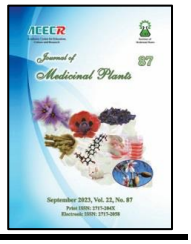
J. Med. Plants 2023; 22(87): 26-38

Institute of Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir



Review Article

Effectiveness of medicinal plant essential oils on drug-resistant bacteria in Iran: A systematic review

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ARTICLE INFO

Keywords:
Herbal medicine
Antibiotic resistant infections
Antimicrobial compounds
Human infection
Systematic review

ABSTRACT

Background: The efficacy of common antibiotics has been hindered by the emergence of drug resistance, including multidrug resistance (MDR). As a result, there is a growing interest in exploring alternative treatments for drug-resistant infections. One promising avenue is the use of herbal medicine, which possesses antimicrobial properties and may serve as a viable supplement or alternative to conventional antibiotics. **Objective:** This study aimed to determine the effectiveness of plant essential oils in Iran against drug-resistant bacteria. **Methods:** In this systematic review, we searched for keywords including herbal medicine, essential oil, *Acinetobacter* spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterococcus* spp. in Scopus, PubMed, Web of Science, and internal databases. Articles published up to October 4, 2023, were considered without any time limit. **Results:** The results showed that the majority of studies conducted were related to thyme, and the most tested bacterial strains were methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Thyme exhibited the highest antibacterial activity, with the lowest minimum inhibitory concentration (MIC) equal to 0.31 µg/ml, followed by *Oliveria decumbens* with a MIC of 0.625 µg/ml. **Conclusion:** Herbal medicines may offer an efficient treatment option for antibiotic-resistant bacteria. However, further studies are needed to investigate potential adverse effects, antibacterial properties, and possible synergistic effects with other medicinal plants.

Abbreviations: MDR, Multidrug-Resistance; MRSA, Methicillin-Resistant *Staphylococcus Aureus*; MIC, Minimum Inhibitory Concentration; ICU, Intensive Care Unit; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-analyses; MBC, Minimum Bactericidal Concentration.

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doi: 10.61186/jmp.22.87.26
Received 26 July 2023; Received in revised form 28 November 2023; Accepted 29 November 2023

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[DOR: 20.1001.1.2717204.2023.22.87.2.7]
[DOI: 10.61186/jmp.22.87.26]

1. Introduction

Microbial resistance is one of the important factors threatening public health, which has been associated with a significant increase in the prevalence and concerns expressed in this field [1, 2]. The emergence of drug resistance has imposed heavy burdens on health care systems, and ICUs have become a place with high potential for strengthening these types of microorganisms [3]. The widespread use of antibiotics in cases such as the treatment of human and animal bacterial infections, as well as their use in non-medical cases, has led to the development of drug resistance in bacterial strains [4]. Sansions Sánchez-López et al. (2019) named *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* strains as the most famous drug resistance strains [5]. Tosi et al. (2019) listed central vein access, pulmonary artery catheterization, urinary tract catheterization, and mechanical ventilation as risk factors for causing infection with drug-resistant microorganisms [3]. Antibiotic-resistant infections and their sensitivity to antibiotics are significant cases in hospital-acquired pathogen infection control programs [6]. According to the statistical estimates published by the World Health Organization, 700,000 people die annually due to antibiotic resistance [7]; therefore, the development of antimicrobial treatment solutions is necessary to prevent the predicted ten million deaths by 2050 [8].

The crisis of drug resistance and the lack of sufficient number of drugs to deal with these infections and the challenges of discovering new antibiotics to deal with these pathogens led researchers to discover new ways to deal with such diseases [9]. With the decrease in the effectiveness of antimicrobial elements and the emergence of drug-resistant pathogenic microbes, public attention to medicinal plants has increased in the past decade as a way to deal with these challenges [10]. Medicinal plants have been used by humans for many years and are of interest for reasons such as fewer side effects, lack of drug resistance, and use in cases such as human infections [11]. The development of antimicrobial compounds from plant sources is one of the main ways to deal with these pathogens, and recent studies have emphasized the antimicrobial properties of medicinal plant components [12]. The available evidence shows the effectiveness of medicinal plants in infectious diseases as a new source of antimicrobial agents that simultaneously have antimicrobial activity and modify antibiotic resistance [13]. Acceptance and good tolerance in long-term use of herbal compounds have been reported in patients, and it seems that these compounds are a reliable source for dealing with microbes [14].

