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Antimicrobial effects of *Lavandula stoechas L.* and *Rosmarinus officinalis* L. extracts on *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

In this study *Lavandula stoechas L.*and *Rosmarinus officinalis* L. extracted with ethanol 96° and the antimicrobial effects of extracts were evaluated on *Escherichia coli* PTCC 1330 and *Staphylococcus aureus* PTCC 1337 by "using the method of Collins" and "disk agar diffusion method". The results show that ethanolic extract was quite effective in 2000 µg/ml concentration on *Escherichia coli* PTCC 1330 and *Staphylococcus aureus* PTCC 1337 and were prevented from growth them on medium. In "disk agar diffusion method", 20, 40, 60 and 80 % alcoholic extract concentrations, was deterrented effect on *Staphylococcus aureus* PTCC 1337. The *Lavandula stoechas L.* and *Rosmarinus officinalis* L. extract presented the more effective impact on the growth of *Staphylococcus aureus* PTCC 1337than *Escherichia coli* PTCC 1330 ($p<0.05$). The results indicate that ethanolic extracts of *Lavandula stoechas L.* have the greatest effect on gram-positive bacterium *Staphylococcus aureus* PTCC 1337. As a result ethanolic extracts of *Lavandula stoechas L.*and *Rosmarinus officinalis* L., have been strong antimicrobial activity against many food pathogen bacteria.

1. Introduction

Food market trends are changing. Consumers demand more high-quality foods with fresh like attributes; consequently less extreme treatments and/or additives are being required. Lipid oxidation and bacterial contamination are the main factors that determine the loss of food quality and shelf-life reduction. Therefore, delaying lipid oxidation and preventing bacterial cross-contamination are highly relevant to food processors. Oxidative processes and bacterial contamination, in turn, contribute to the deterioration in flavor, texture and color of food products (Fernandez-Lopez et al., 2004). The infections risk related to pathogenic germs increases at the present time considering the increased resistance which certain microbes acquire, whose usual antibiotics are ineffective to treat the infectious disease. This is why that many diseases which we controlled formerly, have reappeared and escaped from human control. The antibacterial activities of essential oils from various medicinal plants against micro-organisms were described and proven in experiments by various researchers (Rahman etal., 2002; Inouye etal., 2001).

About 80 percent of the population relies on traditional medicine because western-trained medical personnel are limited or not really accepted by the community, and traditional healers are easily consulted, living in the same community. That points to the demand for Traditional Medicine Practitioners (TMPs) for medicinal plants and the fact that the majority of the people, rural and urban alike, depend largely on herbal medicines for treating a variety of diseases. This reliance is mainly due to the high cost of conventional medicine and inaccessibility of modern health care facilities in most areas (WHO 2002-2005).

Rosemary (*Rosmarinus officinalis* L.) is a spice and medicinal herb widely used around the world. Of the natural antioxidants, rosemary has been widely accepted as one of the spices with the highest antioxidant activity (Peng et al., 2005). Rosemary is known to have antioxidant and antibacterial properties (Shahidi et al. 1992). P u t n am et al. (2006) reported that rosemary essential oil inhibit osteoclast activity and increase bone density *in vitro*. Also, cytotoxic activity of rosemary essential oil has been demonstrated by several authors (E l -Me leigy et al. 2010).

The Lavenders are a genus of about 25 - 30 species of flowering plants in the mint family, Lamiaceae, native to the Mediterranean region south to tropical Africa and to the many regions of Asia. The genus includes annuals, herbaceous plants, sub-shrubs, and small shrubs (Piccaglia et al., 1993). Lavender has been used for centuries as an herbal remedy. Lavender yields a highly effective essential oil with very sweet overtones, and can be used in balms, salves, perfumes, cosmetics, and topical applications. Internally, Lavender essential oil is believed to be of benefit for a multitude of problems, including stress, anxiety, exhaustion, irritability, headaches, migraines, insomnia, depression, colds, digestion, flatulence, upset stomach, liver and gallbladder problems, nervousness, loss of appetite, and as a breath freshener and mouthwash (Kim et al., 2007; Katona et al., 2010).

