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

Azad Khaledi1,2, PhD; Maryam Meskini3, PhD

1Infectious Diseases Research Center, Kashan University of Medical Science, Kashan, Iran; 2Department of Microbiology and Immunology, Faculty of Medicine, Kashan University of Medical Science, Kashan, Iran; 3Department of Mycobacteriology and Pulmonary Research, Pasteur Institute of Iran, Tehran, Iran

Correspondence:
Azad Khaledi, PhD; Department of Microbiology and Immunology, School of Medicine, Kashan University of Medical Sciences, 5th of Qob –e Ravandi Blvd. P.O. Box: 87155-111, Post Code: 87154, Kashan, Iran
Tel: +98 912 7804713
Fax: +98 361 5575057
Email: azadkh99@gmail.com
Received: 22 September 2018
Revised: 27 October 2018
Accepted: 18 November 2018

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 aeruginosa in-vitro.

Keywords: ● Herbal medicine ● Iran ● Pseudomonas aeruginosa ● Systematic review

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%.

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 excessive or improper use leads to antibiotic resistance. Antibiotic-resistant pathogenic bacteria are a serious threat to humans and have affected many patients worldwide. Misuse of antibiotics reduces the effectiveness of the therapy to the extent that higher dosage might be required.

**Pseudomonas aeruginosa** (*P. aeruginosa*) is a common Gram-negative bacteria and a major cause of chronic or acute nosocomial and burn wound infections. A study in China has reported that the most common strains, in descending order, were *Acinetobacterbaumannii*, *Proteus mirabilis*, and *P. aeruginosa*. 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. A similar study in Iran has 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.

*P. aeruginosa* is an opportunist and a gram-negative bacillus which has shown resistance to antibiotics. Recently, a high prevalence of multidrug resistant (MDR) and extensively drug resistant (XDR) bacterial strains have been reported. MDR *P. aeruginosa* strain is defined as 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. In recent years, the spread and sudden outbreak of MDR strains have emerged as an important public health concern to the extent that the World Health Organization (WHO) named 2011 as the year of antibiotic resistance. 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 antibacterial 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. 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 and *Zataria multiflora* Boiss.

*Satureja khuzestanica* Jamzad (SKJ), locally called Marzeh Khuzestani, belongs to the Lamiaceae family. It grows in the south and southwest of Iran and is a shrub with a branched stem of about 30 cm high and dense leaves. In traditional medicine, it is used as an analgesic, anti-inflammatory, anti-thyroid, anti-oxidant, diuretic, appetizer, anti-bacterial, and antiseptic. Most of these features are attributed to carvacrol which is the active ingredient of SKJ essential oil. *Zataria multiflora* Boiss (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.

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*), publication 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. Microscopically, SKJ leaves have an isobilateral amphistomatic structure containing peltate glandular trichomes consisting of a multiseriate stalk (five cells) and an enlarged secretory head composed of 12 cells, capitate glandular trichomes of variable morphology together with two types of non-glandular trichomes. Oval-shaped hygromorphic diacytic stomata with an adaxial stomatal index of 13.54, collateral vascular bundles consisting of xylem, and three layers of sclerenchymatous tissue close to phloem together with cluster, prismatic and raphide calcium oxalate crystals are useful pharmacognostic parameters for the identification of SKJ dried leaves. ZM can be recognized by the orbicular, densely gland-dotted, ovate leaves and the dense white, hairy, round buds on the leaf axils.

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 the 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 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 on gas chromatography-mass spectrometry...
Effects of *Satureja khuzestanica* and *Zataria multiflora* against *Pseudomonas aeruginosa*

(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.

