Original Article

Antimicrobial activity and toxicity of Zhumeria majdae essential oil and its capsulated form

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Abstract

Introduction: Zhumeria majdae is an herbal plant which is only found in Hormozgan province. The aim of this study was to compare the antimicrobial property and toxicity of essential oil (EO) and encapsulated form of the essential oil (EFEO) of Zhumeria majdae.

Materials and Methods: First, the plant was dried, grinded, and its EO extracted by hydrodistallation. Then, the EO was encapsulated by lecithin. Antimicrobial activity of EO and EFEO was evaluated by micro dilution broth method, and minimum inhibitory concentration (MIC) was determined.For toxicity test, serial concentrations of EO and EFEO were incubated with mouse skin cells, and then cell viability was calculated by MTT assay.

Results: Based on atomic force microscopy results, the size distribution of EFEO was between 100-500 nm. Antimicrobial test showed that the MIC50 of both EO and EFEO was similar (0.6%). But, in case of Candida albicans, the MIC90 of EO and EFEO was 5% and 1.25%, respectively. It means that EFEO has greater effect on Candida albicans than EO. The results of MTT assay showed that the toxicity of EFEO was less than EO.

Conclusion: It was found that in some cases, encapsulation could lead to better antimicrobial property and less toxicity. Because of high antimicrobial activity, both EO and EFEO of Zhumeria majdae might be used as powerfully antimicrobial agents.

Keywords: Antimicrobial activity, Toxicity, Essential oil, Zhumeria majdae, Encapsulation

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Introduction

The presence of pathogenic microbes in the environment is very dangerous. It is a fact that their replication and transmission from an infected person to a healthy person can be done easily. So, scientists have impelled to deal with the invisible enemies, and explored various ways for combating.The use of antimicrobial agents is the main solution. These agents minimize the risk of infection or the decay of products by the use of chemicalor by the use of physical agents (X-ray, Gama-ray, e-beam, etc). This leads to reduce bacteria number, particularly pathogenic microbes. However, it must be mentioned that none of antimicrobial agents are suitable for all kinds of microbes. Moreover, all of them are toxic for human. New disinfectants (e.g. antimicrobial agents extracted from plants) must be introduced based on genetic diversity of microbial pathogens and presence of resistant strains [1-3].

Herbal plants are used as long as human history, and the extract of these plants have been used as drug. Although the interest and attention to the useful plants were modest in recent years, but it has recently received great attention.One of the key ingredients of herbal plants is essential oils which available in different parts of them[4, 5].

Zhumeriamajdae(Figure 1) is endemic in Iran and is an exclusive plant of Hormozganprovince, especially in Ghotbabad, Kohgeno, Kohtang, Kohsarechahan, etc[6]. Some studies show that their properties of materials (such as drugs, hormones, oils, etc.)are changed, when they encapsulated[7, 8]. Therefore, in this study, our aim was to evaluate and compare the antimicrobial property of Zhumeria majdae essential oils (EO) and encapsulated form of the essential oil (EFEO).

Figure 1. Zhumeria majdae obtained from Hormozgan province.

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Materials and Methods

Extraction of EOfrom Zhumeriamajdae

First, the plantwas collected from different parts of Hormozgan province, and verified by a botanist. Then, the plant was dried, and grinded. In the next step, 50 gram of grinded plant was added to 200 mL of distilled water and shaken for 2 hours. Finally, the EO was extracted by hydrodistallation method by means of clevenger apparatus[6].

Preparation of encapsulated form of Zhumeria majdae EO

One mL of the plant EO was mixed with 9 mL of diethyl ether. Then, 5 ml of diluted oils (10% v/v) and 1 mL of 5 mg/mL lecithin (Sigma-Aldrich, USA) were added to 5 mL of distilled water, and strongly shaken for 5 minutes in presence of ceramic balls. At this stage, the color of suspension was changed to milky which represents the formation of liposomes and micelles.One droplet of suspension was held on Mica surface, then was dried, and imaged by atomic force microscopy (AFM) (DMT, Denmark).Serial concentrations (10%, 5%, 2.5%, 1.25%, and 0.6%) of the EO and EFEO were prepared in 50% DMSO and distilled water, respectively(9).

Antimicrobial activity

Microdilution method was used to evaluate the antimicrobial property of EO and EFEO. At end, MIC50 and MIC90 were measured for each against each strain. First, standard isolates of Escherichia coli, Staphylococcus aureus, Candida albicans, and Aspergillusniger were provided from Iranian Research Organization for Science and Technology (Table 1).