Although many studies have investigated the effectiveness of native medicinal plants in the field of controlling common microorganisms in drug-resistant infections in Iran [15] [16] [17] so far, no study has been carried out in the field of investigation and screening of native medicinal plants and their effect on drug-resistant microorganisms, so in this study, we intend to investigate the antimicrobial effects of medicinal plants native to Iran against drug-resistant microorganisms.

2. Materials and methods

This systematic review was conducted based on the recommended checklist of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) [18]. The search encompassed all articles published until October 4, 2023, without imposing any time restrictions.

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2.1. Search strategy

The search strategy employed is presented in Table 1. Given the absence of a comparison group in this study, the C or same comparison group was not considered in PICO. The researchers handpicked keywords and subsequently extracted relevant articles, as illustrated in the extraction steps diagram. Resource management was facilitated using Endnote software.

Table 1. Search strategy based on plant essence effective on drug-resistance bacteria

PICO	Search terms	No.
P	*Acinetobacter* spp. OR *Pseudomonas aeruginosa* OR *Klebsiella pneumoniae* OR *Staphylococcus aureus* OR *Enterococcus* spp.	#1
I	Herbal medicine OR medicinal plant OR Essential oil	#2
O	antibacterial activity OR antimicrobial agent OR antimicrobial activity OR antimicrobial resistance OR multidrug resistant OR antibiotic resistance	#3

After conducting a thorough search using PubMed, Scopus, Web of Science, as well as internal databases such as SID, Iran Medex, Magiran, and Google Scholar, duplicate articles were eliminated. Subsequently, two individuals sorted and independently examined the titles and abstracts of the remaining articles. Any articles with unrelated titles and abstracts were excluded, leaving only those with relevant content. Finally, the full text of the selected articles was carefully reviewed.

To be included in the study, articles had to meet specific criteria. Firstly, the study had to be conducted in Iran. Additionally, the article had to focus on the use of plant essential oils. Lastly, the bacteria being investigated had to be drug-resistant. Only articles that fulfilled these inclusion criteria were evaluated for quality. Certain types of studies were excluded from the analysis. This included prospective, observational, and case report studies, conference abstracts, systematic reviews, as well as studies not written in English or Persian. Furthermore, studies related to food, poultry, and animal bacteria were also excluded. By following this rigorous process, we ensured that only relevant articles were included in our study.

2.2. Risk of bias assessment or qualitative assessment

The evaluation of the risk of bias was based on and adapted from previous studies [19-21]. The following parameters were used to evaluate the quality of the articles:

1. Control
2. Sample size calculation
3. Materials used to manufacturer's instruction
4. Single operator
5. Preparation and handling
6. Time interval
7. Blinding of the observer

If any of the mentioned parameters were reported in the article, the word YES was placed in the quality assessment table, and if the mentioned parameters were not observed, the word NO was placed. Articles that reported 1-3 cases were classified as high risk, 4-5 as moderate risk and 6-7 as low risk of bias.

2.3. Extracting the data

After searching, screening, checking for inclusion criteria and then doing quality control, the data of each study based on the name of the first author, the name of the plant, the target bacteria, the type of test (disc/well/MIC), the

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most important results and findings and inhibitor concentration were extracted from the full text of the articles.

3. Results

A total of 914 articles written in English were collected from the main databases, while 245 articles in Persian were obtained from national databases. In total, 1159 articles were reviewed, and eventually, 22 articles were selected for further analysis. These 22 articles specifically focused on testing the antibacterial effect of plant essential oils on resistant bacteria. The selected articles were then summarized, evaluated for quality, and depicted in Figure 1.

