

Antimicrobial Effect of 15 Medicinal Plant Species and their Dependency on Climatic Conditions of Growth in Different Geographical and Ecological Areas of Fars Province

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Article information	Abstract
Article history: Received: 2 July 2011 Accepted: 1 Aug 2011 Available online: 8 Jan 2012	<p>Background: The effects of medicinal plants are variable in different conditions. Here, the antimicrobial effect of 15 medicinal plant species and their dependency on the climatic condition of growth in different geographical and ecological areas of Fars Province were studied.</p> <p>Materials and Methods: In This empirical study, the antimicrobial effect of hydro-alcoholic extract of 15 medicinal plant species was examined against standard bacterial strains comparing to conventional therapeutic antibiotics using disk diffusion assay and serial broth dilution.</p> <p>Results: All Extracts were effective against <i>S.aureus</i> ATCC 25923 growth; also <i>Peganum harmala</i>, <i>Myrtus communis</i>, <i>Mentha pulegium</i>, <i>Mentha</i> spp, and <i>Zataria multiflora</i> extracts were observed to have antimicrobial activity against <i>E.coli</i> ATCC 25922. This antimicrobial activity had partially similar results, comparing to conventional antibiotics</p> <p>Conclusion: Medicinal plants produce various amounts of antimicrobial substances under the climatic and ecological conditions of each zone, which must be considered in manufacturing herbal medicines.</p> <p>Copyright© 2012 Zahedan University of Medical Sciences. All rights reserved.</p>
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Introduction

Using chemicals in medicine production has always drawn researchers' attention; however, chemical medicines' side effects have led to an inclination toward applying essential compounds in the diseases treatment from thousands of years ago. Medicinal plants are used in treating diseases as low-risk, available, and inexpensive natural materials with higher consumption by people comparing to synthetic drugs [1, 2].

Upon recognizing the first antibiotic and developing its use in the treatment, new antibiotics were increasingly developed for infections recovery. Excessive use of these antimicrobial drugs led to medicinal resistance increase against different antibiotics in most bacteria [3, 4]. And, that is why a new wave of expansive global studies has raised and the antibacterial effects of different plants have introduced [1, 2]. Diversity of geographical and climate conditions in Iran has resulted in a diverse and rich source of plant species in our country some of which have medicinal properties including antibacterial activity. Climatic differences of growth in terms of conditions such as average precipitation and annual temperature, altitude from sea level, type of soil, and many other physical, chemical and ecological factors affecting the growth of

plants can lead to qualitative and quantitative differences in morphology and amount of substances produced by plants – which in turn lead to changes in the effects of a plant [5, 6].

This study is aimed at examining the dependency of 15 plant species antimicrobial properties on the growth climate in different zones of Fars Province. These plants included *Peganum harmala*, *Cinamomum zeylanicum*, *Achillea fragrantissima*, *Urtica dioica*, *Saturina hortensis*, *Myrtus communis*, *Mentha pulegium*, *Zingiber officinale*, *Cuminum cyminum*, *Saturina hortensis*, *Plantago psyllium*, *Glycyrrhiza glabra*, *Matricaria chamomilla*, *Mentha* spp., and *Zataria multiflora*. The antimicrobial effects of these plants are demonstrated by the researchers in previous studies [1, 5-10]. The given plants are used by the people of Fars Province to treat some clinical infections.

Materials and Methods

In this empirical-experimental study, the antimicrobial effect of hydroalcoholic extract of 15 medicinal plants species was examined comparing to conventional therapeutic antibiotics against standard bacterial strains.

Herbal Extract Preparation: the plant species were collected from three different zones of Fars Province with various climates on May and were identified and approved by plant products expert. These areas include: Sepidan town and its suburbs (as the cold zone), Shiraz City and its suburbs (as the temperate zone) and Fasa town and its suburbs (as hot and dry zone). Flowers, leaves and stalks of the plants were kept in a cold, dark place and then grounded by electrical mill. About 3g of each plant (typically used as brewed in traditional medicine) was mixed with 6ml ethanol alcohol (60%) and the hydro-alcoholic essence of plants was extracted using soaking technique after 24h. Dried matter of the extract (obtained by SENCO rotary evaporator device) was reached to 50ml volume (about a small tea cup) by distilled water under sterilized conditions for antibacterial assay [11].

