

Antioxidant and Antibacterial Properties of the Essential Oils of Two Iranian Medicinal Plants: *Zhumeria majdae* and *Salvia mirzayanii*

Nafiseh Omidpanah^{1*}, Marzie Valifard², Mansoore Esmaeili¹, Reza Yousefi¹, Ali Moghadam³

¹Department of Biology, College of Sciences, Shiraz University, Shiraz, Iran

²Institute of Biochemistry and Biophysics, the University of Tehran, Tehran, Iran

³Institute of Biotechnology, Shiraz University, Shiraz, Iran

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* Corresponding author:
Nafiseh Omidpanah,
Department of Biology,
College of Sciences,
Shiraz University, 71454,
Shiraz, Iran. Email:
n.omidpanah@gmail.com

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Abstract

Background: The *Lamiaceae* family has been tried to ameliorate symptoms of stomachache and dysmenorrhea in traditional medicine. The extracts and essential oils obtained from these plants have recently attracted much scientific attention. The essential oils of some plants belonging to this family have also been used in food preservation. **Methods:** This study employed pharmaco-diagnostic methods to examine and compare the antioxidant and antibacterial activities of the essential oils obtained from two Iranian plants *Zhumeria majdae* and *Salvia mirzayanii* in *E.coli* infection. **Results:** Our findings suggested that the essential oil extracted from *S. mirzayanii* had more antioxidant potential than *Z. majdae*. By increasing the concentration of both agents, the growth and specific growth activity of *E. coli* was reduced. At 1:200 dilutions, the growth of *E. coli* was inhibited while LC50 occurred at 1:1000 dilutions. Finally, our results showed that both agents had strong antioxidant and antibacterial activities with more significant properties favoring *S. mirzayanii*. **Conclusion:** Plants such as *Z. majdae* and *S. mirzayanii* retain notable medicinal properties and may serve as useful tools when developing natural antibiotics and antioxidant agents against common pathogens including *E. coli*.

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Introduction

Owing to the increasing interest in the human health over the recent years, the antibacterial and antioxidant activity of products have received much attention. The effects of herbal medicinal compounds have been studied in vitro and in vivo

by many researchers (1-2). There is an increasing world-wide interest in finding new and safe antioxidants from natural sources to prevent oxidative deterioration of food and to minimize oxidative damage in living cells (3). Much work has been carried out on the antibacterial and

antioxidant effects of different medical plant species. Medicinal plants are the primary sources of naturally-occurring antioxidants for human and are often shown to have antibacterial and antioxidant effects (4). The aromatic plants have been used traditionally as folk remedies and also to extend the effectiveness of food materials and to inhibit the growth bacteria, fungi and yeasts (5). Most properties of such plants are seen in the essential oils produced by their secondary metabolism (6).

The *Lamiaceae* (*labiatae*) genus which belongs to the mint family consists of 233 to 263 genera and 6900 to 7200 species. Within the *Lamiaceae* species, examples of new antioxidants include phenolic diterpenes, phenolic carboxylic acids, biphenyls, and flavonoids isolated from rosemary, sage, oregano and thyme (7-8). Given the wide use of two genus from this family, *Zhumeria majdae* Rech.F. & Wendelbo and *Salvia mirzayanii* Rech.F. & Esfand, in folk medicine across the Southern Iran, we attempted to compare the antioxidant and antibacterial activities of their essential oils. Both plants are natural flora and their leaves have been used for many years in Iran. The monotypic *Z. majdae* (9), locally known as Mohrekhosh, was recently described as the first member of a new genus, *Zhumeria*. The leaves of this plant have traditionally been used for gastritis relief and as an antiseptic agent for many years. In addition, the anti-nociceptive, anti-inflammatory and signs of acute toxicity of the *Z. majdae* extract were reported (10). *S. mirzayanii* is a wild-growing flowering plant, locally known as Mohretalkh. Several species of *Salvia* are used in folk medicine as antiseptics, astringents and spasmolytic (11). In the current study, the antioxidant and antibacterial activities of the essential oil obtained from *Z. majdae* and *S. mirzayanii* were examined and compared.