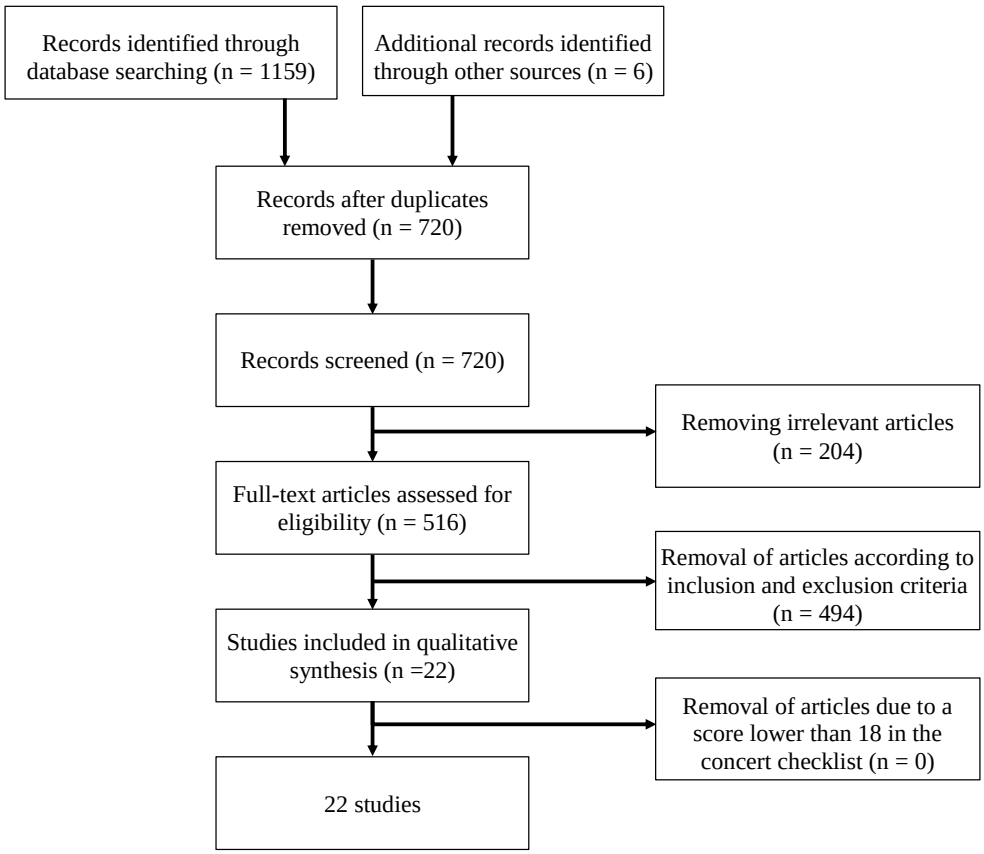

Fig. 1. Flow diagram showing literature search results.

Among these 22 articles, the largest number of studies was related to thyme (total of 8 articles, 3 articles of *Zataria multiflora*, 2 articles of *Thymus daenensis*, 1 article of *Thymus kotschyanus* and 1 article of common *Zataria multiflora*), *Ferula gummosa* (3 articles) and eucalyptus (2 article) (Fig. 2). In these 22 articles in which plant essential oils were tested, generally three methods were used to check the antibacterial activity of essential oils; Agar disk diffusion, agar well diffusion and micro & macro dilution broth, which is the third method to determine the minimum inhibitory concentration or MIC (Minimum Inhibitory Concentration).

The most bacterial strains tested in these 22 articles were related to methicillin-resistant *Staphylococcus aureus* or MRSA strains (8 articles) and multidrug-resistant *Pseudomonas aeruginosa* strains (6 articles) (Fig. 3).

