In Vitro Antibacterial Activities of Essential Oils and Extracts of Six Herbals Against Gram-positive and Gram-negative Bacteria

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Research Article

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Abstract

Background: Today, due to the increasing use of chemical drugs and the spread of microbial resistance to synthetic antibiotics, as well as side effects of drugs, the identification and introduction of plant species with medicinal and antimicrobial properties is very important. In this study, the antimicrobial properties of essential oils and extracts of 6 medicinal plants from Ahvaz region, Iran against 12 Gram-positive and Gram-Negative Bacteria were evaluated.

Methods: The EOs and extracts were extracted using water distillation with Clevenger apparatus. The antimicrobial properties and determination of the minimum concentration of growth inhibition of herbals were investigated by the modified E-test method.

Results: All analyzed extracts and EOs showed antibacterial effects. The antimicrobial activity of *Oliveria decumbens* was strongest herbals with the least MIC ranges (0.008-0.1 mg/ml for EO, 0.9-20 mg/ml for extract), while the antibacterial effects of *Artemisia vulgaris* extract and *Glycyrrhiza glabra* EO with the highest MIC were weaker than the others. According to the effectiveness of plant extracts on bacteria, *Pseudomonas aeruginosa* was resistant to all extracts except *Oliveria decumbens.* In contrast, *Bacillus cereus* was more sensitive than other strains against analyzed EOs and extracts.

Conclusions: It seems that due to the antimicrobial properties of the extracts and essential oils observed in this study, they can be used as an alternative to antimicrobial drugs after more extensive studies.

Introduction

Herbal essential oils (EOs) and extracts have been used for various purposes for thousands of years (1). These aims vary from the use of rosewood and Cedar in perfumes to flavoring drinks with lime fennel or juniper berry oil, and the use of lemongrass oil in food preservation (2, 3). The basis of food storage, herbal medicines, and natural treatments is the antimicrobial properties of plant essential oils and extracts (4, 5). Medicinal and aromatic plants are widely utilized in medicine to combat drug-resistant microorganisms, which are considered as one of the primary causes for the failure of therapy in infectious diseases. Medicinal plants are the primary source of novel medicines and may serve as an alternative to conventional medications (6).

Traditionally, people in different areas of the globe utilize extracts and oils for six plants in this work for diverse purposes. The essential oil of *Oliveria decumbens* contains antibacterial and antifungal properties as well as cytotoxic effects on several cancer cell lines (7). Bioactive substances with varied antioxidant and antibacterial properties discovered in ethanolic extracts and essential oils of *Ocimum basilicum* have significant uses in nutraceutical and pharmaceutical technologies (8). *Glycyrrhiza glabra* has immunological effects in humans (9); *Artemisia vulgaris* oil has good antimicrobial activities (10); and finally *Silybum marianum* herb extract has antibacterial and antiadherent/antibiofilm activity against Gram-negative bacteria (11). In Iran numerous plant extracts and oils, notably for respiratory and gastrointestinal problems, are traditionally utilized as a medicinal plant.

Some investigations have been done only on one extract or one microorganism. While this information is valuable, the results are not directly comparable because each study has a different methodology such as the type of microorganisms, selection of plant extracts, and antimicrobial testing methods (12).

The objective of the current research was to develop directly comparable, quantitative antimicrobial data for extracts that have little information. This study focused on the effect of 6 plant species commonly used in our region, including *Oliveria decumbens, Glycyrrhiza glabra, Ramhormoz Ocimum basilicum, Abadan Ocimum basilicum, Silybum marianum*, and *Artemisia vulgaris* on the most pathogenic bacteria that cause community-acquired and hospital-acquired infections.

Materials And Methods

Medicinal Plants and their collection: The herbals used in this study were Including *Artemisia vulgaris, Silybum marianum, Ramhormaz Ocimum basilicum, Abadan Ocimum basilicum, Glycyrrhiza glabra* and *Oliveria decumbens*. During the flowering season, between December and April 2019, samples were gathered from several areas in Khuzestan (Table 1). The samples were thoroughly cleaned to remove any unusual flora, dust, or other pollutants.

Table 1
Medicinal plants

Scientific name	Plant family	Collection site	Altitude (m)	Collection time	Extracted part
Oliveria decumbens Vent.	Apiaceae	Shushtar	165	February	Flowers and flowering branches
Glycyrrhiza glabra	Fabaceae	Behbahan	300	February	Root
Ramhormoz Ocimum basilicum	Lamiaceae	Ramhormoz	100	March	Purple mass
Abadan Ocimum basilicum	Lamiaceae	Abadan	120	March	Purple mass
Silybum marianum	Asteraceae	Hamidiyeh	120	December	seed
Artemisia vulgaris	Asteraceae	Izeh	300	April	flowering branches

Essential oil extraction: Essential oils were extracted by hydro-steam distillation using the Clevenger equipment from fresh, clean, weighed aerial parts of flowers, flowering branches, seeds, and rhizomes (Table 1) and collected and stored in dark sterile vials (13). Briefly, 100 to 150 g of each plant were placed in a distillation flask (1 L), which was linked to a steam generator through a glass tube and to a condenser. This was recovered in a funnel tube. Essential oil aromatic molecules were released from the plant material and evaporated into hot steam. The heated steam forced the plant material to release the essential oil without burning it. The steam containing the essential oil was then sent through a cooling

system to condense it. The steam was applied for 3 hours. The essential oil was extracted once the recovered mixture had been settled. The produced essential oil was dried by filtering the supernatant essential oil through anhydrous Na2SO4. Following that, the essential oil was gathered in tighter vials and refrigerated. Several dilutions of the oils were done using dimethyl sulfoxide (DMSO) for the antibacterial activity test.