Antibacterial Assay: here, the antibacterial effects were examined using agar-based disk diffusion assay and serial broth dilution. **Agar-based Disk Diffusion Assay:** The applied disks were prepared using blank disks and 100, 200 and 300 µl herbal extracts; then they were subjected to ultraviolet ray for 2h to be sterilized. Using the bottom of Pastor Pipette, sumps were made in Mueller-Hinton agar medium where about 100, 200 and 300 µl of studied herbal extracts were poured. Since the extracts had colors, we could trace the extract diffusion based on color dispersion, as well. To standardize the assay, the antibiogram disks made by Teb-Iran Co. as well as *S.aureus* ATCC 25923 and *E.coli* ATCC 25922 were used

as the standard strains [12]. **Dilution Assay:** serial dilutions 1.2 to 1.64 were prepared from the basic extract (3g in 50ml distilled water) in Mueller Hinton Broth medium. As in disk diffusion assay and in accordance with CLSI standard (using standard turbidity assay of Nim Mcfarland), the bacterium suspension was added to the dilutions.

The media containing bacterium and herbal extracts were incubated at 37°C for 24 h, and Minimum Inhibitory Concentration (MIC) was determined. Then, 1ml non-turbid tube content incubated for 24h with its MIC determined was picked up under completely sterilized conditions, externally cultured on plates containing culture medium and incubated at 37 °C for 24h. The first concentration to inhibit the growth was considered as Minimum Bacterial Concentration (MBC). All assays were carried out in triplicate and mean result was calculated. Statistical analysis was conducted using X2 test.

Results

Results indicated the antibacterial effect of all herbal extracts against *S.aureus* ATCC 25923; also *Peganum harmala*, *Myrtus communis*, *Mentha pulegium*, *Mentha* spp, and *Zataria multiflora* extracts were observed antimicrobial activity against *E.coli* ATCC 25922; besides, *Peganum harmala*, *Myrtus communis*, *Mentha pulegium*, *Mentha* spp, and *Zataria multiflora* extracts simultaneously showed antimicrobial activity against the both given bacteria (Tables 1 & 2).

Table 1. Results of drug sensitivity test about medicinal plants and standard strain *E.coli* ATCC 25922

Extraction Concentration	100 µl			200 µl			300 µl		
	Geographical region			Geographical region			Geographical region		
	Fasa	Sepidan	Shiraz	Fasa	Sepidan	Shiraz	Fasa	Sepidan	Shiraz
Medicinal plant	Inhibitory diameter zone (mm)*								
<i>Achillea millefolium</i>	-	-	-	-	-	-	-	-	-
<i>Urtica dioica</i>	-	-	-	-	-	-	-	-	-
<i>Satureja hortensis</i> leaves	-	8	8	8	10	10	11	13	13
<i>Cinnamomum zelanicum</i>	-	8	8	8	10	10	10	12	12
<i>Peganum harmala</i>	9	11	11	11	13	14	14	15	15
<i>Satureja hortensis</i> seeds	8	10	9	10	11	10	11	13	11
<i>Myrtus communis</i>	10	14	12	13	16	15	15	17	17
<i>Mentha pulegium</i>	9	11	13	11	13	14	13	15	16
<i>Zingiber officinale</i>	-	7	10	7	9	11	10	13	13
<i>Cuminum cyminum</i>	-	9	9	8	10	11	10	12	13
<i>Plantago psyllium</i>	-	-	-	-	-	-	-	-	-
<i>Glycyrrhiza glabra</i>	-	8	7	7	10	8	9	11	11
<i>Chamaemelum nobile</i>	-	-	-	-	-	-	-	-	-
<i>Mentha Piperita</i>	11	13	13	10	15	15	13	17	18
<i>Thymus vulgaris</i>	12	13	12	13	14	14	15	15	16

*1- The diameter of the inhibition zone 15mm≤, the same effect as Gentamicin, Nitrofurantoin and Tobramicina, 2- the diameter of the inhibition zone 16mm≤, the same effect as Cotrimoxazole and Imipenem, 3- the diameter of the inhibition zone 17mm≤, the same effect as Amoxicillin, 4- the diameter of the inhibition zone 18mm≤, the same effect as Sefctacidium, 5- the diameter of the inhibition zone 21mm≤, the same effect as Ciprofloxacin, 6- the diameter of the inhibition zone 23mm≤, the same effect as Cefotaxime.