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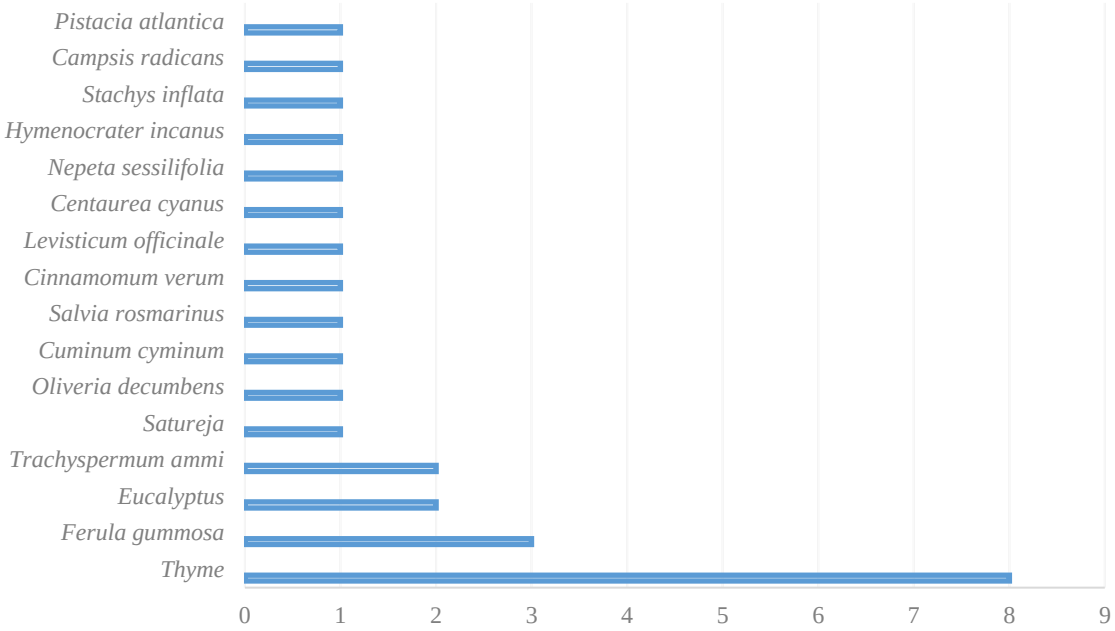

Fig. 2. Number of studies in each plant species.

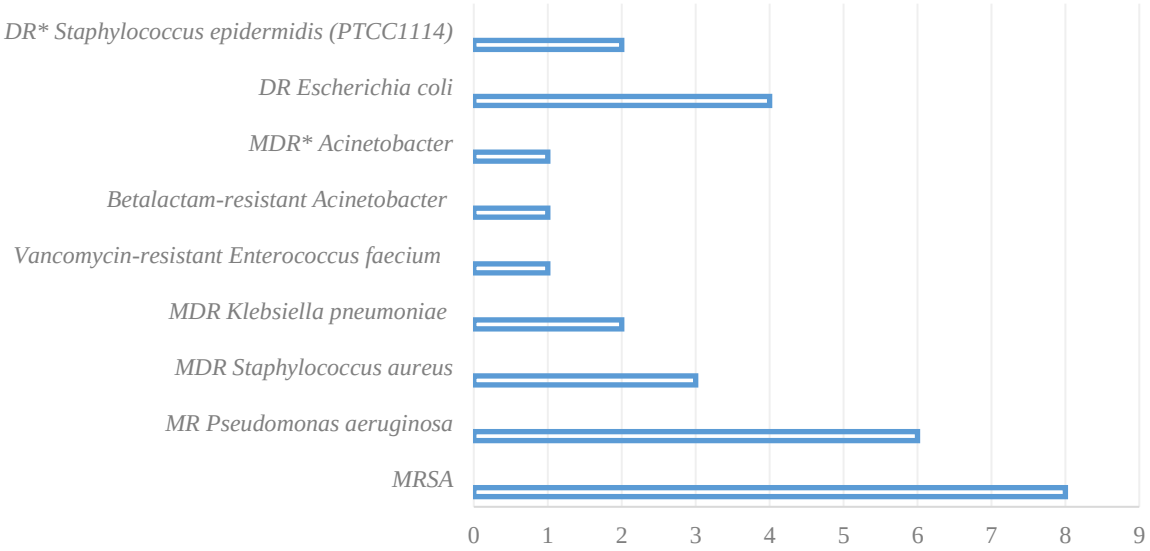

Fig. 3. Number of studies for bacterial strains. (*DR: Drug resistance, *MDR: Multidrug resistance)

The highest antibacterial activity is related to the thyme plant with the lowest MIC equal to 0.31 µg/ml (0.31 µg/ml), followed by *Oliveria decumbens* plant with the lowest MIC equal to 0.625 µg/ml (0.625 µg /ml) (Table 2).

