

A Systematic Review of the Effects of *Satureja Khuzestanica* Jamzad and *Zataria Multiflora* Boiss against *Pseudomonas Aeruginosa*

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What's Known

- Several studies have examined the anti-bacterial effect of both *Satureja khuzestanica* Jamzad (SKJ) and *Zataria multiflora* Boiss (ZM).
- Previous studies have shown carvacrol and thymol as the active ingredients of SKJ and ZM.

What's New

- We reviewed studies published during 2000-2017.
- The minimum inhibitory concentration value of essential oils from SKJ and ZM for bacterial strains was in the range of 0.31-450 and 2-8,000 µg/mL, respectively. The carvacrol content of SKJ was reported between 38.33-97.89% and the amount of thymol in ZM ranged between 25.70-64.87%.

Abstract

Background: The use of antibiotics is common, but its excessive or improper use leads to antibiotic resistance. Consequently, scientists have been interested in exploring traditional medicine to identify a new source of anti-bacterial agents. The present study aimed to conduct a systematic review to determine the anti-bacterial activity of *Satureja khuzestanica* Jamzad (SKJ) and *Zataria multiflora* Boiss (ZM) essential oils against *Pseudomonas aeruginosa* *in vitro*.

Methods: A comprehensive literature search for studies published on SKJ and ZM was carried out. Various keywords (*S. Khuzestanica*, *Marzeh-e-Khuzestani*, *Z. Multiflora*, *Z. Multiflora* Boiss; *avishan-e-shirazi*, shirazi thyme, thymol, carvacrol; *P. aeruginosa*, Iran, antibacterial effect; traditional medicine, phytomedicine, herbal medicine) were used to search both international and Iranian databases (Google Scholar, Science Direct, Web of Science, MEDLINE; PubMed, Scopus, Cochrane Library, Academic Search; Journal Storage, Magiran, Irandoc, Scientific Information Database (SID), and IranMedex). The selected articles were published during 2000-2017 and were written in English or Persian.

Results: Seventeen articles were included in the review. The main ingredients of SKJ and ZM plants were carvacrol and thymol. The potential anti-bacterial activity of essential oils from these plants was confirmed. The carvacrol content, as the major active ingredient of SKJ was between 38.33-97.89%. The major ingredients of ZM were carvacrol (16.8-82.7%) followed by thymol (25.70-64.87%). The minimum inhibitory concentration (MIC) value of SKJ and ZM essential oils against bacterial strains was in the range of 0.31-450 and 2-8,000 µg/mL, respectively.

Conclusion: The present review study confirmed the anti-bacterial activity of SKJ and ZM, particularly against *Pseudomonas in vitro*.

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Keywords • Herbal medicine • Iran • *Pseudomonas aeruginosa* • Systematic review

Introduction

Pathogenic bacteria are microorganisms that can cause infectious diseases in humans.¹ Antibiotics were first introduced in the 1940s and led to a major advancement in the pharmaceutical industry to fight against pathogenic microorganisms.² Nowadays, the use

of antibiotics is common, but their excessive or improper use leads to antibiotic resistance.³ Antibiotic-resistant pathogenic bacteria are a serious threat to humans and have affected many patients worldwide.^{4,5} Misuse of antibiotics reduces the effectiveness of the therapy to the extent that higher dosages might be required.^{3,6}

Pseudomonas aeruginosa (*P. aeruginosa*) is a common Gram-negative bacteria and a major cause of chronic or acute nosocomial and burn wound infections.⁷ A study in China reported that the most common strains, in descending order, were *Acinetobacter baumannii*, *Proteus mirabilis*, and *P. aeruginosa*.⁸ A systematic review and meta-analysis showed that Gram-negative pathogens (*P. aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter* spp., and *Proteus* spp.) were the most common Gram-negative bacteria isolated from clinically infected burn wounds.⁹ A similar study in Iran reported *P. aeruginosa*, *Acinetobacter*, and *Klebsiella* as the most common Gram-negative and *Staphylococcus aureus* as the most common Gram-positive organisms isolated from Iranian burn patients.¹⁰