MATERIALS AND METHODS

Plant material

Two species of *Lamiaceae* family, *Z. majdae* and *S. mirzayanii* were purchased during July 2014 at the grocery in Laar city, Fars province, Iran.

Chemicals

Pentane, Trolox (Hoffman-La Roche) (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid), NaCl, agar culture, yeast extract were purchased from Merck chemical company. ABTS (2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), Potassium persulfate and ethanol were purchased from Sigma Company.

Extraction of the essential oil

The dry aerial parts of the plants were collected and crumbled. Then, 100 g of each plant were soaked in 1000 mL distilled water and placed in 2000 mL flask of the flavoring apparatus. To avoid mixing distilled water and essential oil, 1 mL of pentane solvent were added to the collecting duct. After 2 hours, the mixture of pentane and essential oil were collected. Nitrogen gas was utilized to evaporate pentane and to isolate the essential oil. The segregated essential oil was maintained in 4 °C and darkness until use.

Determination of antioxidant activity

ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS•+) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 hours before use. Oxidation of the ABTS was commenced immediately, whereas the absorbance was not maximum and remained stable until over 6 hours. The radical was stable in this form for more than two days when stored in the dark at room temperature (12). The ABTS•+ solution was diluted with ethanol to an absorbance of 0.70 at 734 nm. The final concentration for ABTS•+ was 15 mM. Then, 500 µL distilled water, 500 µL ethanol, 15 µL ABTS.+ solution and 20 µL of different essential oil dilutions was added to the cuvette and the absorbance reading was done at 734 nm every 30 seconds for 5 min. Trolox was prepared in ethanol for use as a stock standard. Fresh working standards were prepared daily on dilution using ethanol. All determinations were carried out at least three times and in triplicate, for each treatment, and at each separate concentration of the standards and samples. The percentage inhibition of absorbance at 734 nm was calculated and plotted as a function of concentration of antioxidants and of Trolox for the standard reference data. Finally, the concentration-response curve for 5 sequentially and separately prepared stock standards of Trolox was illustrated.

Determination of the antibacterial activity

The essential oil of *Z. majdae* and *S. mirzayanii* were dissolved and diluted in ethanol to produce stock solution then, serial twofold dilutions were made in the well of microtitre plate. Finally, serial concentration of essential oils in the wells determined as 1:20000, 1:2000, 1:1000, 1:200, 1:80, and 1:20. Ethanol was used as a control for each serial concentration. Dh5α, an antibiotic

sensitive strain of *Escherichia coli* (*E. coli*) was grown in LB agar media containing 1% Trypton, 0.5% NaCl and 0.5 % yeast extract. The anti-bacterial activity of the essential oil was assessed on microplate-base assay. In each well, 200 μ L of bacteria and 10 μ L of different concentrations of the essential oil were inoculated into the wells. The mixture was shaken at 37 $^{\circ}$ C for 8 hours and the optical density at 600 nm was measured every 30 min until the end of the experiment using the ELISA Reader Expert 96. The specific growth rate was calculated from the plot of OD 600 versus time as the following (13) where μ is specific growth rate, OD 600 is the OD of bacterial turbidity and Δt represent the time difference ($\mu = \text{Ln OD600} / \Delta t$).

The LC50, lethal dose at which 50% of the bacterial population killed in a given period of time by essential oil concentrations was calculated and shown in a curve. The obtained results were the mean of the three independent measurements.

RESULTS

The antioxidant activity

The antioxidant activity of different essential oils was compared with scavenging of ABTS free radicals with the standard solution, Trolox. Figure 1 shows the interaction of Trolox with ABTS•+ at 734 nm for five dilutions (5, 10, 15, 20 and 25 μ M). Results demonstrated that the Trolox reaction with ABTS•+ was complete in 60 seconds.

