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Antimicrobial Activity of Saudi Mint on some Pathogenic Microbes

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The methanol extract of *Mentha pulegium* exhibited significant effect on some pathogenic microbes. Four of them were Gram positive bacterial isolates (*Bacillus subtilis, Klebsiella* sp., *Sarcina maxima* and *Staphylococcus aureus*), four Gram negative bacteria (*Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* and *Salmonella arizonae*) and one unicellular fungus (*Candida albicans*) were evaluated for there resistance against these extracts. The bacterial isolates showed sensitivity at well concentrations of 2 and 3 mg/disc. *Klebsiella* sp. and blood MRSA showed high clear zone at 3mg/disc to record 19mm. *C. albicans* showed sensitivity at disc potencies of 2 and 3 mg. on the other hand *Pseudomonas aeruginosa* was the most resistant microbe that was not showed any inhibition zone.

Key words: Antimicrobial Activity, Methanol Extract, *Mentha pulegium*, KSA.

The increasing technological skills in chemical, pharmacological and microbiological researches nowadays, has lined the way for efficient screening of higher plants for active compounds (Yakubu and Mukhtar, 2011). In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases (Service, 1995; Iwu *et al.,* 1999). In addition to this, antibiotics are sometimes associated with adverse effects. Therefore, there is a need to develop alternative antimicrobial medicines for the treatment of infectious diseases from other sources such as plants (Cordell, 2000). Natural products of higher plants may be a new source of antimicrobial agents possibly with novel mechanisms of action (Barbour *et al*., 2004). Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts. Most of their properties are due to essential oils produced by their secondary metabolites (Adam *et al.,* 1998). Essential oils and extracts from several plant species are able to control microorganisms related to skin, dental caries and food spoilage, including Gram negative and Gram-positive bacteria (Sartoratto *et al.,* 2004).

Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that medicines derived from plant are safe and dependable, compared with costly synthetic drugs that have adverse effects (Iwu *et al.,* 1999; Gordon & David, 2001).

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To keep out potential invaders, plant produces a wide range of selective antimicrobial compounds either in a constitutive or inducible manner (Cuilel, 1994). Secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medical agents. *Mentha*, the genus of Labiatae family, includes 20 species that spread all over the world.

Leaves, flowers, and the stems of *Mentha* spp. are frequently used due to their antiseptic properties (Derwich, Benziane, & Boukir, 2010b) and in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavor (Gulluce *et al*., 2007; Moreno, Bello, Primo-Yufera, & Esplugues, 2002). Several studies have investigated the chemical composition of the *Mentha pulegium* EOs grown in different geographical regions (Derwich *et al*., 2010b; Hajlaoui *et al*., 2009; Lorenzo *et al*., 2002; Mahboubi & Haghi, 2008). The plant oil has also proved to have antibacterial activity (Derwich *et al*., 2010b; Hajlaoui *et al*., 2009; Mahboubi & Haghi, 2008).

M. pulegium flowering aerial parts have been traditionally used for its antiseptic for treatment of cold, sinusitis, cholera, food poisoning, bronchitis and tuberculosis (Zargari, 1990).

The aim of this study was to evaluate the antimicrobial activity of Iranian *Mentha pulegium* L. against different microorganisms in order to validate its traditionally used.

MATERIALS AND METHODS

Collection of the plant material

Mentha pulegium (Medina Mint) was collected from the market in Medina Menawara, KSA.

Extraction of plant material

The whole plant except roots were washed gently and thoroughly with distilled water and dried at room temperature. This was finely powdered using a clean mortar and pestle and sieved to get a fine powder. 20 g of the powder soaked in 100 ml 80% methanol and shacked for 72 hours with constant shaking at regular intervals. The solution was then filtered and the solvent were evaporated in oven at 60°C and extracts were stored in a refrigerator at 4°C until needed for further analysis (Adoum *et al*., 1997).

The organic extract of the plant were checked for its antimicrobial activity against pathogenic bacteria inoculated in Mueller-Hinton and the fungus organism in Saborauds dextrose (agar plates). Mint extract were inoculated in the wells in pre-seeded agar plates at concentrations 0, 1, 2 and 3mg/ hole. Reference standard of different antibiotics were inoculated in the same Petri plates as a positive control. The plates were kept undisturbed for 2 to 3 h and then incubated for 24 h at 37°C aerobically. Finally, the plates were examined for the presence of inhibition zones by the help of Digital caliper in mm (Reinheimer *et al*., 1990).