Table 2. Results of drug sensitivity test about medicinal plants and standard strain *S.aureus* ATCC 25923

Extraction Concentration	100 µl			200 µl			300 µl		
	Geographical region	Fasa	Sepidan	Shiraz	Fasa	Sepidan	Shiraz	Fasa	Sepidan
Medicinal plant	Inhibitory diameter zone (mm)*								
<i>Achillea millefolium</i>	8	9	10	9	11	11	11	13	13
<i>Urtica dioica</i>	8	9	10	10	11	11	12	13	13
<i>Satureja hortensis</i> leaves	9	11	13	10	13	16	11	15	17
<i>Cinnamomum zelanicum</i>	9	9	10	11	10	11	13	11	13
<i>Peganum harmala</i>	7	18	19	9	19	22	11	21	23
<i>Satureja hortensis</i> seeds	-	9	10	-	11	12	-	13	15
<i>Myrtus communis</i>	13	13	15	15	16	16	17	18	18
<i>Mentha pulegium</i>	9	11	12	10	14	15	12	16	17
<i>Zingiber officinale</i>	-	9	11	-	11	12	-	14	15
<i>Cuminum cyminum</i>	-	9	11	-	11	12	-	13	14
<i>Plantago psyllium</i>	-	-	-	-	-	-	-	-	-
<i>Glycyrrhiza glabra</i>	7	13	16	9	15	17	11	17	19
<i>Chamaemelum nobile</i>	7	-	9	9	7	11	11	9	14
<i>Mentha Piperita</i>	10	12	14	12	14	16	14	16	17
<i>Thymus vulgaris</i>	7	11	12	10	12	13	11	14	15

*1- The diameter of the inhibition zone 15mm≤, the same effect as Oxaciline, 2- the diameter of the inhibition zone 15mm≤, the same effect as gentamicin, and vancomycin, 3- the diameter of the inhibition zone 17mm≤, the same effect as Amikacin, 4- the diameter of the inhibition zone 19mm≤, the same effect as Tetracycline, 5- the diameter of the inhibition zone 20mm≤, the same effect as Cefoxitin and Imipenem, 5- the diameter of the inhibition zone 21mm≤, the same effect as siprofloxacine, 7- the diameter of the inhibition zone 23mm≤, the same effect as erythromycin, 8- the diameter of the inhibition zone 23mm≤, the same effect as peniciline.

Dilution 1.8 of *Myrtus communis*, *Mentha* spp. and *Glycyrrhiza glabra* extracts and dilution 1.4 of *Peganum harmala*, *Satureja hortensis* and *Mentha pulegium* extracts under serial dilution assay are observed to inhibit the growth. The results were confirmed by culturing the content of the tubes with the given dilutions on a nutrient medium (nutrient agar).

Based on the results of the herbal extracts antibiogram against *S.aureus* ATCC 25923, *Myrtus communis* had the highest antimicrobial effect and *Plantago psyllium* had the least antimicrobial effect.

Also regarding zonal total efficacy, the plants of Shiraz (26 plants), Sepidan (21 plants) and Fasa (5 plants) were respectively recorded as the most effective plants. With *E.coli* ATCC 25922, maximum inhibitory effect was observed with *Myrtus communis* and minimum inhibitory effect was observed with *Cinnamomum zeylanicum*, *Achillea fragrantissima*, *Zingiber officinale*, *Cuminum cyminum*, *Satureja hortensis*, *Plantago psyllium*, *Glycyrrhiza glabra* and *Matricaria chamomilla*. Regarding total efficacy, the plants of Shiraz and Sepidan (7 plants), and Fasa (2 plants) respectively showed maximum inhibitory effect.

Discussion

Results demonstrated that antimicrobial effects of the plant species under study collected from various zones of Fars Province are to a significant extent zone-dependent so that the maximum effect has respectively been observed in Shiraz, Sepidan and Fasa plants; that is, since all these plants are capable of growing in most zones of the country and all were collected from the mountain hills

and heights of the given zones, temperate climate provides a more suitable context for optimized propagation and growth of the plant species under study in terms of producing essential substances (in particular secondary metabolites of plant) comparing to cold and hot climates [1,5,13-15].

Producing high quality medicines is among the most significant subject of the medicinal plants production, and this would not be feasible unless we detect and utilize appropriate plant species (in terms of producing the effective substances) affected by suitable ecological conditions including the amount of sunlight in different hours of day-and-night, mean annual temperature of a geographical zone, soil quality, height from the sea level, annual humidity and precipitation percent, and physico-chemical indices [5,6].

Notwithstanding, based on the expansion of arable areas of the country and potential capability of the herbal medicines as an alternative to synthetic medicines, we can prospect an outstanding regional and global status for medicinal plants export and pharmaceutical industry of the country by conducting comprehensive and deliberate studies on the medicinal plants effects of each zone in-vitro and in-vivo.

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