Figure 1

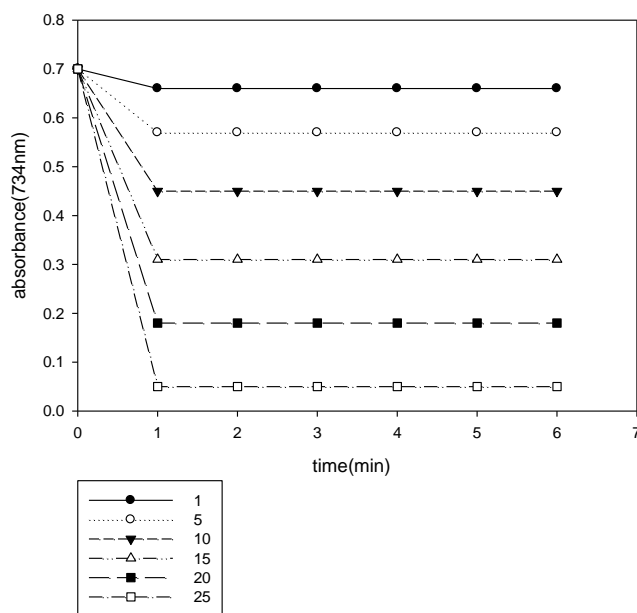


Figure 1: The effects of time on the suppression of ABTS•+ absorbance. The vertical axis represents the absorbance of ABTS free radicals in 734 nm and the horizontal axis indicates the duration of ABTS scavenge by Trolox in different Trolox concentrations. The numbers 5, 10, 15, 20 and 25 show the concentration of Trolox solution interacting with ABTS.

The percentage inhibition of absorbance at 734 nm was calculated and plotted as a function of concentrations of Trolox for the standard reference

data. The concentration response curves for five sequentially and separately prepared stock standards of Trolox are illustrated in Figure 2.

Figure 2

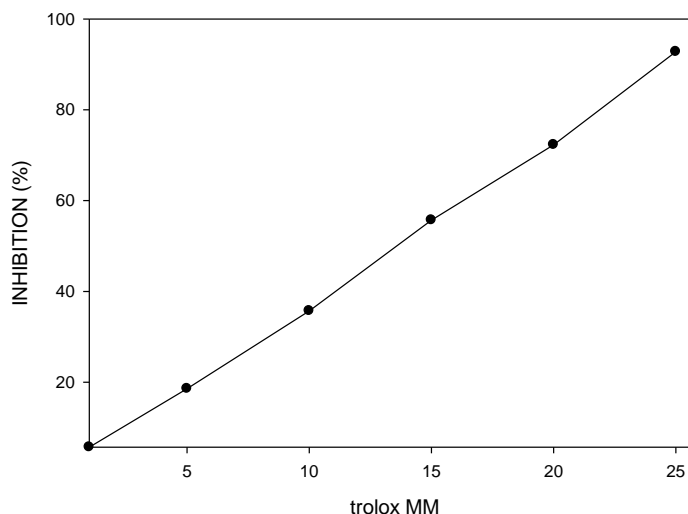


Figure 2: The concentration-response curve for the absorbance at 734 nm for ABTS•+ as a function of concentration of standard Trolox solution. (Five separately-prepared stock standard solutions \pm SD). The percentage inhibition of absorbance for ABTS free radicals at 734 nm and the different Trolox concentrations interacting with ABTS are represented in the vertical axis and horizontal axis, respectively.

Figure 3A

Figures 3A and B show the effects of interaction period of the *Z. majdae* and *S. mirzayanii* essential oils on the suppression of the ABTS radical cation absorbance at 734 nm for the three dilutions (1:10, 1:20 and 1:40). Our results demonstrated that reaction of *S. mirzayanii* essential oil dilutions with ABTS•+ was completed at 5 min for 1:10 dilution, but not for 1:20 and 1:40 dilutions. (Figure 3A). Figure 3B illustrates that reaction with ABTS •+ for the concentrations of *Z. majdae* was not completed by 5 min needing further time as compared to *S. mirzayanii*.