The aim of this study was evaluated of antimicrobial effects of alcoholic of *Lavandula stoechas L*. and *Rosmarinus officinalis L*. leaves against *E. coli* and *Staphylococcus aureus* of the important food pathogen.

2. Materials and methods

2.1. Preparation plant

In this study *the Lavandula stoechas L. and Rosmarinus officinalis L.* purchased from local markets in Mashhad, Iran and the species were identifying in the herbarium of Ferdowsi Mashhad University.

2.2. Preparation of alcoholic extracts from *Lavandula stoechas L.* and *Rosmarinus officinalis L.*

Maceration method was used to prepare extracts. The amount 50 gram of *Lavandula stoechas L*. and *Rosmarinus officinalis L*. powder was added to 250 ml ethanol 96 degree. The alcoholic extract mixture was preserved at laboratory temperature for 24 hours and was stirred every few hours with a glass rod. (Ahmad and beg., 2001).

2.3. Determination dry weight of alcoholic *Lavandula stoechas L.* and *Rosmarinus officinalis L.* extracts

At first the weight of a tube were measured, and then 1ml of alcoholic extracts were poured in it. The contents of the tube were dried at room temperature. After drying the extract, the tubes were weighed again.

Weight differences are equivalent weight of 1ml alcohol extract. Average of three replicates, was calculated as the dry weight of the extract (Sattari et al, 2005).

2.4. Source of microorganisms

The bacterial strain used was *Escherichia coli* PTCC 1330 and *Staphylococcus aureus* PTCC 1337 for each test, to evaluating the antimicrobial effects, fresh medium was prepared.

2.5. Preparation of microbial suspension

To preparing microbial suspensions, requires 24-hour culture from each microorganism. So, 24 hours before experiments, microorganisms were inoculated from storage medium to nutrient agar medium slope. After 24 hours, the cultures were washed by Ringer solution and microbial suspensions were prepared. Then some of the bacterial suspension was poured in sterile tubes containing Ringer solution and its turbidity, was measured by spectrophotometer at 530 nm wavelength. It was diluted by Ringer solution until the solution turbidity equalizes with 5% McFarland standard solution. Suspension should have contains 1.5×10^8 CFU / ml (Valero and Salmeron., 2003).

2.6. Evaluation of antimicrobial activity

Adding extracts to the culture medium "according of the method of Collins *et al.* (1995)" and "disk agar diffusion method" were done and to evaluated the antimicrobial effects of alcoholic *Lavandula stoechas L*. and *Rosmarinus officinalis L*. extracts. Then 0.2 gram of ethanol extract, were added to 5 ml of sterile distilled water. Then it was stirred with vortex system to assist being steady. Then 1 ml of this solution was added to sterile plates. The final concentration of the extract was 2000 µg / ml (Babayi et al., 2004). In the next step, Mueller Hinton agar (Merck-Germany) medium were sterile and used for bacteria, were added to the plates, and placed at room temperature, so the medium was prepared. One loop of each standard strain culture media was cultured inoculums on these medium. The plates were incubated for 48 hours at 37 ° C. The culture with extract and without bacteria was used as control (Babayi et al., 2004; Collins et al., 1998). The disk agar diffusion method, at first a loop of each standard strain culture media was cultured on the plates, and then paper discs (from Whatman filter with 6 mm diameter), plates were saturated with 100 µl of the test compound allowed to dry and was introduced on the upper layer of the seeded agar plate with 20, 40, 60 and 80 % extract concentrations, were prepared in distilled water and was treated with *Lavandula stoechas L*. and *Rosmarinus officinalis L*. extract and placed in culture medium by Sterile loop. Then it was fixed on the media with a light little pressure. After incubation time, the diameter of free zone was measured exactly by using a ruler in millimeters. All experiments were performed with 3 replicates.

2.7. Statistical analysis

SPSS software was used for statistical analysis. The Tukey test was used for Samples comparison. One-way ANOVA with equal replications was used in order to determine the samples with significant average differences.

3- Results

The results of the antimicrobial effects of alcoholic extracts, by "using the method of Collins *et al.* (1995)" were show on in Tables 1, 2 and Figure 1).