P. aeruginosa is an opportunist and a Gram-negative bacillus, which has shown resistance to antibiotics.¹¹ Recently, a high prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacterial strains have been reported.¹² MDR *P. aeruginosa* strain is defined by the resistance of the bacterium to three common antibiotic classes, whereas XDR refers to organisms resistant to carbapenem, amikacin, and colistin as well as the three common antibiotic classes.¹³ In recent years, the spread and sudden outbreak of MDR strains have emerged as an important public health concern¹⁴ to the extent that the World Health Organization (WHO) named 2011 as the year of antibiotic resistance.¹⁵ Consequently, due to the broad range of antibiotic resistance and subsequent complications, scientists have become interested in exploring traditional medicine to identify a new source of anti-bacterial agents. A review study over a period of 30 years (1981-2010), on natural products as sources of new drugs, reported that about 80% of the world's population resort to herbal medicine as part of their treatment.¹⁶ Traditional medicine is also popular in Iran, since the region has eleven different climates and over 7,500 herbaceous species; a suitable platform for the acquisition of rare medicinal plants. Currently, 25% of the existing drugs are derived from such plants. Iran is one of the countries with a variety of medicinal plants with different characteristics, such as *Satureja khuzestanica* Jamzad (SKJ)

and *Zataria multiflora* Boiss (ZM).¹⁷

SKJ locally called Marzeh Khuzestani, belongs to the Lamiaceae family. It grows in the south and southwest of Iran and is a subshrub with a branched stem of about 30 cm high and dense leaves.¹¹ In traditional medicine, it is used as an analgesic, anti-inflammatory, anti-thyroid, anti-oxidant, diuretic, appetizer, anti-bacterial, and antiseptic agent. Most of these features are attributed to carvacrol, which is the active ingredient of SKJ essential oil.^{18, 19} ZM locally called avishan-e-shirazi (shirazi thyme), is a thyme-like plant from the Lamiaceae family. It is a wild plant that grows in Iran, Pakistan, and Afghanistan. This plant is known to be effective as an antiseptic, carminative, stimulant, analgesic, diuretic, anesthetic, anti-spasmodic, diaphoretic, and anti-bacterial agent.²⁰

Due to the importance of SKJ and ZM in herbal medicine and the existence of numerous studies regarding their effects against *Pseudomonas*, there was a need for a review study. Hence, the present study aimed to conduct a systematic review to determine the anti-bacterial activity and the minimum inhibitory concentration (MIC) of SKJ and ZM essential oils against *P. aeruginosa* *in vitro*.

Materials and Methods

Search Strategy

To identify all relevant published studies on SKJ and ZM, we searched Google Scholar, Science Direct, Web of Science, MEDLINE, PubMed, Scopus, Cochrane Library, Academic Search, and Journal Storage databases. In addition, four Persian scientific search engines including Magiran, Irandoc, Scientific Information Database (SID), and IranMedex were searched. The keywords used for the search were *S. khuzestanica*, *marzeh-e-khuzestani*, *Z. multiflora*, *Z. multiflora* Boiss, *avishan-e-shirazi*, shirazi thyme, *Z. bracteata* Boiss, *Z. multiflora* var. *elatior* Boiss, thymol, carvacrol, *P. aeruginosa*, Iran, Anti-bacterial effect, herbal plant, traditional medicine, phytomedicine, herbal medicine, medicinal plants, plant extracts, essential oils, culinary herbs, folk medicine, and herbal essence. We also searched the references cited in these articles to find other relevant studies. The search was conducted in accordance with the MOOSE checklist and covered all original observational, descriptive, and analytical studies during 2000-2017 both in English and Persian.

Inclusion and Exclusion Criteria

Of the obtained articles/abstracts, those with the following features were included in the study:

full-text articles published during 2000-2017, studies on *P. aeruginosa* microorganism, and studies on the effect of SKJ and ZM against *P. aeruginosa*. The exclusion criteria were studies that did not address microbiology (especially in relation to *Pseudomonas*), publications prior to 2000, irregular articles (abstract only, case reports, meeting reports, congress articles), articles with unclear results or data analysis, articles reported in languages other than English or Persian, and duplicated publications (articles, review papers, meta-analysis, systematic reviews).

Quality Assessment of Included Articles

The selected articles were assessed based on the quality of the study, design, methods, context, findings, data analysis, and interpretations. All articles not meeting the pre-defined standard in each of the mentioned items were excluded.

Data Extraction

Following a careful and detailed study of the full-text articles, information such as the name of the first author, publication year, location where the plant was collected, plant parts used and drying conditions, essential oil analysis, the source of isolates, abundant compounds, carvacrol and thymol content, and general remarks were extracted. The information was then entered in an abstract format.