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Table 1. Standard microbial strains which used in this study

Species	**Error! No text of specified style in document.**ATCC
E.coli	25922
S.aureus	25923
C.albicans	10231
A.niger	16888

Both of bacteria were cultured on nutrient agar (Invitrogen, UK)and incubated for 24 hours at 35 °C, and both of fungi were cultured on Sabouraud dextrose agar (Invitrogen, UK) for 24 hours at 25 °C. Next, a single colony of each strain was separately added to Mueller Hilton broth (Invitrogen, UK) to adjust to ½ McFarland.At next, 100 µL of serial concentrations of EO and EFEO was separately exposed to 100 µL of bacterial and fungal suspension, and incubated for 24 hours. The incubation temperature was 35 °C and 25 °C for bacterial and fungal isolates, respectively. After incubation, the optical density (OD) of wells caused by the growth of fungi and bacteria was determined by spectrophotometer (Novin Gostar, Iran) at 405nm. To calculate MICvalues, the difference of OD (ODt0-ODt24) in negative control was considered as 100%growth or 0% death. In the next step, the difference ofOD (ODt0-ODt24) in each well was measured, and MIC50 and MIC90 were extracted from Formula 1. Since solvents had antimicrobial property, the inhibition percentage of test wells was detracted from the inhibition percentage of each solvent(10).

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Formula 1.

The inhibition percentage = (ODt0-ODt24) test×100/(ODt0-ODt24) control

Controls

Some controls were considered in this study was as following:

1. Microbial suspensions were separately incubated with solvents including distilled water, DMSO, and diethyl ether.

2. Bacterial isolates were incubated with 5 µg/mL ciprofloxacin.

3. Fungal isolates were incubated with 3 µg/mL nystatin.

Toxicity test

To evaluate the toxicity of phytol, MTT assay was used. First, serial concentrations (62.5, 125, 250, 500, 1000 µg/mL) of phytol were prepared. Then, 100 µL of each concentration was added to 100 µL of mouse skin suspension, and incubated for 12, 24 and 36 hours at 37 °C. Mouse skin suspension was prepared according to standard protocol [11], and adjusted at 10000 cells/mL. After incubation, 25 µL of 5 mg/mL MTT (Sigma-Aldrich, USA) was added to each well and then incubated at 37 °C for 3 hours. Finally, the optical density of each well was read by the ELISA reader at 492 nm. Such as antimicrobial test, in control well, 100 µL of distilled water was added to 100 µL of mouse skin suspension. Finally, the percentage of cell viability was calculated [11].

Results and discussion

Figure 2 shows AFM image of EFEO. As shown, globular particles at micrometer to nanometer scale were observed. The size distribution of EFEO was between 100-500 nm. Table 2and Table 3 show the MIC of EO and EFEO of Zhumeria majdae against Escherichia coli, Staphylococcus aureus, Candida albicans, and Aspergillusniger, respectively. As seen, the MIC50 of both EO and EFEO was similar (0.6%). In case of MIC90, EO and EFEO had same value against Escherichia coli, Staphylococcus aureus, and Aspergillusniger. But in case of Candida albicans, the MIC90 of

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EO and EFEO were 5% and 1.25%, respectively.It means that EFEO has greater effect on Candida albicans than EO. On the other hand, EFEO and EO have same effect on other microorganisms.

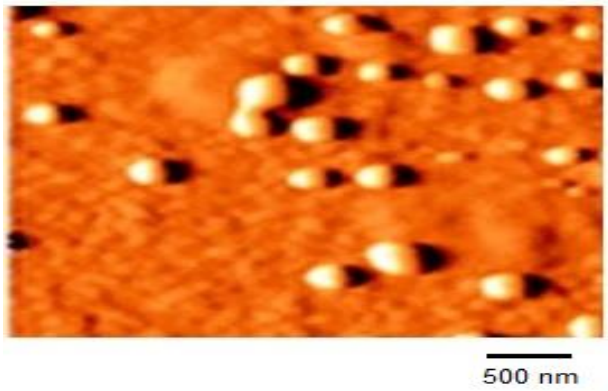

Figure 1. The AFM image of EFEO

Table 2. The MIC50 and MIC90 of Zhumeria majdae EO against microbial strains

Type of microorganism	MIC_{90} (%v/v)	MIC_{50} (%v/v)
Aspergillus niger	2.5	0.6
Candida albicans	5	0.6
Escherichia coli	1.25	0.6
Staphylococcus aureus	1.25	0.6