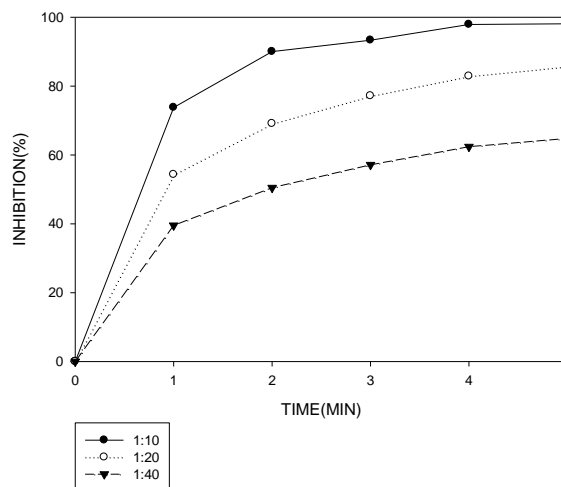


Figure 3B

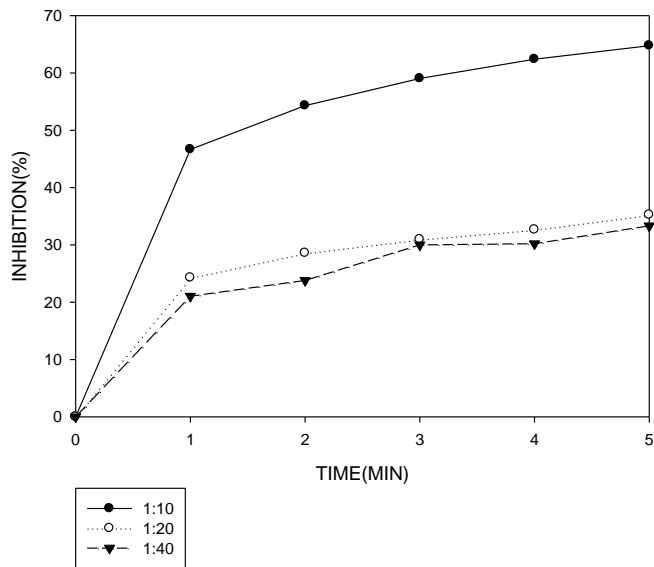


Figure 3: Effect of interaction period of *S. mirzayanii* and *Z. majdae* essential oils on the suppression of ABTS•+ absorbance at 734 nm for three dilutions. The vertical axis shows suppression percentage of the ABTS radical cation absorbance at 734 nm and the horizontal axis shows interaction period of *S. mirzayanii* (3A) and *Z. majdae* (3B) essential oils. Three dilutions of essential oils were shown as 1:10, 1:20 and 1:40.

Figure 4A

The extent of inhibition of the ABTS•+ absorbance is plotted as a function of essential oil concentration in order to determine the TEAC that can be assessed as a function of time. The essential oil concentrations of *S. mirzayanii* and *Z. majdae* (1:40, 1:20 and 1:10) were shown the percentage inhibition of radical cation absorbance at 734 nm as 7.1, 9.8, 17.9 μM for *Z. majdae* (Figure 4A) and 17.8, 22.8, 26.1 μM for *S. mirzayanii* (Figure 4B). Trolox respectively was calculated in terms of Trolox equivalent antioxidant capacity at each specific time point.