Preparation of pathogen microorganisms

Ten pathogenic bacteria [*Escherichia coli* (pus), *Enterobacter cloacae* (Blood), *Klebsiella pneumoniae* (Pus), *Klebsiella Sp.*, *Proteus mirabilis* (Sputum), *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Salmonella choleraesuis*, *Salmonella typhi, Staphylococcus aureus*)] and one fungal species (*Candida albicans*) were selected for testing their responses to the treatment by the investigated disinfectant. These isolates were delivered from the culture collection sector of microbiology, Al-Jouf University, and from Al Sedairy hospital laboratories. Test microorganisms were inoculated to nutrient broth (Merck, Darmstad) and incubated at appropriate incubation conditions until the concentration reached 10^7 to 10^8 cfu/ml.

Determination of antimicrobial activity

The antimicrobial activity of the disinfectant was determined by using the well diffusion method. A total of four hand disinfectants were used, they were hexamide (30%), ethyl alcohol (70%), sterillium and fast clean jel. Each material was checked for antimicrobial activity against pathogenic microbes in inoculated Mueller-Hinton agar plates. Inoculating the micro-flora on the untreated and the treated hands in nutrient agar plates by finger print technique. A volume of 50 and 100µl for each disinfectant solution was inoculated in the wells in pre-seeded agar plates. The plates were kept undisturbed for 2 to 3 h and then incubated for 24 h at 37°C aerobically, and at 28°C for the fungal species. Finally, the plates were examined for the presence of inhibition zones by the help of Digital caliper in mm (Reinheimer *et al*., 1990).

RESULTS AND DISCUSSION

The search for new products to control either pathogenic or food spoilage microorganisms is a promising area of research. Natural compounds produced by the secondary metabolism of plants, as EOs, are a potentially important source of new types of food preservatives. In spite of the increasing research in this field, more studies about the antimicrobial activity or chemical composition of many of them are still required (Ait-Ouazzou *et al*., 2012).

The current investigation revealed different sensitivities depending on the bacterial strain. *Mentha pulegium* extract inhibited the growth of the tested microbes except *Pseudomonas sp.*, *Klebsiella Sp* 1 and *Proteus* sp. and recorded inhibition zones ranged between 10mm at concentration 2mg to 26mm at concentration of 3mg. The highest inhibition was observed for *Candida albicans* (26 mm) whereas the most resistant isolates were *Pseudomonas* sp. and *Proteus* sp. (Table 1 and Figure 1). The methanol extract of *M. pulegium* was very effective to inhibit the growth of most tested bacteria in comparison of commercial antibiotics (Table 2). The antibacterial activity of this material might be due to the presence of pulegone, menthone and neo-menthol. Duru *et al*. (2004) have demonstrated the strong antimicrobial activity of pulegone against a set of bacteria, including *S. typhimurim* and *E. coli*.

Similarly, Angioni *et al*. (2003) also

Table 1. Effect of *Mentha pulegium* methanol extract on the test microbes

Bacterial isolates	Inhibition zone diameter (mm)		
	1mg	2mg	3mg
Wound Methicillin resistance *Staphylococcus aureus*	0	15	19
S. aureus	0	11	14
Escherichia coli	0	0	17
E.coil wound swab	0	12	15
Pseudomonas sp.	0	0	0
Salmonella typhi	0	10	18
Klebsiella Sp 1	0	0	0
*Klebsiella Sp*2	0	14	19
Proteus sp.	0	0	0
Candida albicans	0	22	26

Table 2. Effect of the antibiotic against the studied bacterial strains

	Wound MR *Staphylococcus aureus*	*S. aureus*	*E.coil*	*E.coil* wound swab	*Pseudomonas sp.*	*Salmonella typhi*	*Klebsiella Sp*1	*Klebsiella Sp2*
AMC	-	32	-	35	18	15	25	20
VA	26	-	10	23	-	19	25	-
TE	26	29	-	25	21	23	23	
AM	10	20	-	17	12	25	17	13
CX	-	-	-	30	-	-	32	-
PB	-	25	14	17	12	13	25	-
CDZ	-	35	19	22	20	-	22	32

* AMC = AMOXICLIN CLAVULANIC ACID conc 30.
* TE= TETRACYCLINE. conc 30.
*CX= CLOXACILLIN. conc 1.
* CDZ= CEFODIZIME . conc 30.
* VA= VANCOMYCIN. conc 30.
* AM = AMPICLLIN. conc 10.
* PB= POLYMIXIN B. conc 300.