Table 1
Antimicrobial effects of 2000µg/ml alcoholic *Lavandula stoechas L*. and *Rosmarinus officinalis L*.extract concentrations, on *E.coli* (using the method of Collins et al. (1995)).

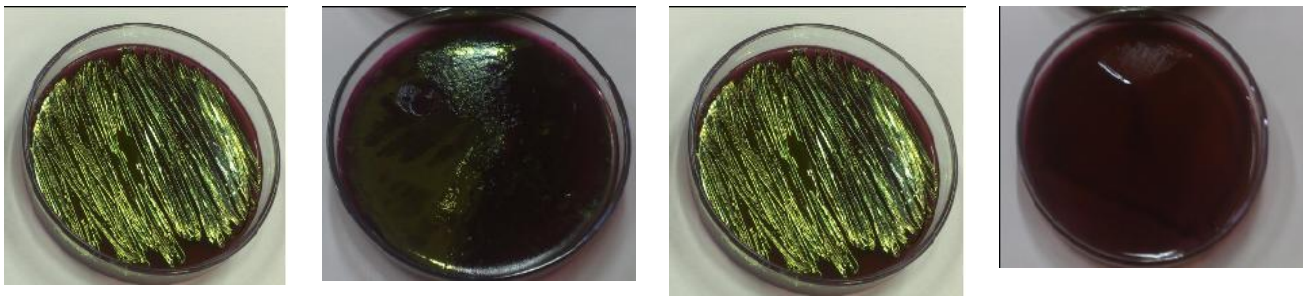
Extract	***E.coli***
Lavandula stoechas L.	+
Rosmarinus officinalis L.	+

(+) in Table showed no bacterial growth on culture and antibacterial ctivity of alcoholic *Lavandula stoechas L*. and *Rosmarinus officinalis L*.

Table 2
Antimicrobial effects of 2000µg/ml alcoholic *Lavandula stoechas L*. and *Rosmarinus officinalis L*.extract concentrations, on *S. aureus* (using the method of Collins et al. (1995)).

Extract	***S. aureus***
Lavandula stoechas L.	+
Rosmarinus officinalis L.	+

(+) in Table showed no bacterial growth on culture and antibacterial activity of alcoholic *Lavandula stoechas L*. and *Rosmarinus officinalis L*.



A: Control *Escherichia coli*

B: Antimicrobial effects of 2000µg/ml *Rosmarinus officinalis L*.extract concentrations

C: Control *Escherichia coli*

D: Antimicrobial effects of 2000µg/ml *Lavandula stoechas L*. extract concentrations

Fig. 1. Antifungal activity of alcoholic *Rosmarinus officinalis L*. *Lavandula stoechas L*. and extract on *Escherichia coli* (using the method of Collins et al. (1995)).

The results showed 2000 µg/ml concentration of both alcoholic extracts, were quite effective on reduce of growth *E.coli* and *S. aureus* and were had prevent growth over the medium.

The results of the antimicrobial effects of alcoholic *Lavandula stoechas L*. and *Rosmarinus officinalis L*. extracts, by "the agar diffusion method" are presented in Tables 3 and 4.

Table 3
Average diameter (mm) of microbial free zone area of by alcoholic *Rosmarinus officinalis L*. extract, on *E. coli* and *S. aureus* (disk agar diffusion method).

Microorganism		***E. coli***		
***Rosmarinus officinalis L*.concentration**	**20**	**40**	**60**	**80**
Average diameter (mm) of microbial free zone area	-	-	7.1±0/5774	8.7 ±0/2887
Microorganism		*S. aureus*		
Rosmarinus officinalis L.concentration	20	40	60	80
Average diameter (mm) of microbial free zone area	7.3±0/5774	8.7±0/5	11 ±0/5	14.4±0/2887