The risk of bias assessment summary of included studies are shown in table 3. Most studies showed a moderate risk of bias.

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Table 2. Characteristics of included studies.

Plant	Bacteria	Name Strains (unit)	Inhibition zone (mm) [range]	Part	MIC [range]	MIC unit	Reference
Zataria multiflora	MRSA	EH1, EH2	-		0.66	mg/ml	[22]
		EH3, EH10	-		2.64		
		EH6, EH8, EH9	-	Arial	1.32		
		EH10	-		2.64		
Zataria multiflora	*K. pneumoniae*	UI 10, UI 14	21.3, 23.0		0.125	µg/ml	[23]
		UI 9, UI 17, UI 43	20.7, 22.7, 22.3	Arial	0.25		
		UI 13, UI 33	29.7, 24.7		0.031		
		UI 41	20.7		0.50		
		UI 46, UI 54	23.7		0.062		
Oliveria decumbens	MRSA	ATCC 6538	-		0.312	µg/ml	[24]
		ATCC 6633, ATCC 10231	-		0.625		
		MRSA	-	Aerial	0.625		
		ATCC 8739	-		1.25		
		ATCC 16404	-		2.5		
		ATCC 9027	-		>10		
Eucalyptus camaldulensis	Standard strains	ATCC 33591, ATCC 33150	-		12.5	mg/ml	[25]
		PTCC 1399, PTCC 1709	-	Leaf	12.5		
		PTCC 1156	-		25		
Trachyspermum ammi	*P. aeruginosa* & MRSA	For all 16 MRSA strains	[27-60]	Fruit	<0.02	µg/ml	[26]
Satureja khuzistanica	*P. aeruginosa*	PAO1	-		6	µg/ml	[27]
		PA9	-		10		
		PA11	-	Aerial	8		
		PA13	-		9		
		PA41	-		7		
		PA42	-		12		
Thymus kotschyanus	MSRA	ATCC33591	-		2.5	µg/ml	[28]
		MRSA1, MRSA2, MRSA3	-		2.5		
		MRSA4, MRSA5	-	Aerial	2.5		
		MRSA6	-		5.0		
		MRSA7, MRSA8	-		2.5		
Zataria multiflora	*S. aureus*	MRSA 31	-		1	µg/ml	[29]
		MRSA 3, MRSA 4, MRSA 6	-	Aerial	0.5		
		MRSA 32, MRSA 34, MRSA 26	-		0.5		
Levisticum officinale	*E. faecium* (Vancomycin resistant clinical strain)	-	-	1.ifs: *		mg/ml	[30]
		-	-	Root	3.2		
		-	-	Aerial	8		
		-	-	2.IFS: *			
		-	-	Root	3.2		
		-	-	Aerial	3.2		
Thymus vulgaris	MRSA	ATCC 33592	12		18.5	µg/ml	[31]
		M14, M6, M5, M4, M3	27,24,35,28,28		18.5		
		M1,M2,M7,M8,M9,M10	22,20,25,21,24,23		37		
		M11, M12, M13	31,29,21		37		
Eucalyptus globulus		ATCC 33592	8	Aerial	85.6		
		M1, M2, M3, M5, M8, M10, M12	13, 16, 20, 30, 17, 10, 21		34.24		
		M7, M9, M11, M14	15, 22, 23, 19		85.6		
		M6, M13	18, 15		51.36		
		M4	19		8.56		

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Table 2. Characteristics of included studies (Continued)