Results

The morphology of SKJ and ZM is shown in figure 1. In the isobilateral amphistomatic structure of SJK leaves, there are both glandular, peltate and capitate, and two types of

non-glandular trichomes. The peltate glandular trichomes comprise of a five-cell multiseriate stalk and a 12-cell enlarged secretory head. On the other hand, the capitate trichomes show variable morphologies. Dried leaves of SKJ are recognized by their oval-shaped hygromorphic diacytic stomata which have an adaxial stomatal index of 13.54. Other morphological features are collateral vascular packs consisting of xylem, as well as a three-layer sclerenchymatous tissue near phloem. Another feature includes prismatic alongside with raphide calcium oxalate crystals. The oval-shaped leaves with an orbicular thickly gland-dotted structure, along with the dense white, hairy, and round buds on the leaf axils are main features characterizing ZM.

As shown in figure 2, initially 117 studies were identified through English and Persian database searching. Based on the exclusion criteria, 32 articles were excluded due to duplication, and thus 85 articles remained for eligibility evaluation. A further three articles were excluded due to their format, such as study design, book, congress, thesis, publication prior to the year 2000, or written in other languages. In the next step, the abstracts of 82 articles were screened of which 49 were excluded due to subject irrelevance. Subsequently, 33 full-text articles were evaluated from which 16 were excluded due to the incompleteness of the data. Finally, 17 articles on the studies related to inhibitory effects on *P. aeruginosa* (SKJ: 7 studies, ZM: 10 studies) were included in the present meta-analysis.

As described in table 1, the majority of the Iranian researchers collected the SKJ plants from Khuzestan and Lorestan provinces. Based



Figure 1: The morphology of *S. khuzestanica* (A) and *Z. multiflora* (B)

on gas chromatography-mass spectrometry (GC-MS) analysis, the rate of carvacrol, as the major active component of SKJ, was reported between 38.33-97.89%.²² The major ingredients of ZM were carvacrol (16.8-82.7%) followed by thymol (27.5-64.87%). The MIC value of SKJ and ZM essential oils for bacterial strains was

in the range of 0.31-450 and 2-8000 µg/mL, respectively. Based on the results, SKJ inhibited the growth of *P. aeruginosa* less than ZM. The ZM plants were collected from the provinces of Fars, Guilan, Lorestan, Hormozgan, Yazd, Khorasan Razavi, Golestan, Sistan-Baluchestan, Kerman, and Isfahan (table 2). The major ingredients of ZM were carvacrol followed by thymol in various concentrations.

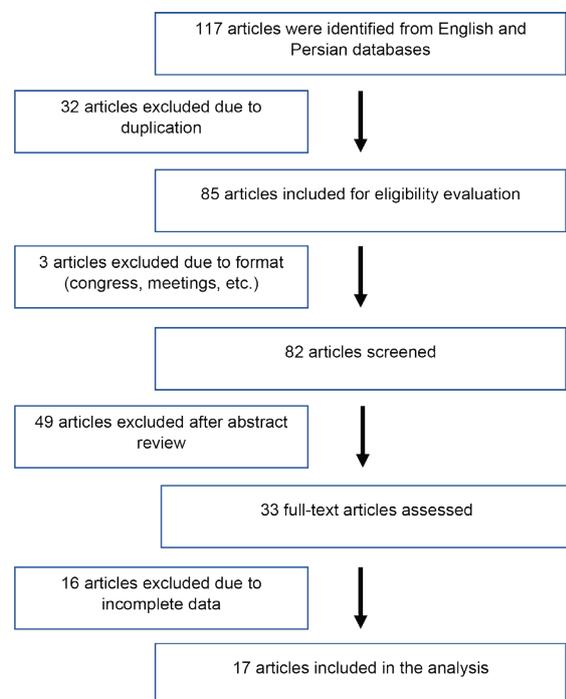


Figure 2: The Moose flowchart for article screening.

Discussion

The review of the articles showed that the level of carvacrol, as the major active component of SKJ, was between 38.33-97.89%.²² Major ingredients of ZM were carvacrol (16.8-82.7%)^{31, 34} followed by thymol (25.7-64.87%).^{30, 33} The abundant presence of these ingredients explains their anti-bacterial effect of SKJ and ZM. The MIC values in *Satureja khuzestanica* essential oil (SKEO) and *Zataria multiflora* essential oil (ZMEO) for bacterial strains were in the range of 0.31-450 and 2-8,000 µg/mL, respectively. Therefore, SKJ inhibited the growth of *P. aeruginosa* less than ZM. Variations in the quantitative composition of carvacrol and thymol compounds in the Lamiaceae family were likely due to the geographical area, genetic diversity, climatic conditions, the existence of different chemotypes, and/or ecological differences.³⁷ Note that essential oil yield is associated with