(-) in Table showed no inhibitory effects was shown

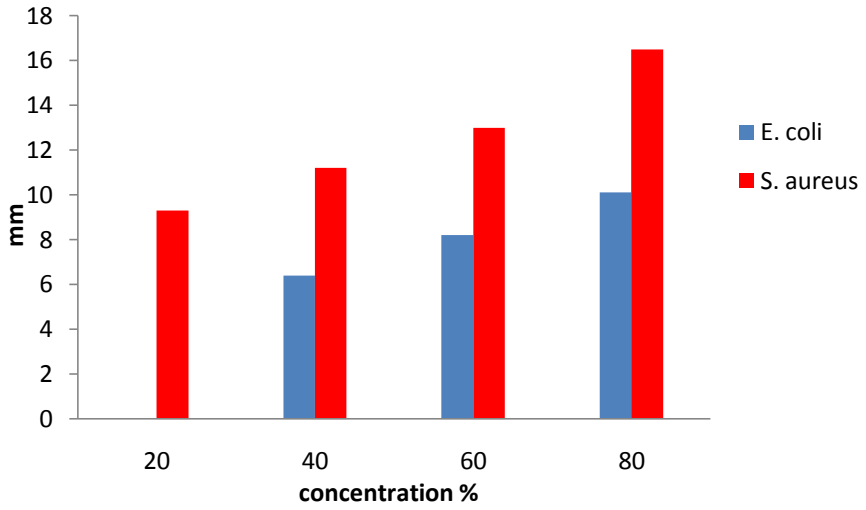


Fig. 2. Antimicrobial activity of alcoholic *Rosmarinus officinalis L*. extract, on *E. coli* and *S. aureus*.

Table 4
Average diameter (mm) of microbial free zone area of by alcoholic *Lavandula stoechas L*.extract, on *E. coli* and *S. aureus* (disk agar diffusion method).

Microorganism	***E. coli***			
***Lavandula stoechas L*.concentration**	**20**	**40**	**60**	**80**
Average diameter (mm) of microbial free zone area	-	6.4±0/5774	8.2±0/5	10.1 ±0/5
Microorganism		*S. aureus*		
Lavandula stoechas L. concentration	20	40	60	80
Average diameter (mm) of microbial free zone area	9.3±0/5774	11.2±0/5	13 ±0/2887	16.5±0/5

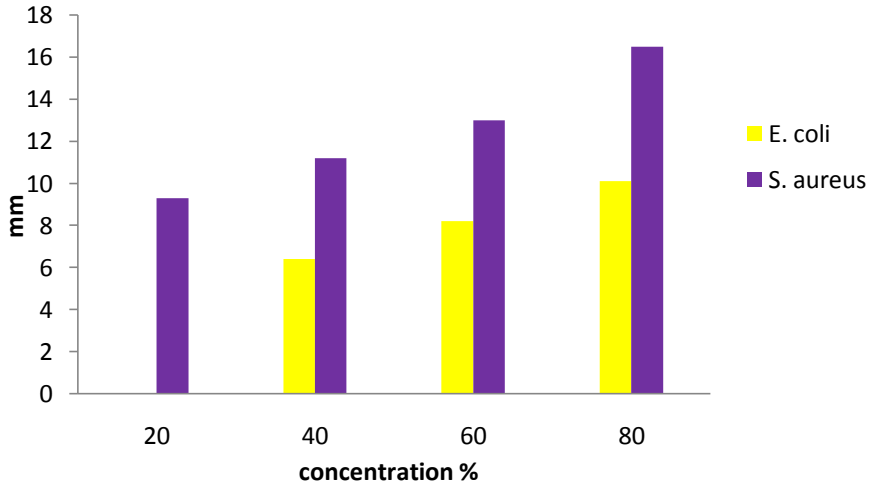


Fig. 2. Antimicrobial activity of alcoholic *Lavandula stoechas L*. extract, on *E. coli* and *S. aureus*.

