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ORIGINAL ARTICLE

EVALUATION OF ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF LAVENDER (*LAVANDULA STOECHAS*) FLOWERS AND LEAVES

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ABSTRACT: Lavender (Davendula vera officinals) grown in most parts of the world as a wild plant, especially in the south of France, the Mediterranean area and in Toronto, Canada. The aim of present study was to evaluate the antibacterial activity of methanol extract of lavender flowers and leaves against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Bacillus cereus. In present study we used of Minimum Inhibitory Concentration and Ager Well Diffusion methods to evaluate the antibacterial effects of lavender. Comparison among dilutions 30, 50, 100 and 400mg/ml of methanol extract of lavender against tested genera showed that S.aureus and B.cereus have the maximum susceptibility against extract. While, E.coli had the minimum susceptibility and P.aeruginosa showed no inhibition pattern. Comparison of antibacterial effects of leaves and flowers showed that inhibitory activity was more powerful in flowers than leaves. However, this activity in leaves was more than flowers against B.cereus. Results obtained from variance test showed that diameter of induced shadow of S.aureus, B.cereus and E.coli is not the same against different methanol extract of flower and leaves ($P<0.001$). Also, According to the Duncan test the mean value of diameter of S.aureus, B.cereus and E.coli induced by methanol extract of flowers and leaves at the dilution 400 mg/ml was more than others statistically ($P<0.001$) and this value in dilution 50 mg/ml was not significant than 30 and 100 mg/ml. in conclusion can state that the antibacterial activity of leaves and flowers of lavender in gram positives is more powerful than gram negatives. It seems that the antibacterial activity of this plant is due to its secondary metabolites and phenolic compounds.

KEYWORDS: Antibacterial Activity, Methanol Extract, Lavender, Flowers, Leaves.

INTRODUCTION

In recent years, more public attention to plant originated drugs has been paid, mainly due to side effects of chemicals and more trending of humans to maintaining a healthy condition. Problems in modern drug systems such as high costs, the use of non-renewable resources such as fossil fuels and environmental pollution in the pharmaceutical industries and ultimately the human inability to make some drugs that normally exist in medicinal plants have doubled the importance of this subject (Ahmad *et al.,* 2006; Akbarinia *et al.,* 2007; Akbarinia *et al.,* 2006; Evandri *et al.,* 2005; Jones, 2011; Niroumanesh *et al.,* 2008; Noorhosseini Niyaki *et al.,* 2011; Azadbakht, 1999; Duke *et al.,* 2002; Gordon, 1990; Howard, 1987). Materials and drugs derived from natural sources, especially from flowers and whole plants, have a long history. In fact, flowers and whole plants are the main sources of many drugs for a large part of the world's population, particularly in developing countries. Despite the rise of pharmaceutical chemistry in the early twentieth century, producing many types of pharmaceutical addiction drug molecules extracted from plants began and allowed treatment of incurable or life-threatening diseases. The strategies used by traditional doctors to prevent the progression of diseases or in holding the healthy conditions of patients were varying, but the effects of herbal medicines used in the human body were the same. Now hundreds of species of medicinal plants with power and precision are used, mostly acting on a specific operating system and are appropriate to treating certain types of diseases within the body (Ahmad *et al.,* 2006; Akbarinia *et al.,* 2007; Akbarinia *et al.,* 2006; Evandri *et al.,* 2005; Jones, 2011; Niroumanesh *et al.,* 2008; Noorhosseini Niyaki *et al.,* 2011).

Lavender (Davendula vera officinals) grown in most parts of the world as a wild plant, especially in the south of France, the Mediterranean area and in Toronto, Canada. Growing conditions are largely dependent on environmental conditions and different types of soil. Lavender is a perennial plant of low height,

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with narrow long leaves. Lavender (Lavandula) is such a romantic flower that every gardener eventually succumbs to the urge to grow it. Undeterred by the fact that it is a native of the Mediterranean and a lover of dry, sunny, rocky habitats, we try it anyway, hoping it will adapt. After all, England can hardly be considered dry or particularly sunny, yet English gardeners are renowned for growing lavender plants (Ahmad *et al.,* 2006; Akbarinia *et al.,* 2007; Akbarinia *et al.,* 2006; Evandri *et al.,* 2005; Jones, 2011; Niroumanesh *et al.,* 2008; Noorhosseini Niyaki *et al.,* 2011).