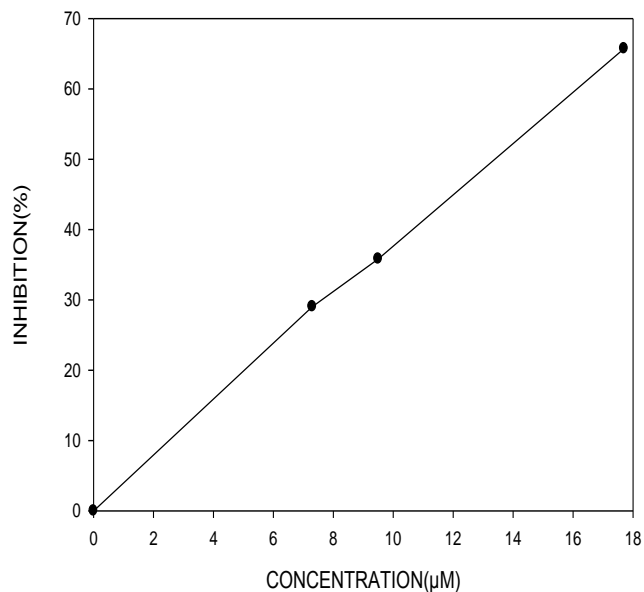


Figure 4B

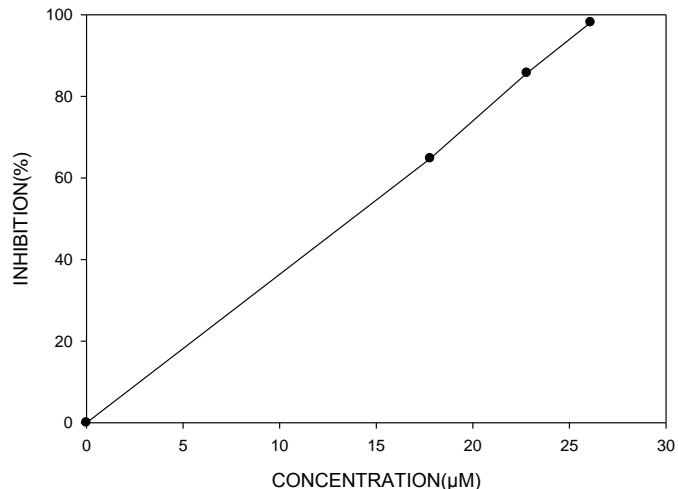


Figure 4: The effects of concentration of the *S. mirzayanii* and *Z. majdae* essential oil on the inhibition of the $ABTS \bullet +$. The vertical axis shows percentage inhibition of absorbance of the radical cation at 734 nm while the horizontal axis represents the concentration of the *S. mirzayanii* (4A) and *Z. majdae* (4B) essential oils.

The TEAC values were obtained from the capacity of an individual antioxidant or a mixture to inhibit the $ABTS \bullet +$ at defined time point, relative to Trolox. As a screen for relative antioxidant activities of the essential oils, the antioxidant activity referred to measurement at more than 5 min time point and seemed to be appropriate.

The antioxidant activity can also be expressed in terms of the total contribution to the antioxidant activity over the time range studied to determine the

area under the curve (AUC). The AUC was derived by plotting the gradient of the percentage inhibition/concentration as a function of reaction time. The AUC method is an alternative way to describe the antioxidant activity of compounds when considering the varied rates of reaction of the antioxidants with $ABTS \bullet +$.

As shown in Figure 5, the ratio between AUC for the reaction of essential oil and that for Trolox gives the relative antioxidant activity.

Figure 5

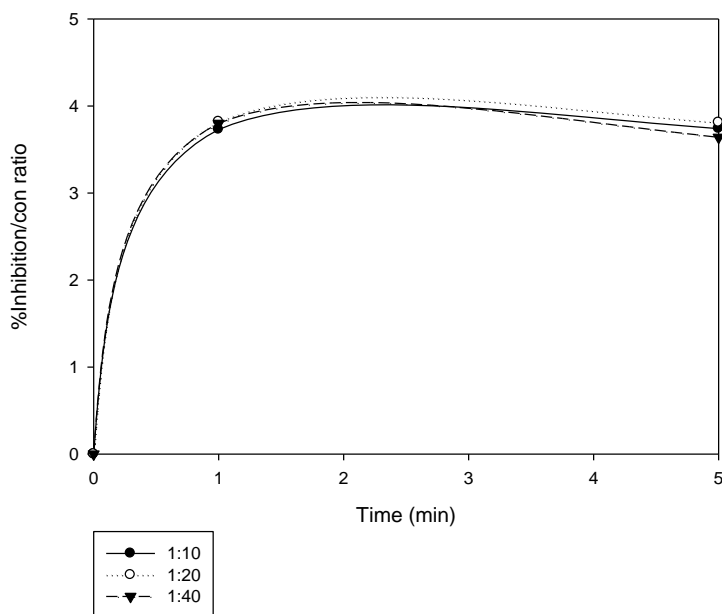


Figure 5: Variation profile of gradient of the percentage inhibition vs. concentration plot of each antioxidant at 1 min and 4 min used to measure the area under the curve (AUC) for the *S. mirzayanii* (A) and *Z. majdae* (B) essential oil. The vertical axis shows percentage of inhibition to concentration ratio for the two essential oils and the horizontal axis shows the duration of reaction between essential oils and ABTS free radicals. The antioxidant activity derived from the AUC plot is calculated from the ratio of the area under the curve for the specific antioxidant in question to that for Trolox. Three dilutions of essential oils were shown as 1:10, 1:20 and 1:40.