Ethanolic extract preparation: Samples were washed, air dried for 7–8 days, and ground into powder before being put in the flask of the Soxhlet apparatus for extraction using ethanol with increasing order of polarity to extract the phytoconstituents individually at 20°C for 3–4 hours. The extracts were then filtered using Whatman No.1 filter sheets. Following that, decreased pressure was used to evaporate and dry the filtrates, which were then kept at -20°C in labeled, sterile, screw-capped vials.

Microorganisms and their maintenance: 12 Standard strains, including *Staphylococcus aureus* (*S. aureus*) PTCC 1113, *Escherichia coli* (*E. coli*) PTCC 1533, *Bacillus cereus* PTCC 1015, *staphylococcus epidermidis* PTCC 1435, *Pseudomonas aeruginosa* PTCC 1558, *Helicobacter pylori* PTCC 5211, *Acinetobacter baumannii* PTCC 1855, *Enterococcus faecalis* PTCC 1237, *Enterobacter cloacae* PTCC 1003, *Shigella dysenteriae* PTCC 1188, *Klebsiella pneumoniae* PTCC 1290 and *Corynebacterium diphtheria* ATCC 27010 obtained from the Persian Type Culture Collection. The subculture of bacteria using a panel of laboratory control strains obtained from the Persian Type Culture Collection. All bacteria were stored in trypticase soy broth containing 25% (v/v) glycerol for the time of the investigation.

Antimicrobial activity of plants: The best factor for evaluation of the antimicrobial activity of plants and comparison of their antimicrobial effect is a measurement of minimum inhibitory concentration (MIC). In this study, the modified E-test was used (14). A 5% concentration of each plant extracts and essential oils was prepared and diluted using the double dilution method. Then the 10^8 CFU/ml suspension of each bacteria was impregnated on the plate of sterile Mueller-Hinton agar (MHA, Merck) media by sterile cotton swab and permitted to stay in contact for 1 minute. Eight blank sterile paper discs were put on culture medium. Ten microliters of each dilution were impregnated in each disc. The plates were incubated at 37°C for 24 hours (15). All tests were done fourfold and the mean of results was calculated.

Results

In this research the antimicrobial effects of 6 plant specieses were evaluated against 12 bacterial strains. The results of the growth inhibition effect of plant extracts and essential oils on the studied bacteria are shown in Tables 2 and 3. Among the plant EOs, *Oliveria decumbens* EO had the highest inhibitory activity and *Glycyrrhiza glabra*, *Ocimum basilicum* and *Ocimum basilicum* essential oils had the least inhibitory effect on bacterial strains. However, all studied strains were sensitive against each essential oil. According to the effectiveness of plant extracts on bacteria, *Pseudomonas aeruginosa* was resistant to all extracts except *Oliveria decumbens.*

Table 2

(Minimum inhibitory concentration values of herbal essential oils against pathogenic bacteria (µg/ml

Plants Bacteria	*Oliveria decumbens*	*Glycyrrhiza glabra*	*Ramhormoz Ocimum basilicum*	*Abadan Ocimum basilicum*	*Silybum marianum*	*Artemisia vulgaris*
Staphylococcus aureus	22	500	100	110	50	80
Staphylococcus epidermidis	20	450	120	100	56	85
E. coli	20	400	110	95	55	76
Pseudomonas aeruginosa	100	1200	1100	1200	200	220
Helicobacter pylori	18	420	230	220	60	75
Acinetobacter baumannii	12	420	220	240	60	80
Enterococcus faecalis	20	480	280	310	55	65
Enterobacter cloacae	22	510	310	300	65	55
Shigella dysenteriae	16	380	180	205	45	55
Klebsiella pneumoniae	24	480	350	365	65	85
Corynebacterium diphtheriae	28	560	120	180	50	75
Bacillus cereus	8	210	84	95	38	50

Table 3

(Minimum inhibitory concentration values of herbal extracts against pathogenic bacteria (mg/ml

