6mm in diameter) were impreganted with 10µl of the oil then placed on the inoculated plates aseptically. They were incubated for 24h at 37°C. The diameters of the inhibition zone was calculated in millimeters to classify the EOs as follows: resistant (< 8 mm), moderately sensitive (+) (a 8–14mm diameter), sensitive (++) (a 14–20mm diameter), and very sensitive (+++) (> 20mm).

RESULTS

Seven components in the essential oil of *Cymbopogon citratus*, representing 94.33 % of the oil, were identified while There are three components were unknown representing 5.67%. Monoterpene hydrocarbons were The dominant compounds represented 86.64% and including geranial (44.36%), neral (38.57%), myrcene (3.71%). Table (1)

Table1. Chemical composition of *Cymbopogon citratus* essential oil

Peak no.	Component	Area %
1	Myrcene	**3.71**
2	unknown	**4.01**
3	Limonene	**1.23**
4	unknown	**0.90**
5	unknown	**0.76**
6	Linalool	**2.70**
7	Neral	**38.57**
8	Geranial	**44.36**
9	Geranyl acetate	**2.22**
10	□-caryophellene	**1.53**

Twelve components were identified in the essential oil of *Rosmarinus officinalis*, representing 93.03% of the total oil and seven unknown components were representing 6.97%. The major constituents were camphor (18.48%), □-pinene (14.09%), □-terpineol (13.73%) and 1,8-cineole (12.90%). Table(2)

Table2. Chemical composition of *Rosmarinus officinalis* essential oil

Peak no.	Component	Area %
1	□-pinene	**14.09**
2	camphene	**4.52**
3	□-pinene	**3.47**
4	Limonene	**1.59**
5	1,8-cineole	**12.90**
6	unknown	**0.65**
7	unknown	**1.42**
8	Linalool	**4.37**
9	camphor	**18.48**
10	□- terpineol	**13.73**
11	borneol	**11.51**
12	unknown	**2.08**
13	unknown	**2.13**
14	bornyl acetate	**6.43**
15	Eugenol	**0.46**
16	unknown	**0.37**
17	□-caryophellene	**1.11**
18	unknown	**0.29**
19	unknown	**0.40**

Table3. The antimicrobial activity of essential oil of *Cymbopogon citratus* and *Rosmarinus officinalis* against carbapenems resistant *K.pneumoniae* strains isolated from ICUs of Assiut and Sohag university hospitals

	inhibition zone diameter					
Carbapenems resistant K.pneumoniae strains	*Rosmarinus officinalis L*			*Cymbopogon citratus*		
	1/2	1/4	1/8	1/2	1/4	1/8
TraumaICU-Assiut university hospital						
strain No 1	5	0	0	0	0	0
strain No 2	21	15	0	0	0	0
Chest ICU- Assiut university hospital						
strain No 3	15	12	3	0	0	0
strain No 4	5	0	0	0	0	0
strain No 5	0	0	0	0	0	0
strain No 6	0	0	0	0	0	0
strain No 7	16	6	0	0	0	0
Trauma ICU- Sohag university hospital						
strain No 8	10	0	0	0	0	0
strain No 9	11	0	0	0	0	0
strain No 10	0	0	0	0	0	0
strain No 11	3	0	0	0	0	0
strain No 12	0	0	0	0	0	0
strain No 13	0	0	0	0	0	0
strain No 14	0	0	0	0	0	0

The essential oil of *Cymbopogon citratus* had antibacterial activity for carbapenems resistant *Klebsiella peumoniae* strains at some of the used dilutions. The strain No 2 was highly sensitive to oil at 1/2 (inhibition zone:21 mm) and sensitive at 1/4 (inhibition zone:15 mm), strain No 7 and strain No 3 were sensitive at 1/2 (inhibition zone: 16, 15 mm). The essential oil of *Rosmarinus officinalis* had no antibacterial effect at all used dilutions. Table(3)

DISCUSSION

Nosocomial infection continues to be a major public health concern throughout the world because of the associated mortality and socioeconomic costs (Jroundi et al., (2007). The essential oils as plants preparations highlighted as new antimicrobial agents for treatment of nosocomial infections Zenati *et al.,* (2014). In this study high antimicrobial activity of *Cymbopogon citratus* essential oil to carbapenems resistant *K.pneumonia* strains was due to essential oil was characterized by the presence of two dominating components of monoterpene hydrocarbons (geranial 44.36% and neral 57%) Abdellatif et al (2014).

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