Table 3. The MIC50 and MIC90 Zhumeria majdae EFEO against microbial strains

Type of microorganism	MIC_{90} (%v/v)	MIC_{50} (%v/v)
Aspergillus niger	2.5	0.6
Candida albicans	1.25	0.6
Escherichia coli	1.25	0.6
Staphylococcus aureus	1.25	0.6

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The cell viability of skin cells when exposed to Zhumeria majdae EO and EFEO is shown in Figure 2 and Figure 3, respectively. The results revealed that the toxicity of both EO and EFEO was dose-dependent, i.e. the minimum concentration caused maximum cell viability and viscera. Such pattern was observed for all incubation times. In case of Zhumeria majdae EO, significant difference ($P<0.05$) was observed between cell viability at concentration of 5%, 2.5%, and 1.2% vs. cell viability at concentration of 0.6% and 0.3%.Another finding was that the toxicity of EFEO was time-dependent, and toxicity of EO had not such pattern. In case of EFEO, the decrease of incubation time led to increase of cell viability. As seen, the toxicity of Zhumeria majdae EFEO was less than Zhumeria majdae EO.

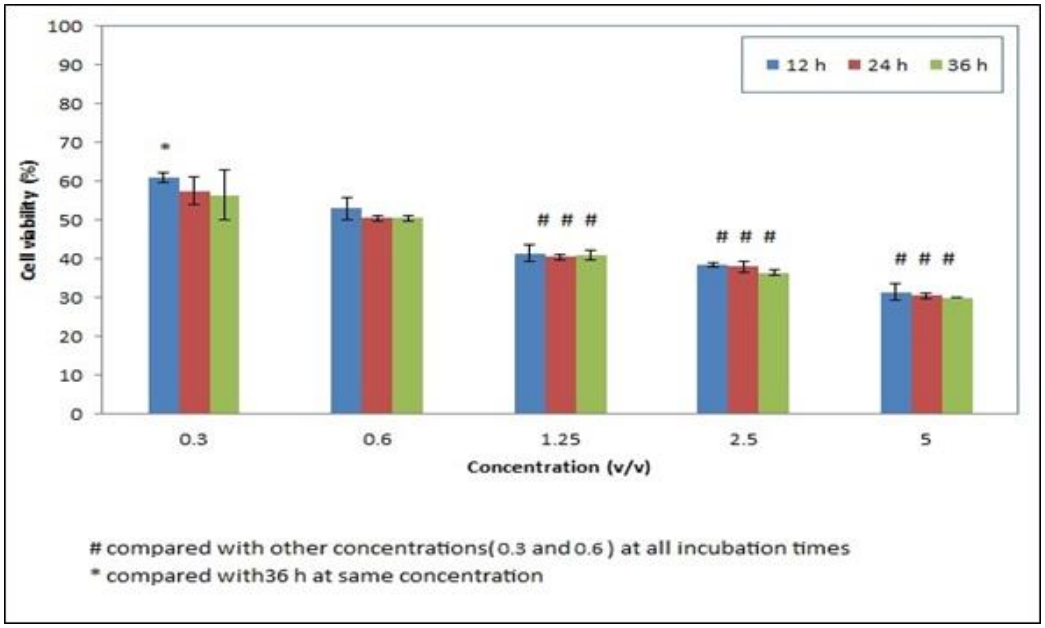

Figure 2. The cell viability after exposed to Zhumeria majdae EO, by MTT assay

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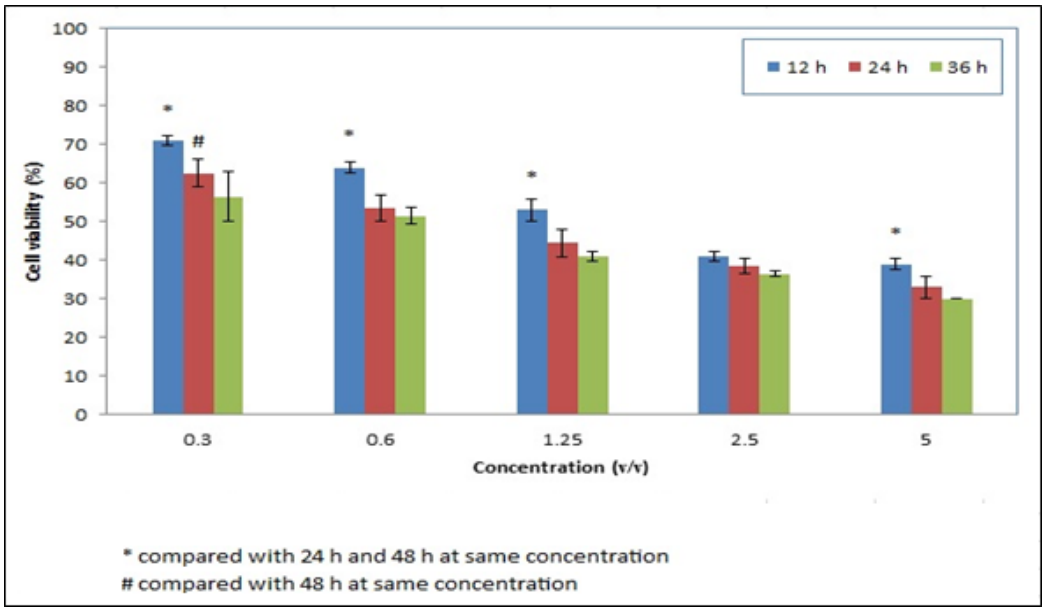