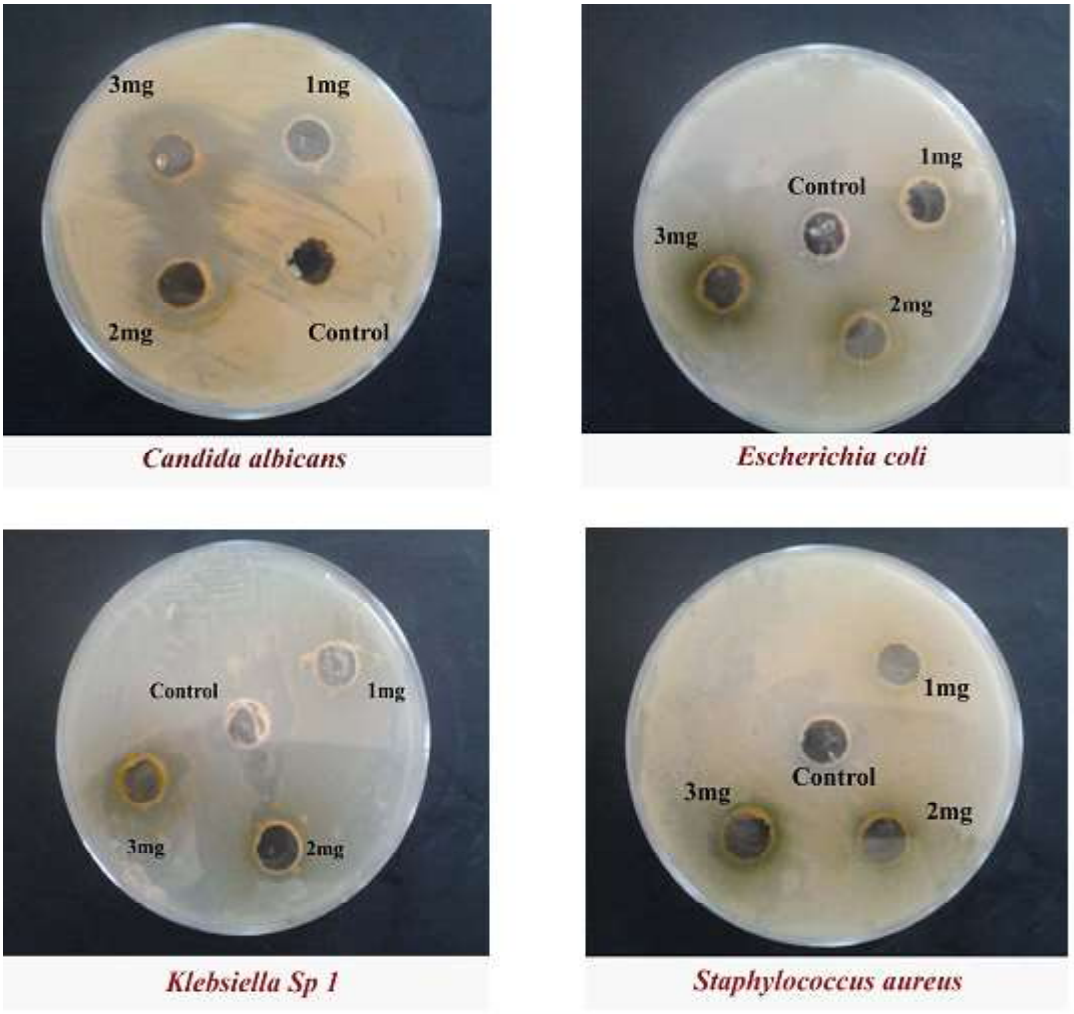

Plate 1. Inhibition zone of *Mentha pulegium* against the tested microbes.

reported that EOs from the leaves of *Juniperus phoenicea* exhibited weak activity against *S. aureus* and no activity against *E. coli* or *P. aeruginosa*. On the other hand, Derwich *et al*. (2010a) found that *E. coli* was the most sensitive strain tested to the EO of *J. phoenicea* with the strongest inhibition zone (34 mm), whereas inhibition zones of 24 mm were measured for *S. aureus*. Cytotoxicity of this material appears to include a bacterialmembrane damage that occurs when the essential oil passes through the cell wall and cytoplasmic membrane, and disrupts the structure of their different layers of polysaccharides, fatty acids and phospholipids (Bakkali *et al*., 2008).

Our results also indicate that *M. pulegium* could be an effective inhibitor of many of the strains studied. Similar results have been found in previous studies, which have reported this EO to have a potent antimicrobial activity by the disk diffusion assay (Hajlaoui *et al*., 2009; Mahboubi & Haghi, 2008).

CONCLUSION

In conclusion, the increasing antibiotic resistance of pathogens that associated with infectious diseases as well as undesirable of side effects of antibiotics suggested the use of *Mentha pulegium* L. extract as antibiotic or alternative. In

this way, *M. pulegium* extract has a huge potential as alternatives to synthetic preservatives in food industry. Further studies should evaluate the safety and toxicity of *M. pulegium* extracts to human consumption before considering their use for food preservation or medicinal purposes.

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