The calculation of AUC is derived from both essential oil concentration and reaction time and is therefore an overall measure of the abilities of essential oil to scavenge free radicals compared to the standard Trolox during the specific time range, taking into account the variation with regard to the time variable.

The antibacterial activity

The antibacterial activity of essential oils has shown to be different in various concentrations. Figures 6 and 7 illustrate the effect of different concentrations of *S. mirzayanii* (Figures 6A and B) and *Z. majdae* (Figures 7A and B) essential oils on the antibiotic sensitive strain *E. coli* DH5 α bacteria. Results showed that with increasing *S. mirzayanii* essential oil's concentration, the bacteria proliferation (Figures 6A and 7A) and specific growth activity was decreased (fig. 6B & 7B).

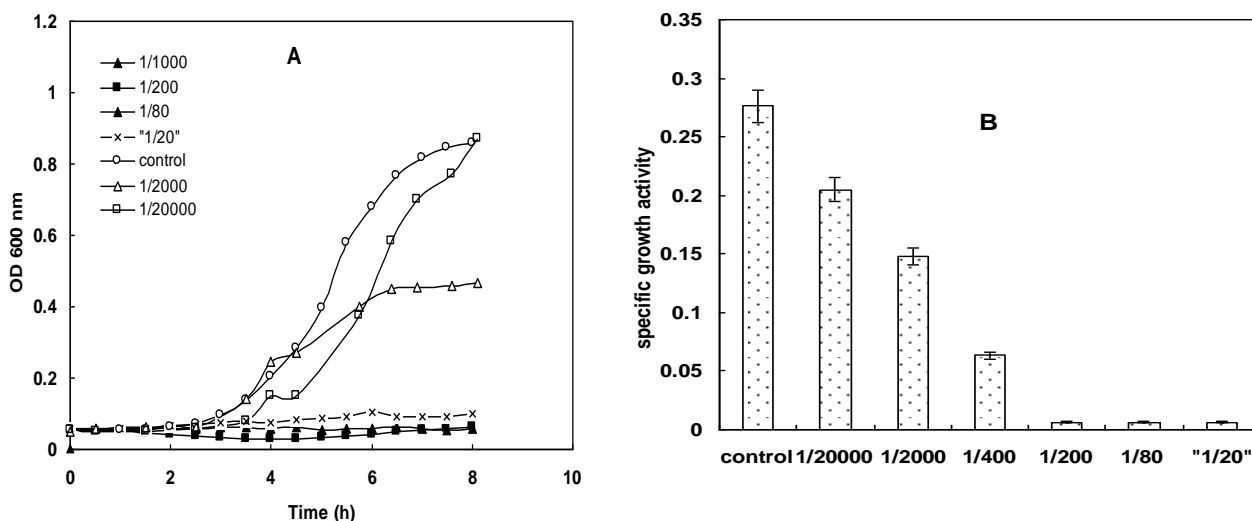


Figure 6: The effects of different concentration of *S. mirzayanii* essential oil on *E. coli* growth (A) and effects of different dilutions of *S. mirzayanii* essential oil on *E. coli* specific growth activity (B). The vertical axis shows the optical density (OD) of bacterial medium in 600 nm while the horizontal axis indicates the duration of interaction between the essential oils and the bacteria. The ratios 1/2000, 1/2000, 1/1000, 1/200, 1/80 and 1/20 were the various applied concentrations of the *S. mirzayanii* essential oil.