Lavender scent is very pleasant. It has a bitter taste and smell and due to its conditioning is used in perfumery. Distillation of lavender essential oil from flowers that come from this plant has yellow or greenish yellow fluid, which has a pleasant smell. Lavender essential oil is most commonly and is used in aromatherapy. However, lavender flowers also provide a fragrant aromatic herbal remedy in tea or tincture form, which is useful for nausea, motion sickness, flatulence, colic, bloating, gut dysbiosis and irritable bowel syndrome. Lavender is also well known for its soothing and calming properties. It is useful with other herbs for mild anxiety, depression, tension, restlessness and for insomnia as it helps improve sleep quality. In this study, we review some main uses of lavender plant in Iranian traditional medicine and instructions of usage by mainly rural peoples (Azadbakht, 1999; Duke *et al.,* 2002; Gordon, 1990; Howard, 1987). The aim of present study was to evaluate the antibacterial activity of methanol extract of lavender flowers and leaves against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Bacillus cereus.

MATERIAL AND METHODS

Lavender was gathered from Marand and Hashrood zones during year 2012. Water distillation method and Klunjer system was used for 2 hours in order to extraction. Obtained extracts were stored in small dark vials at 2°C following rinsing with sodium sulfate.

2.1. GC system

GC analysis was conducted by means of gas chromatography system (model 9A) made by Shimudzo company equipped with DB-5 bar, the height of 30 meters, internal diameter of 0.25 mm and thin layer thickness of 0.15 micrometer. Oven temperature was kept at 40°C for 5 minutes; then the temperature increased to 250°C with the speed of 4°/minute. The temperature of injection and detector sections was 260°C; also the gas carrying helium with the speed of 32cm/s was used.

2.2. GC/MS system

GC/MS analysis was conducted by means of Varian system (model 3400) equipped with DB-5 bar, the height of 30 meters, internal diameter of 0.25 mm and thin layer thickness of 0.25 micrometer. Oven temperature increased from 60 to 250°C with the speed of 4°/minute. The gas carrying helium with the speed of 1.1mm/minute as well as 70ev ionization energy was used. Identification of the extract constituents was done by means of mass spectrum and inhibition index. In order to obtain various extracts the following methods are used:

2.3. Liquid extract

50 g of dried and powdered herb and 300 ml water were mixed and kept in refrigerator for 72 hours. Then, the mixture was filtered.

2.4. Methanol extracts

50 g of dried and powdered herb and 300 ml of 80% Methanol were mixed and kept in refrigerator for 72 hours. Then, the mixture was filtered.

2.5. Etheric extracts

50 g of dried and powdered herb and 300 ml diethyl ether were mixed and kept in refrigerator for 72 hours. Then, the mixture was filtered.

2.6. Genera selection

Microbial tests was conducted using bacteria i.e. Staphylococcus aurous ATCC:25923, Pseudomonas aeruginosa ATCC:27853, E-coli ATCC:25922 and Bacillus cereus ATCC:1247.

2.7. Media preparation and Culturing of microorganisms

In present study we used of Minimum Inhibitory Concentration and Ager Well Diffusion methods to evaluate the antibacterial effects of lavender. For this mean, 4-5 colonies of bacteria were sampled and were moved to a tube with mueller hinton broth (MHB) medium. Then samples were moved into the incubator at 37ºC for 2-4 hours in order to achieve the standard turbidity of 0.5 McFarland. After dilution, 500 µl of suspension (1.5×10^6 cfu/ml) was moved onto the mueller hinton agar (MHA) using a sterile swap. Then wells with 6 mm diameter and 2.5cm distance from each other were made on the medium. In continue, 100ml of extract with different dilution (30, 50, 100 and 400 mg/ml) was injected into the each well. We used of

DMSO 5% and chloramphenicol as negative and positive controls, respectively. At the end, mediums were incubated at 37ºC for 24 hours. Then the induced diameters of shadow was measured and recorded.