Plant	Bacteria	Name Strains (unit)	Inhibition zone (mm) [range]	Part	MIC [range]	MIC unit	Reference
Thymus daenensis	MRSA	-	-	Aerial	25	mg/ml	[32]
Ferula gummosa	*Pseudomas aeruginosas*	-	-	Gum	[12.5-50]	mg/ml	[33]
Cuminum cyminum	MDR *S. aureus*	Clinical isolates	-		[1.25-5]	µg/ml	[34]
		ATCC 33591	-	Seed	5		
		SA1199B	-		5		
Ferula gummosa	*Acinetobacter baumannii*	ATCC 19606	[10-16]	Oleo-gum-resin	[26.86-146.6]	mg/ml	[35]
Rosmarinus officinalis	Standard strains & clinical isolate	ATCC 2973				mg/ml	[36]
		ATCC 10031	[7-9.6]	Leaf	[0.06-0.16]		
		PTCC 1182					
Thymus vulgaris	*Acinetobacter baumannii*	-	-	-	[0.0125-0.5]	µg/ml	[37]
Centaurea depressa.	*S. aureus, S. epidermidis, E. coli*	-	13.33		-		[38]
		-	14.66	Aerial	-		
Centaurea cyanus		-	16		-		
		-	13.5		-		
		-	11.6	Aerial	-		
		-	13.5		-		
Cinnamomun verum	Standard strains	-	-	Wood	[0.0019-0.1]	mg/ml	[39]
Ferula gummosa		-	-	Root	[0.01-0.05]		
Pistacia atlantica.	*P. aeruginosa*	ATCC 9027	-	Oleor-esin	37.8	µg/ml	[40]
Trachyspermum ammi	*S. aureus, E. coli*	ATCC 1431	-	Seed	[0.06-64]	µg/ml	[41]
		PTCC 1399	-		[1-64]		
Thymus daenensis	*P. aeruginosa E. coli S. aureus K. pneumoniae S. epidermidis*	ATCC 27853	-		125	µg/ml	[42]
		ATCC 10536	11		125		
		ATCC 29737	39		125		
		ATCC 10031	18		125		
		ATCC 12228	9		125		
Nepeta sessilifolia		ATCC 27853	-		125		
		ATCC 10536	-		500		
		ATCC 29737	10		500		
		ATCC 10031	-		125		
		ATCC 12228	9	Aerial	250		
Hymenocrater incanus		ATCC 27853	-		16		
		ATCC 10536	-		250		
		ATCC 29737	-		500		
		ATCC 10031	-		63		
		ATCC 12228	11		500		
Stachys inflata		ATCC 27853	-		16		
		ATCC 10536	-		500		
		ATCC 29737	9		>1000		
		ATCC 10031	-		125		
		ATCC 12228	-		500		
Campsis radicans	*P. aeruginosa*	-	17.33	Bark	-		[43]
	E. coli	-	13.44		-		

*ifs: In Fruiting Stage, *IFS: In Flowering Stage

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Table 3. Judgments about each risk of bias item for each included study.

In vitro articles	Blinding of the observer		Time interval		Sample preparation and handling		Single operator		Materials used to manufacturer's instruction		Sample size calculation		Control	
First author (year)	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Eftekhar (2012)	*		*			*	*			*	*			*
Eftekhar (2011)	*		*			*	*			*	*		*	
Haji mahdipour (2010)	*		*			*	*			*	*			*
Heidari (2017)	*			*		*	*			*	*			*
Hosseinkhani (2016)	*			*		*	*			*	*			*
Islamieh (2020)	*			*		*	*			*	*			*
Khanavi (2011)	*		*		*		*			*	*		*	
Mahboubi (2010)	*			*		*	*		*		*			*
Miran (2018)	*			*		*	*		*		*			*
Touhidipour (2010)	*			*		*	*		*		*			*
Saidi (2016)	*		*			*	*			*	*			*
Sattarian (2016)	*		*			*	*			*	*			*
Sharifi (2021)	*		*			*	*			*	*			*
Afshar (2016)	*		*			*	*			*	*			*
Al Zuhairi (2020)	*		*			*	*			*	*			*
Norbakhsh (2019)	*			*		*	*			*	*		*	
Mobaiyen (2015)	*			*		*	*			*	*			*
Soleimani (2016)	*			*		*	*		*		*			*
Modareskia (2022)	*		*			*	*			*	*			*
Ghahfarrokhi (2022)	*		*			*	*			*	*			*
Ghavam (2022)	*		*			*	*			*	*			*
Ramtin (2022)	*			*		*	*			*	*		*	

4. Discussion

Plant essential oils have many properties that inhibit the growth or destruction of microbes. Among these properties are 1. The hydrophobic property that causes the essential oil to penetrate into the lipids of the bacterial cell membrane, and the cell structure collapses when ions and other cell contents are released. 2. The phenolic substances in the essential oil damage the cytoplasmic membrane of the bacterial cell. 3. The carbonic group of essential oil is attached to cell proteins and prevents the role of amino acid and decarboxylase. 4. The reactions of aldehydes with SH groups prevent the growth of microorganisms and 5. The presence of alphatriptin in essential oil prevents the growth of microbes [44].