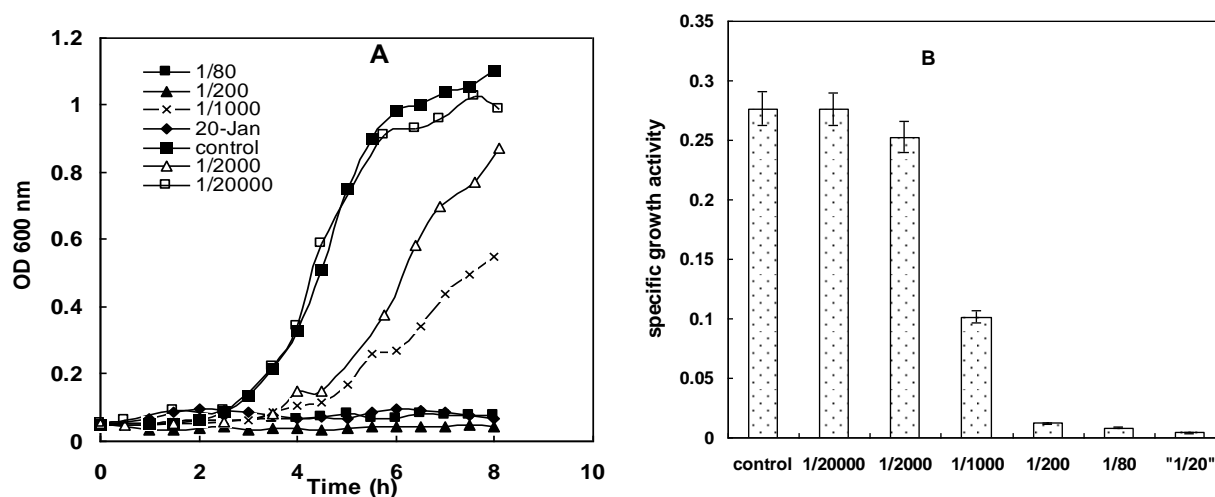


Figure 7: The effects of different dilutions of *Z. majdae* essential oil on growth of *E. coli* (A) and the effects of different dilutions of *Z. majdae* essential oil on the specific growth activity of *E. coli* (B). The vertical axis shows the optical density (OD) of bacterial medium in 600 nm and the horizontal axis indicates the duration of interaction between the essential oils and the bacteria. The ratios 1/2000, 1/2000, 1/1000, 1/200, 1/80 and 1/20 were the various applied concentrations of the *Z. majdae* essential oil.

At the 1:200 dilution, the growth of *E. coli* was inhibited while the LC50 was achieved at 1:1000 dilution.

Our results showed that both essential oils have strong antibacterial activity. The ratio of *S.*

mirzayanii essential oil lethal dose to *Z. majdae* essential oil is 1.8 X.

Discussion

In order to prolong the storage stability of foods and reduce the damage to living cells, synthetic

antioxidants are used in industrial processes. However, according to toxicology and nutrition viewpoints, the side effects of some synthetic antioxidants such as BHT have been clearly demonstrated (14). Therefore, governmental authorities and consumers are concerned about safety of their food and the potential effects of the synthetic additives on health (15). Most plant species, especially the *Lamiaceae* family have been shown powerful antioxidant properties (16). The range of traditional applications of the *Salvia* herbs in domestic medicine seems to be very extensive. They have been used as a medication against perspiration and fever, as a carminative, spasmolytic, antiseptic/bactericide, astringent, gargles or mouth-washes against mouth, tongue and throat inflammation, a remedy for skin and hair problems, against rheumatism and sexual debility, and in treating mental and nervous system disease conditions and even as an insecticide (17-18). Many studies have indicated the antioxidant, antibacterial and antiviral activities of some *Salvia* species (9-19). The essential oil and methanol extract of *Z. majdae* have shown strong antioxidant activities almost equal to BHT [14]. Diterpenes such as labdane has been detected in the root of *Z. majdae* holding antioxidants properties (20). Labdane diterpens has been detected as a potential radical scavenger (21).