Figure 3. The cell viability after exposed to Zhumeria majdae EFEO, by MTT assay

It must be mentioned that antimicrobial property of the EO of Zhumeria majdae is not new finding. Previously, this had been reported by other researchers. For the first time, in this study, toxicity of Zhumeria majdae EO was investigated. Mahboubi et al evaluated the antimicrobial activity of Zhumeria majdae EO against some gram negative, gram positive, yeast and fungi. The EO exhibited similar levels of antimicrobial activity against different microorganisms, but some microorganisms were more sensitive. i.e it had significant power against Klebsiella pneumoniae with MIC and MBC values 0.5 and 1 µl ml-1. The EO showed antimicrobial property against Staphylococcus aureus, Bacillus cereus, Escherichia coli, Enterobacter aerugenes, Bacillus subtilis, Proteus vulgaris, Aspergillus flavus, Aspergillus niger, Salmonella typhi, Pseudomonas aeruginosa, and Bacillus subtilis[10]. Soltanipour et al collected Zhumeria majdae from Sarchahanmountaiof Hormozgan province and the EOs were analyzed with GC/MS. Then, antimicrobial activity of different concentrations of EOs was tested.GC/MS analysis showed that there were 22 different compounds in EOs of leaves of Zhumeria majdae. This study showed antibacterial activity of the EO on Escherichia

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coli and Staphylococcosaureous[12]. Mahboubi et al tested antimicrobial activity of thirteen EOs against some bacteria, fungi and yeast using disc diffusion and micro broth dilution assays. In disc diffusion assay, Zataria multiflora and Satureja hortensis oils showed the highestantimicrobial activity. The MIC values of Zataria multiflora and S. hortensis oils were lower than of 1 µl/ml [13].In another study, they evaluated the antimicrobial activity of the EOs from Mentha piperita and Zhumeria majdae, Ziziphora tenuior against extended spectrum β-lactamases (ESBLs) of Kelebsilla pneumoniae. This study showed that the antimicrobial activity of Ziziphora tenuior EOs wasclose to that of Zhumeria majdae EO. The Mentha piperita oil was less effective than the Zhumeria majdae. They declared that the antimicrobial effects of the EOs were increased when they were used incombination [14].

Here, we found that antifungal property of the EFEO of Zhumeria majdae was increased compared with the EO of Zhumeria majdae. Interstingly, EFEO and EO had same antimicrobial activity against other microorganisms. There are some reports that shows encapsulated EO have different antimicrobial property. For example, Van Vuuren et al encapsulated the EO from Artemisia afra, Eucalyptus globulus and Melaleuca alternifoliaby diastearoylphosphatidylcholine and diastearoylphosphatidylethanolamine.

Fractional inhibitory concentrations (FICs) were calculated in order to determine the antimicrobial activity. With the exception of A. afra, microbial growth was inhibited at lower concentrations for the encapsulated formulations in comparison with the non encapsulated EO [7].Lemongrass (Cymbopogomcitratus) EO was micro encapsulated by Leimann et al. in this study, the composition and the antimicrobial properties of the encapsulated oil were determined, demonstrating that the process of microencapsulation did not deteriorate the encapsulated essential oil [15]. In the study of Liolios et al, the chemical composition of the EO from Origanumdictamnus was analyzed by GC–MS. The EO was successfully encapsulated in phosphatidyl choline-based liposomes and the antimicrobial activities were tested. All tested

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compounds presented enhanced antimicrobial activities after the encapsulation [9].

Conclusion

It is concluded that in some cases, encapsulation can lead to better antimicrobial property and less toxicity. It seems that the types of EO, microbial strain, size of liposomes are main parameters. The authors propose that because of high antimicrobial activity of both EO and EFEO,they may be used as new antimicrobial agents for using in foodstuffs.

Acknowledgments

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