Table 1: Characteristics of the effect of *Satureja khuzestanica* against *Pseudomonas aeruginosa*. In all studies, the aerial part of the plant was used and air-dried at room temperature

Article	Plant location	Analysis	Source of isolates	Carvacrol content (%)	General remarks
Moghaddam ²¹	Kuzestan, Lorestan	GC-MS	Collection	90.9	The inhibition zones and MIC values for bacterial strains sensitive to SKJ were 12-32 mm and 19-312 µg/mL, respectively.
Amiri ²²	Lorestan	GC-MS	Collection	97.89	The MIC of the extract was 450 ug/mL and the growth inhibition zone was 45 mm (in diameter). Results showed a strong inhibitory effect of SKJ against bacteria.
Saei-Dehkordi ²³	Khuzestan	GC-MS	Reference strains	53.8	A synergetic phenomenon (FICI ≤0.5) was observed in the majority of combinations. Essential oil resulted in a reduction in the inhibitory doses of the anti-microbials <i>in vitro</i> .
Yousefzadi ²⁴	Southern Iran	GC-MS	Reference strains	92.87	The oil exhibited considerable anti-microbial activity against the majority of the tested bacteria, particularly the inhibition zone of carvacrol against <i>P. aeruginosa</i> was 0.129 mm.
Bahrami ²⁵	-	GC-MS	Reference strains	84	SKEO showed potential anti-microbial activity against food microorganisms. Anti-microbial activity of SKEO for <i>P. aeruginosa</i> was observed (MIC=340 µg/mL, inhibition zone=11.5±0.1 mm).
Abbasi ²⁶	-	-	Clinical strains	-	The result of agar dilution method revealed that SKJ had strong inhibitory effects against MDR <i>P. aeruginosa</i> strains (MIC=160 µg/mL).
Abbasi ¹¹	Lorestan	GC-MS	Clinical strains	90.88	The inhibition zone of SKEO against <i>P. aeruginosa</i> was 25 mm (diameter). The MIC value was 0.31 µg/mL. It caused inhibition of expression of <i>exoA gene</i> in <i>P. aeruginosa</i> .

GC-MS: Gas chromatography-mass spectrometry; MIC: Minimum inhibitory concentration; SKEO: *Satureja khuzestanica* essential oil

Table 2: Characteristics of the effect of *Zataria multiflora* against *Pseudomonas aeruginosa*. In all studies, the aerial part of the plant was used and air-dried at room temperature

Article	Plant location	Analysis	Source of isolates	Abundant compounds (%)	General remarks
Faezeh ²⁷	-	GC-MS	Reference strains	-	The MIC of ZMEO for <i>P. aeruginosa</i> was 2.6-4.2 µg/mL.
Heidary ²⁸	Fars, Guilan	GC-MS	Clinical strains	-	MIC of ZM extract (methanol and acetone) for IMP-producing <i>P. aeruginosa</i> strains was 6,250 µg/mL. ZM extract had high anti-bacterial effects against β-lactamase producing <i>P. aeruginosa</i> isolates.
Fallah ²⁹	Fars	GC-MS	Clinical strains	-	ZM extract had a high anti-bacterial effect against IMP-producing <i>P. aeruginosa</i> strains in 6,250 µg/mL concentration.
Saei-Dehkordi ³⁰	Fars, Lorestan, Hormozgan, Isfahan, Yazd	GC-MS	Clinical strains	Thymol: 27.05-64.87	MIC of <i>Z. multiflora</i> extract for <i>P. aeruginosa</i> strains was between 2,000-8,000 µg/mL.
Zomorodian ³¹	Fars	GC-MS	Reference and clinical strains	Carvacrol: 82.7	MIC for <i>P. aeruginosa</i> strains was between 2–128 µg /mL.
Owlia ³²	Research Institute of Medicinal Plants (Tehran, Iran)	GC-MS	Reference strains	Carvacrol: 37	MIC of <i>Z. multiflora</i> extract for <i>P. aeruginosa</i> strains was 64.128 µg/mL.
Mahammadi Purfard ³³	Arsenjan (Fars Province)	-	Reference strains	Carvacrol: 29.48, Thymol: 25.7	MIC showed a reduction of bacteria growth in tested compared to control wells.
Aida ³⁴	Khorasan Razavi	GC-MS	Reference strains	Thymol: 42.46, Carvacrol: 16.85	Minimum inhibitory and bactericidal concentrations of ZMEO were MIC=25 µg/ mL and MBC=100 µg/mL.
Saeidi ³⁵	Zabol, Kerman	-	Reference strains	-	The MIC and MBC for <i>P. aeruginosa</i> strains were 1,250 µg/mL.
Varposhti ³⁶	Golestan	-	Clinical strains	-	ZMEO completely inhibited biofilm formation at a concentration of 4 µg/mL.