Plants Bacteria	*Oliveria decumbens*	*Glycyrrhiza glabra*	*Ramhormoz Ocimum basilicum*	*Abadan Ocimum basilicum*	*Silybum marianum*	*Artemisia vulgaris*
Staphylococcus aureus	2.5	23	25	28	45	65
Staphylococcus epidermidis	1.8	20	22	26	50	62
E. coli	1.8	24	24	26	45	60
Pseudomonas aeruginosa	20	-	-	-	-	-
Helicobacter pylori	1.2	28	30	28	46	68
Acinetobacter baumannii	1.4	32	30	32	40	70
Enterococcus faecalis	2.2	22	24	30	50	65
Enterobacter cloacae	2.5	35	38	42	55	60
Shigella dysenteriae	1.4	36	34	34	50	55
Klebsiella pneumoniae	2.8	38	40	41	48	65
Corynebacterium diphtheriae	3.2	36	38	36	42	62
Bacillus cereus	0.9	12	14	15	38	45

Discussion

So far, wild or cultivated medicinal herbs have been used as important sources of medicine. Traditional botanical science offers valuable methods for discovering new medicinal herbs and herbal remedies (16, 17). Today, the use and application of plants require comprehensive information about understanding their properties and characteristics (16). The scientific study of medicinal plants as potential sources of new antimicrobial compounds is crucial (18).

The results of this study showed that the essential oils and extracts of these plant samples have antibacterial properties. Essential oils appear to have more antimicrobial effects than extracts.

Antibacterial activity of different EOs and extracts of medicinal plants on different microorganisms in different regions has been reported.

In 2020, Khoshbakht et al. from Bandar Abbas examined *Oliveria decumbens'* antibacterial activity of essential oil on seven microbial strains. MIC values were in the range of 0.0625–2 mg/ml (7), while in this study the minimum inhibitory concentration of essential oil and extract of *O. decumbens* was 0.008-0.1 mg/ml and 0.9–20 mg/ml respectively. In another study conducted in 2018 in Kazerun, *Glycyrrhiza glabra* extract had antibacterial effects on strains such as *S. aureus*, *B. cereus, E.coli, P. aeruginosa*, and the MIC range was 6.25–100 mg/ml, The MIC for B. cereus strain was 12.5 mg/ml (19). Also, in the present study, *Glycyrrhiza glabra* essential oil and extract showed antimicrobial effects on gram-positive and gram-negative bacterial strains, and MIC on *B. cereus* was 12 mg/ml. In the study of Manandhar et al. from Nepal, the antibacterial activity of *Artemisia vulgaris* extract on some gram-positive and gram-negative bacterial strains was investigated. The study results showed that the extract of this species did not have any effect on strains (10). Also, in this study, *Artemisia vulgaris* extract did not affect *Pseudomonas aeruginosa* strain. In Turkey, Evren et al. studied the antimicrobial effect of *Silybum marianum* extracts. This species showed antimicrobial activity on gram-positive and gram-negative bacteria and created MIC with a range of 0.06–0.2 mg/ml (11). In contrast, the extracts of this plant in our study showed antimicrobial activity with MIC of about 55 – 38 mg/ml. According to a study in Romania, the essential oil of plant species of *Ocimum basilicum* showed better effects on gram-positive bacteria (20). Also, in our study, gram-positive bacterial strains were more sensitive to *Ocimum basilicum* extract and essential oil. Besides, In this study, essential oils and extracts of aerial parts of two types of basil species were investigated, and *Ramhormoz Ocimum basilicum* showed better antimicrobial effects than *Abadan Ocimum basilicum*. According to a study in 2020 in Cyprus, the effect of geographical conditions and altitude of the cultivation site can affect the type of compounds in the plant and their amount and thus antimicrobial activity, which due to the diversity of antimicrobial effect of different species in this study, our research consistent with the results of these researchers (21).

According to previous studies in different places and the findings of this study, it was found that the essential oils and extracts of the studied herbal species can show the antimicrobial effect on gram-positive and -negative bacteria and inhibit the growth with different MIC. This difference can be due to several reasons, including different methods of oil extraction, genetic factors, seasonal and environmental factors at the time of sampling. It seems that due to the antimicrobial properties of some extracts and essential oils that were observed in this study, they can be used as an alternative to antimicrobial drugs after more extensive studies.

Limitation of the study

In this study, the composition of extracts and essential oils does not determine.

Conclusion

The findings of this study showed that all essential oils and extracts of the studied plant species have strong antimicrobial activity. Among the species, *Oliveria decumbens* extract and EO had the most inhibitory effect. These differences are related to the composition of EOs and extracts, as well as the susceptibility of the bacterial strains against different plants.

It is noteworthy that in recent years, there have been many reports of bacterial resistance to antibiotics, and measures should be taken to combat them. Therefore, conducting additional research on the studied plants in this research and their active ingredients can be helpful in this regard. These plant extracts could be a potential source of new antibacterial agents.

Abbreviations

EO: Essential oil

MIC: Minimum Inhibitory Concentration

CFU: colony forming unit

Declarations

Ethics approval

This project was confirmed by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (No. IR.AJUMS.REC.1395.479).

Consent for publication

The authors all agree with the publication of the data in this article.

Availability of data and material

All data is available

Competing Interests

No conflict of interest declared.

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Authors' contributions

A.H. worked experimentally, S.A. and M.A. wrote the main manuscript text and F.H. prepared tables 1-3. All authors reviewed the manuscript.

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