RESULTS

Comparison among dilutions 30, 50, 100 and 400mg/ml of methanol extract of lavender against tested genera showed that S.aureus and B.cereus have the maximum susceptibility against extract. While, E.coli had the minimum susceptibility and P.aeruginosa showed no inhibition pattern. Comparison of antibacterial effects of leaves and flowers showed that inhibitory activity was more powerful in flowers than leaves. However, this activity in leaves was more than flowers against B.cereus (tables 1 and 2).

Table 1: mean ± SD of inhibition zone induced by methanol extract of leaves against tested microorganisms

Dilution of extract / Genera	30	50	100	400	DMSO	Chloramphenicol
Staphylococcus aurous	9±0.817	10±0.817	11±0.817	19±0.817	7	21
Bacillus cereus	10±0.817	13±0.0	13±0.0	22.5±0.75	7	19
E.coli	7	7	7	10±0.817	7	26
Pseudomonas aeruginosa	7	7	7	7	7	24

Table 2: mean ± SD of inhibition zone induced by methanol extract of flowers against tested microorganisms

Dilution of extract / Genera	30	50	100	400	DMSO	Chloramphenicol
Staphylococcus aurous	9±0.817	11±0.817	11±0.817	20±0.817	7	26
Bacillus cereus	7	10±0.75	10±0.75	20±0.817	7	19
E.coli	7	7	7	11±0.817	7	26
Pseudomonas aeruginosa	7	7	7	7	7	24

Results obtained from variance test showed that diameter of induced shadow of S.aureus, B.cereus and E.coli is not the same against different methanol extract of flower and leaves (P<0.001). So that, based on Duncan test the mean value of diameter of S.aureus induced by methanol extract of leave at the dilution 400 mg/ml was more than others statistically (P<0.001) and this value in dilution 50 mg/ml was not significant than 30 and 100 mg/ml. also, based on Duncan test the mean value of diameter of B.cereus induced by methanol extract of leave at the dilution 400 mg/ml was more than others statistically (P<0.001) and there was no significant difference among dilution 50 and 100 mg/ml (P>0.001).

According to the Duncan test the mean value of diameter of S.aureus and B.cereus induced by methanol extract of flowers at the dilution 400 mg/ml was more than others statistically (P<0.001) and this value in dilution 50 mg/ml was not significant than 30 and 100 mg/ml.

According to the Duncan test the mean value of diameter of E.coli induced by methanol extract of flowers and leaves at the dilution 400 mg/ml was more than others statistically (P<0.001) and this value in dilution 50 mg/ml was not significant than 30 and 100 mg/ml (tables 3 and 4).

Table 3: MIC and MKC of methanol extract of leaves against tested microorganisms

Extract / Genera	MIC (mg/ml)	MKC (mg/ml)
Staphylococcus aurous	12.5	25
Bacillus cereus	6.25	12.5
E.coli	100	200
Pseudomonas aeruginosa	N	N

Table 4: MIC and MKC of methanol extract of flowers against tested microorganisms

Extract / Genera	MIC (mg/ml)	MKC (mg/ml)
Staphylococcus aurous	12.5	25
Bacillus cereus	6.25	12.5
E.coli	25	50
Pseudomonas aeruginosa	N	N

DISCUSSION

Comparison among dilutions 30, 50, 100 and 400mg/ml of methanol extract of lavender against tested genera showed that S.aureus and B.cereus have the maximum susceptibility

against extract. While, E.coli had the minimum susceptibility and P.aeruginosa showed no inhibition pattern. Comparison of antibacterial effects of leaves and flowers showed that inhibitory activity was more powerful in flowers than leaves. However, this activity in leaves was more than flowers against B.cereus.

Results obtained from variance test showed that diameter of induced shadow of S.aureus, B.cereus and E.coli is not the same against different methanol extract of flower and leaves ($P<0.001$). Also, According to the Duncan test the mean value of diameter of S.aureus, B.cereus and E.coli induced by methanol extract of flowers and leaves at the dilution 400 mg/ml was more than others statistically ($P<0.001$) and this value in dilution 50 mg/ml was not significant than 30 and 100 mg/ml.