GC-MS: Gas chromatography–mass spectrometry; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; IMP: Imipenem; ZMEO: *Zataria multiflora* essential oil

the concentration of essential elements and the amount of organic matter in the environment.³⁸

P. aeruginosa has a high level of intrinsic resistance to most anti-bacterial agents due to the presence of an outer membrane barrier. The compounds carvacrol, gamma-terpinene, p-cymene, and thymol possess high hydrophobic properties to overcome such outer membrane barrier. The discharge of lipids from the bacterial cell wall is their functional mechanism. However, increased permeability of the membrane might also be the result of an ion ejection and electron imbalance.³⁹ The anti-microbial activity of phenolic compounds might be due to the location of the hydroxyl group in these compounds, which is vital for better efficacy of these natural ingredients.⁴⁰ The review of the articles showed that SKJ and ZM have anti-bacterial effects against both susceptible and resistant *P. aeruginosa* strains. Despite some difference in MIC values, all studies indicated the anti-microbial activity of SKEO and ZMEO in various ranges and degrees. Consistent with studies in Iran, a study in India reported a significant correlation between the anti-microbial activity and carvacrol from SKJ and ZM.⁴¹ Another study showed that SKJ could lead to a decrease

in drug resistance and inhibition of *exoA* gene expression in *P. aeruginosa*.¹¹ SKJ, due to its acceptable anti-bacterial effect, is recommended as an alternative disinfectant to control the nosocomial resistant micro-organisms. However, further comprehensive studies and clinical trials are required to approve the use of such natural disinfectants in health care and pharmacopeia systems.²²

The use of herbal essences in combination with currently available anti-microbial agents is a promising option. Their synergistic and additive effects could be potentially effective in the fight against pathogens. Consequently, lower doses of toxic agents would have to be used to control micro-organisms.⁴¹ In line with the reviewed articles, another study showed that the combination of essential oils with a standard dose of antibiotics, due to their synergistic interaction, may have a potential benefit in controlling pathogens or inhibiting their growth. In other words, the efficacy of the combined anti-microbial agents leads to the use of lower doses of toxic antibiotics, and consequently a significant reduction in the inhibitory concentrations of anti-microbial agents *in vitro*.^{42, 43} In fact, the use of natural herbs is

beneficial in eliminating the side effects of anti-oxidant and anti-bacterial agents.²⁵ Based on the reviewed articles, it can be concluded that the anti-bacterial effect of essential oil is higher than extracts, which is attributed to their phenolic components (e.g., carvacrol and thymol).

Overall, in spite of some differences in MIC, all articles acknowledged the anti-bacterial/anti-Pseudomonal characteristics of both plants; attributed to their phenolic components (carvacrol, thymol). In those studies, antibiotic susceptibility testing (disk diffusion method) and MIC were used *in vitro*. The anti-microbial effect of carvacrol and thymol is due to the permeability of the cell membrane. They can disrupt the cations of the mucous membrane and consequently its vital activities.⁴⁴ Thymol inhibits the activity of ATPase and increases the non-specific permeability of the bacterial cell membrane. It not only prevents the formation of a microbial population, but also increases the permeability of the bacterial membrane, which makes the bacteria more susceptible and vulnerable. Carvacrol inhibits the neutrophilic elastase enzyme and the production of Prostaglandin E2 (PGE2), F1, and F2.⁴⁵ However, further studies are required to better understand the mechanisms of their anti-microbial activity against pathogenic micro-organisms as well as their safety, effectiveness, and toxicity.

The main limitation of the present study was the lack of access to all articles, including unpublished research. Additionally, we did not contact the authors of the included studies to obtain further data, nor approached experts in this field to obtain clarifications. Furthermore, the review did not include articles in other languages than Persian and English.

Conclusion

Overall, this review study confirmed the anti-bacterial activity of SKJ and ZM, particularly against *Pseudomonas in vitro*. Further studies are required to evaluate the use of plant extracts as natural anti-bacterial agents in packaged food, effects of herbal essential oils as complementary medicine, or as a replacement for synthetic antibiotics. Proper interaction between the essential oils and standard drug dosage could be a challenge *in vivo*.

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Conflict of Interest: None declared.

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