Iran stands as one of the most abundant regions globally in terms of the variety and quantity of medicinal plants. By conducting scientific research and investigating their impact on pathogenic microorganisms, as well as cultivating, expanding, and utilizing these plants, Iran can effectively contribute to the treatment and overall health of society. The utilization of medicinal plants for disease treatment has seen a significant increase in recent years. Among the conducted studies, the *Zataria multiflora* plant

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has shown a remarkable antimicrobial effect on bacteria isolated from clinical samples. This suggests that the essential oil derived from this plant has the potential to effectively combat infections caused by these bacteria.

Furthermore, the volatile compounds found in *Cuminum cyminum* and *Oliveria decumbens* have proven to be effective against methicillin-resistant *Staphylococcus aureus* bacteria, with a minimum inhibitory concentration (MIC) of less than 5 µg/ml.

In a study conducted by Eftekhar et al. (2011), the effect of this plant on *Klebsiella pneumoniae* bacteria resistant to multiple drugs and sensitive to imipenem (MDR) was examined. The results showed that the MIC and minimum bactericidal concentration (MBC) values were 0.015-2.0 mg/ml for ATCC strains and 0.03 to 0.5 mg/ml for the clinical isolates [23].

Another study by Mahboubi and Bidgoli (2010) investigated the effect of *Zataria multiflora* on methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria. The results indicated that at a concentration range of 0.25-1 µg/ml, *Zataria multiflora* essential oil inhibited the growth of the mentioned bacteria [29]. Similarly, Noorbakhsh et al. (2019) reported a minimum inhibitory concentration of 0.5 µg/ml for thyme on multidrug-resistant *Acinetobacter baumannii* bacteria, which aligned with the findings of Mahboubi et al.'s study [37]. In Hajimehdipour et al.'s (2010) study, the MIC against methicillin-resistant *Staphylococcus aureus* was 0.625 µg/ml in *Oliveria decumbens* [24].

It is important to note that the results of these studies have varied due to several factors, including the concentration of essential oil compounds, the volume of the tested solution, the concentration of the inoculated solution, the temperature of bacterial incubation, and the method employed to determine the MIC. These variables have a significant impact on the outcomes of the research.

In investigations conducted on various Gram-positive and Gram-negative bacteria isolated from clinical samples, it is evident that the essential oils derived from medicinal plants exhibit a greater efficacy against Gram-positive bacteria compared to Gram-negative bacteria. This disparity arises from the polysaccharide membrane present in Gram-negative bacteria, which hinders the penetration of plant essential oils. Consequently, the antimicrobial effect of these oils is diminished [45].

5. Conclusion

In conclusion, the use of herbal medicines, such as *Cuminum cyminum* and *Oliveria decumbens*, presents a promising treatment option for antibiotic resistant bacteria. However, further research is necessary to fully comprehend their antibacterial properties, potential adverse effects, and possible synergistic effects when combined with other medicinal plants. This comprehensive understanding will contribute to the development of effective treatments in the face of antibiotic resistance.

Author contributions

IKY, MS, MTM and MVE searched for the articles, and wrote the first draft. ZTK designed the study and contributed to the writing process and analysis. FM was the study supervisor, contributed to all aspect of the study, and provided the final manuscript. SS contributed to the study process. All authors read and approved the paper.

Conflicts of interest

The authors of this study hereby declare that they no conflicts of interest to disclose.

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Acknowledgment

This research represents the culmination of a project carried out at Qom University of Medical Sciences, under the Code of IR.MUQ.REC.1401.068. The project received support from both Qom University of Medical Sciences and the National Elite Foundation. Consequently, the researchers would like to extend their heartfelt gratitude and appreciation to all individuals who contributed to this study.