Gram-negative bacteria have an outer lipopolysaccaride wall that can work as a barrier against toxic agents (Gaunt *el al.,* 2005). T. serpyllum was proven to have good antimicrobial effect against MRSA, Strep. pyogenes and C. albicans. As with S. aromaticum, T. sepyllum consists of phenolic structures (eg. eugenol and carvacrol), which have been confirmed by many studies to have a good antimicrobial activity (Kalemba *et al.,* 2003). Essential oils with predominant alcoholic compounds have in previous studies been shown to be slightly less active than compounds containing phenolic structures (Dorman *et al.,* 2000).

One explanation could be the fact that the antimicrobial activity of volatile compounds results from the combined effect of direct vapour absorption on microorganism and indirect effect through the medium that absorbed the vapour (Inouye *et al.,* 2000; Inouye *et al.,* 2001).

Another explanation is that some fungi are more susceptible to essential oils than bacteria. The exact mechanism of action of essential oil volatile on fungi is unclear but the majority of reports agree that oil volatiles result in morphological changes to the hyphae (Cavanagh, 2007).

Different lavender species have variable antibacterial effects, depending on the concentration of specific chemical constituents (Lis-Balchin *et al.,* 1998). The essential oil of L.angustifolia has bacteriostatic and bactericidal activity against methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus aecium66. Linalool and cineole exhibit antibacterial activity against 17 and 16 out of 18 strains of gram-positive and gram-negative bacteria tested, respectively (Pattnaik and Subramanyam, 1997). In another study, the essential oil of L. angustifolia inhibited the growth of B. megaterium by 50% as compared to control cultures; the oil was also found to inhibit the growth of P. aeruginosa by 75% and to delay the growth of S. aureus and M. lysodeikticus (Larrondo *et al.,* 1995). In one study, some clones of L. angustifolia showed increases in phenolic content and/or rosmarinic acid synthesis when challenged by a strain of Pseudomonas. The authors hypothesized that the upregulation of these metabolites conferred tolerance to the plants against the bacterium (Al-Amier *et al.,* 1999). In another study, four samples of the essential oil of L. hybrida had inhibitory activity against five strains of non-tubercular rapid growth mycobacteria. The samples all had high concentrations of linalool, linalyl acetate, and eucalyptol (Gabbrielli *et al.,* 1988).

Kunicka-Styczyńska *et al.,* (2009) verified the antimicrobial activity of commercial essential oils: lavender, tea tree and lemon as the components of a preservative system in oil in water body milks. They concluded that in all combinations of essential oils with the synthetic preservative, a synergistic effect of the preservative system components was observed, which made it possible to reduce the usable level of the synthetic preservative up to 8.5 times.

In one study by Kirmizibekmez *et al.,* (2009) it has been revealed that the flower essential oil of lavender was found to be relatively more active than the leaf oil towards the tested pathogenic microorganisms. They also found that Methicillin-resistant Staphylococcus aureus was more susceptible to the flower oil (MIC = 31.2 microg/mL). The oils, evaluated for their free radical scavenging activity using a TLC-DPPH assay, were inactive at a concentration of 2 mg/mL, which is compatible with our study.

Results of Dadalioglu and Evrendilek (2004) revealed that all essential oils of Turkish oregano (Origanum minutiflorum), bay laurel (Laurus nobilis), Spanish lavender (Lavandula stoechas L.), and fennel (Foeniculum vulgare) exhibited a very strong antibacterial activity against the tested bacteria ($P < 0.05$). Gas chromatography-mass spectrophotometry analyses revealed that carvacrol (68.23%), 1,8-cineole (60.72%), fenchone (55.79%), and trans-anethole (85.63%) were the predominant constituents in Turkish oregano, bay laurel, Spanish lavender, and fennel essential oils, respectively that is according to the our statement.

Gören *et al.,* (2002) analyzed the composition of essential oil of the leaves of Lavandula stoechas

ssp. stoechas, by means of capillary GC-MS. They showed that the main components of L. stoechas ssp. stoechas oil were pulegone (40.4%), menthol (18.1%), menthone (12.6%). The essential oil of the plant was evaluated for antibacterial and panel cytotoxic activities, which is in line with our results. In conclusion can state that the antibacterial activity of leaves and flowers of lavender in gram positives is more powerful than gram negatives. It seems that the antibacterial activity of this plant is due to its secondary metabolites and phenolic compounds.

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