4. Discussion

Based on the results ethanolic extract of *Lavandula stoechas L*. and *Rosmarinus officinalis L*. in this study have significant antimicrobial activity on the studied microorganisms. The results show that *Lavandula stoechas L*. and *Rosmarinus officinalis L*. alcoholic extracted at all concentrations (20, 40, 60 and 80%) had the inhibitory effect on *S. aureus* .The results show that *Lavandula stoechas L*. extracted at concentrations (40, 60 and 80%) had the inhibitory effect on *E. coli*, However, 20% concentration extracts, have no significant antimicrobial effect on *E. coli* and it is not able to prevent the growth of bacteria on culture. The results show that *Rosmarinus officinalis L*. extracted at concentrations (60 and 80%) had the inhibitory effect on *E. coli*, However, (20 and 40 %) concentration extracts, have no significant antimicrobial effect on *E. coli* and it is not able to prevent the growth of bacteria on culture. Antimicrobial effect of the extracts was different, depending on the type of microorganisms, thus, the gram-positive bacterium *Staphylococcus aureus*, was higher sensitivity compared to gram-negative bacteria *E. coli* (Table 3, 4) and showed inhibitory effects at lower concentrations of *Lavandula stoechas L*. and *Rosmarinus officinalis L*. extracts. Alcoholic extract of *Rosmarinus officinalis L*. extract was more effective *Lavandula stoechas L.* (Table 3, 4 and Figure 2,3).

Gram-positive bacteria are more sensitive than gram-negative bacteria to alcoholic *Lavandula stoechas L*. and *Rosmarinus officinalis L*. *extracts,* Due to differences in cell structure of gram-negative and gram-positive bacteria, because gram-positive bacteria have more mucopeptide in their cell wall composition while gram-negative bacteria have only a thin layer of mucopeptide and most of their cell structure is lipoprotein and lipo-polysaccharides. Thus, gram-negative bacteria are more resistant (Tassou and Nychas., 1995; Ghalem et al., 2008). These points were consistent with the results obtained in this study. Several mechanisms are discussed to explain the antimicrobial effect. Kotzekidou et al., 2008 found that the antimicrobial compounds in the plant extract, have interaction with the phospholipids' two layers membrane, and affect the permeability of the bacterial cell membrane, and released the intracellular components. In many studies, the mechanism of the cell wall is considered. They have reported that cell wall and cell membrane affected and changed their permeability cause Release of intracellular contents, which can be associated with impaired membrane function, such as electron transfer, enzyme activity or nutrient uptake.

The results indicated that the rosemary extracts showed antibacterial activity, according to Weckesser et al. (2007), mainly against the Gram-positive bacteria (S. aureus and B. cereus). The extracts also exhibited an effect against the Gram-negative bacteria (E. coli and P. aeruginosa). However, this effect was less efficient than that presented against the Gram-positive bacteria, since a higher MIC value was obtained with the Gram-negative bacteria. A similar behaviour was reported by Panizzi et al. (1993).

Therefore, using *Lavandula stoechas L*. and *Rosmarinus officinalis L*. as a natural antimicrobial compounds in vitro requires further research on mechanism of the pharmacy plant on the microorganisms.

Lavenders' antimicrobial activity is usually attributed to their terpenic compounds (Lis-Balchin, 1998; Hammer, 1999). In the essential oils of *L. stoechas* subsp. *luisieri*, the presence of necrodane derivatives have been reported as the characteristic and dominant compounds, but chemotypes are mentioned (Matos et al., 2009; Gonzalez-Coloma et al., 2011). Recent studies also report a variable chemical composition in *L. pedunculata*, but concerning the presence of essential oils fenchone-1, 8-cineole- and camphor-rich (Zuzarte et al., 2010).

The plants are a reserve of biologically active substances. Essential oils can be a significant source of a great diversity of chemical species equipped with antimicrobial capacity, the oil of *Lavandula stoechas* and *Cistus ladaniferus* can have application in therapy of the infectious diseases is like substituent of certain antibiotics or like complementary agents used in synergy with the synthesis substances. Essential oils can also have application in food industries not only like aromatizing but also like preservative of foodstuffs.

In conclusion, it can suggest that *Lavandula stoechas L*.and *Rosmarinus officinalis* L. extract in In-vitro have considerable antimicrobial ability over the studied strains. In addition, more studies are needed in In-Situ be done, to identifying the effective dose of the extract on the microorganisms, and finally introduce the extract as a natural and novel antimicrobial compound.

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