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How to cite this article: Khahan-Yazdi I, Shabani MA, Tajvidi-Monfared MH, Vahidi Emami H, Mojab F, Shams S, Taheri Kharameh Z. Effectiveness of medicinal plant essential oils on drug-resistant bacteria in Iran: A systematic review. ***Journal of Medicinal Plants*** 2023; 22(87): 26-38. doi: 10.61186/jmp.22.87.26

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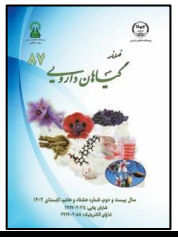

مقاله مروری

اثربخشی اسانس گیاهان دارویی بر باکتری‌های مقاوم به دارو در ایران: یک مطالعه مرور سیستماتیک

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اطلاعات مقاله

گل‌واژگان:
گیاه دارویی
عفونت‌های مقاوم به آنتی‌بیوتیک
ترکیبات ضد میکروبی
عفونت انسانی
مرور سیستماتیک

چکیده

مقدمه: اثربخشی آنتی‌بیوتیک‌های رایج به دلیل ظهور مقاومت دارویی، از جمله مقاومت چند دارویی (MDR) مختل شده است. در نتیجه، علاقه فزاینده‌ای به بررسی درمان‌های جایگزین برای عفونت‌های مقاوم به دارو ایجاد شده است. یکی از راه‌های امیدوارکننده استفاده از داروهای گیاهی دارای خواص ضدمیکروبی می‌باشد که ممکن است به عنوان یک مکمل یا جایگزین مناسب برای آنتی‌بیوتیک‌های معمولی عمل کنند. **هدف:** این مطالعه با هدف تعیین اثربخشی اسانس گیاهان دارویی بومی ایران بر باکتری‌های مقاوم به دارو انجام شد. **روش بررسی:** در این مرور سیستماتیک، با استفاده از کلیدواژه‌هایی شامل داروی گیاهی، اسانس، اسینتوباکتر، سودوموناس آئروژینوزا، کلبسیلا پنومونیه، استافیلوکوکوس اورئوس، گونه‌های انتروکوکوس در پایگاه‌های اطلاعاتی Scopus، PubMed، Web of Science و پایگاه‌های داخلی منتشر کننده مقالات جستجو شد. تاریخ ۴ اکتبر ۲۰۲۳ بدون محدودیت زمانی در نظر گرفته شد. **نتایج:** نتایج نشان داد که بیشترین تعداد مطالعات انجام شده مربوط به آویشن و بیشترین گونه باکتری مورد آزمایش مربوط به سویه‌های استافیلوکوکوس اورئوس یا استافیلوکوکوس اورئوس مقاوم به متی‌سیلین (MRSA) بوده است. بیشترین فعالیت ضد باکتریایی مربوط به گیاه آویشن با کمترین غلظت بازدارنده حداقلی (MIC) معادل ۰/۳۱ میکروگرم در میلی‌لیتر و پس از آن لعل کوهستان با کمترین MIC برابر با ۰/۶۲۵ میکروگرم در میلی‌لیتر است. **نتیجه‌گیری:** داروهای گیاهی ممکن است یک گزینه درمانی کارآمد برای باکتری‌های مقاوم به آنتی‌بیوتیک باشد. اثرات نامطلوب بالقوه و خواص ضدباکتریایی همراه با اثرات هم‌افزایی احتمالی با سایر گیاهان دارویی نیازمند مطالعات بیشتر برای روشن شدن است.

مخففها: MDR، مقاومت چند دارویی؛ MRSA، استافیلوکوکوس اورئوس مقاوم به متی‌سیلین؛ MIC، حداقل غلظت مهارکنندگی؛ ICU، بخش مراقبت‌های ویژه؛ PRISMA، موارد ترجیحی در گزارش مقالات مروری منظم و فرا تحلیل‌ها؛ MBC، حداقل غلظت باکتری‌کشی

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تاریخ دریافت: ۴ مرداد ۱۴۰۲؛ **تاریخ دریافت اصلاحات:** ۷ آذر ۱۴۰۲؛ **تاریخ پذیرش:** ۸ آذر ۱۴۰۲

doi: 10.61186/jmp.22.87.26

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