The comparison between the antioxidant activity determined from AUC and TEAC methods shown that the *S. mirzayanii* essential oil retains more powerful total antioxidant capacity than *Z. majdae*. *S. mirzayanii* was shown to complete the scavenging reaction in the lower time and concentration. Previous studies reported that phenolic compound in *S. mirzayanii* plant exhibit antioxidant activity by inactivating lipid free radicals or by preventing the decomposition of hydroperoxides into free radicals (22). The phenolic compounds exhibit considerable free radical scavenging activities through their reactivity as hydrogen- or electron-donating agents, and metal ion chelating properties. Flavonoids are a secondary class of phenolic in plants possessing powerful antioxidant properties (23). The essential oils of *S. mirzayanii* have been studied and the results have indicated that major components of the essential oil are 5-neo-cedranol (15.48%), 1,8-cineol (11.23%), α -terpinyl acetate (9.64%), γ -cadinene (6.64%), bicyclogermacrene (6.47%), δ -cadinene (5.46%), Globulol (5.12%), α -cadinol (4.58%), epi- α -Cadinol (4.20%), Linalool (3.35%), 7-epi- α -selinene (3.23%), Linalyl acetate (2.55%) and α -guaiene (2.23%) (24). There seem to be at least twenty six identifiable compounds in the *Z. majdae* essential oil. The oil profile exhibits that linalool is the main

compound (53.28%). Additionally, other major compounds were camphor (26.15%) and D-limonene (4.17%) (14). It appears that the antioxidant activities of plants extract are partly due to total content of polyphenol and flavonoid (25).

Recent studies on the essential oils of many plants from *Lamiaceae* family have shown that these plants hold a broad range of biological activities, notably their antibacterial potency (26). The antibacterial effect of essential oils from aerial parts of *Mentha piperita*, *M. spicata*, *Thymus vulgaris*, *Origanum vulgare*, *O. applii*, *Aloysia triphylla*, *Ocimum gratissimum*, and *O. basilicum* have already been articulated in the literature (5). The effect of the essential oil of *Z. majdae* on some microorganisms such as *Klebsiella pneumonia*, *Staphylococcus aureus*, *Vibrio cholera*, *Bacillus cereus*, *B. subtilis*, *Proteus vulgaris*, *Aspergillus flavus* and *A. niger* are thus far reported (27). Several studies have focused on the antibacterial activity of the essential oils of *Salvia* species. Similarly, the essential oils of *S. officinal* and *S. triloba* showed antibacterial activity (28-29). Kabouche et al. (30) reported the essential oil obtained from roots of *S. jaminiana* has antibacterial activity. Likewise, the essential oil of *S. tomentosa* showed antibacterial activity against eight microorganisms (31). 5-neo-cedranol, α -terpinyl acetate, 1, 8-cineol, bicyclogermacrene, δ -cadinene, Globulol, α -cadinol, tau-cadinol, 7-epi- α -selinene, Linalyl acetate, Linalool, β -Elemene, γ -cadinene and α -guaiene are proposed to be the main constituent of *S. mirzayanii* essential oil considered as an antibacterial agents (32). In addition this, the antibacterial activity of linalool and camphor has been reported previously (33-34) and it seems that linalool and camphor could be potentially responsible for the antibacterial effects of our currently examined essential oil. Nevertheless, other potential components, as well as a possible interaction between the substances could also affect the antibacterial activities in our experiment. In fact, the antibacterial activity of essential oils may be due to the presence of synergy, antagonism or additive effects of the other components of the oil, which possess various potential effects (32).

Conclusion

Our results provided some added insights about the two plants with potent antioxidant and antibacterial activity, and hence may provide materials for bioassay-guided fractionation to determine the active constituents of such plants' extracts and essential oils. The essential oils of these two plants, *S. mirzayanii* and *Z. majdae*,

contain components considered as potential industrial sources for preparing effective drugs.

In the present study, Dh5 α , an antibiotic-sensitive strain of *E. coli* was used for the antibacterial experiments. It has some mutation such as *recA* which eliminates the homologous recombination making the strain somewhat sickly. Although this strain is antibiotic-sensitive, it might not always be necessarily a proper case for antibiotic susceptibility tests. Thus, it would be beneficial to test the essential oils and extracts of these two medicinal plants on other pathogens. In addition, a standard antibiotic needs be used as a positive control to more efficiently compare the effect of the essential oil compounds in future research attempts.

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