**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 the concentration of essential elements and the

**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.

<table>
<thead>
<tr>
<th>Article</th>
<th>Plant location</th>
<th>Analysis (GC-MS)</th>
<th>Source of isolates</th>
<th>Carvacrol content (%)</th>
<th>General remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moghaddam21</td>
<td>Kuzestan, Lorestan</td>
<td>GC-MS Collection</td>
<td>90.9</td>
<td>The inhibition zones and MIC values for bacterial strains sensitive to SKJ were 12-32 mm and 19-312 μg/mL, respectively.</td>
<td></td>
</tr>
<tr>
<td>Amiri22</td>
<td>Lorestan</td>
<td>GC-MS Collection</td>
<td>97.89</td>
<td>The MIC of the extract was 450 μg/mL and the growth inhibition zone was 45 mm (in diameter). Results showed a strong inhibitory effect of SKJ against bacteria.</td>
<td></td>
</tr>
<tr>
<td>Saei-Dehkordi23</td>
<td>Khuzestan</td>
<td>GC-MS Reference strains</td>
<td>53.8</td>
<td>A synergetic phenomenon (FICI ≤0.5) was observed in the majority of combinations. Essential oil resulted in a reduction in inhibitory doses of the anti-microbials in vitro.</td>
<td></td>
</tr>
<tr>
<td>Yousefzadi24</td>
<td>Southern Iran</td>
<td>GC-MS Reference strains</td>
<td>92.87</td>
<td>The oil exhibited considerable anti-microbial activity against the majority of the tested bacteria, particularly the inhibition zone of carvacrol against <em>P. aeruginosa</em> was 0.129 mm.</td>
<td></td>
</tr>
<tr>
<td>Bahrami25</td>
<td>-</td>
<td>GC-MS Reference strains</td>
<td>84</td>
<td>SKEO showed potential anti-microbial activity against food microorganisms. Anti-microbial activity of SKEO for <em>P. aeruginosa</em> was observed (MIC=340 μg/mL, inhibition zone=11.5±0.1 mm).</td>
<td></td>
</tr>
<tr>
<td>Abbasi26</td>
<td>-</td>
<td>Clinical strains</td>
<td>-</td>
<td>The result of agar dilution method revealed that SKJ had strong inhibitory effects against MDR <em>P. aeruginosa</em> strains (MIC=160 μg/mL).</td>
<td></td>
</tr>
<tr>
<td>Abbasi27</td>
<td>Lorestan</td>
<td>GC-MS Clinical strains</td>
<td>90.88</td>
<td>The inhibition zone of SKEO against <em>P. aeruginosa</em> was 25 mm (diameter). The MIC value was 0.31 μg/mL. It caused inhibition of gene expression of exoA in <em>P. aeruginosa</em>.</td>
<td></td>
</tr>
</tbody>
</table>

GC-MS: Gas chromatography-mass spectrometry; MIC: Minimum inhibitory concentration; SKEO: *Satureja khuzestanica* essential oil
amount of organic matter in the environment.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 anti-microbial activity and carvacrol from SKJ and ZM.\textsuperscript{41} Another study showed that the SKJ could lead to a decrease in drug resistance and inhibition of *exoA* gene expression in *P. aeruginosa*.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 inhibitory concentrations of anti-microbial agents in vitro.\textsuperscript{42, 43} In fact, the use of natural herbs is beneficial in eliminating

<table>
<thead>
<tr>
<th>Article</th>
<th>Plant location</th>
<th>Analysis</th>
<th>Source of isolates</th>
<th>Abundant compounds (%)</th>
<th>General remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faezeh\textsuperscript{27}</td>
<td>-</td>
<td>GC-MS</td>
<td>Reference strains</td>
<td>-</td>
<td>The MIC of ZMEO for <em>P. aeruginosa</em> was 2.6-4.2 μg/mL.</td>
</tr>
<tr>
<td>Heidary\textsuperscript{28}</td>
<td>Fars, Guilan</td>
<td>GC-MS</td>
<td>Clinical strains</td>
<td>-</td>
<td>MIC of ZM extract (methanol and acetone) for IMP-producing <em>P. aeruginosa</em> strains was 6,250 μg/mL. ZM extract had high anti-bacterial effects against β-lactamase producing <em>P. aeruginosa</em> isolates.</td>
</tr>
<tr>
<td>Fallah\textsuperscript{29}</td>
<td>Fars</td>
<td>GC-MS</td>
<td>Clinical strains</td>
<td>-</td>
<td>ZM extract had a high anti-bacterial effect against IMP-producing <em>P. aeruginosa</em> strains in 6,250 μg/mL concentration.</td>
</tr>
<tr>
<td>Saei-Dehkordi\textsuperscript{30}</td>
<td>Fars, Lorestan, Hormozgan, Isfahan, Yazd</td>
<td>GC-MS</td>
<td>Clinical strains</td>
<td>Thymol: 27.05-64.87</td>
<td>MIC of <em>Z. multiflora</em> extract for <em>P. aeruginosa</em> strains was between 2,000-8,000 μg/mL.</td>
</tr>
<tr>
<td>Zomorodian\textsuperscript{31}</td>
<td>Fars</td>
<td>GC-MS</td>
<td>Reference and clinical strains</td>
<td>Carvacrol: 82.7</td>
<td>MIC for <em>P. aeruginosa</em> strains was between 2–128 μg/mL.</td>
</tr>
<tr>
<td>Owlia\textsuperscript{32}</td>
<td>Research Institute of Medicinal Plants (Tehran, Iran)</td>
<td>GC-MS</td>
<td>Reference strains</td>
<td>Carvacrol: 37</td>
<td>MIC of <em>Z. multiflora</em> extract for <em>P. aeruginosa</em> strains was 64.128 μg/mL.</td>
</tr>
<tr>
<td>Mahammadi Purfard\textsuperscript{13}</td>
<td>Arsenjan (Fars Province)</td>
<td>-</td>
<td>Reference strains</td>
<td>Carvacrol: 29.48, Thymol: 25.7</td>
<td>MIC showed a reduction of bacteria growth in tested compared to control wells.</td>
</tr>
<tr>
<td>Aida\textsuperscript{34}</td>
<td>Khorasan Razavi</td>
<td>GC-MS</td>
<td>Reference strains</td>
<td>Thymol: 42.46, Carvacrol: 16.85</td>
<td>Minimum inhibitory and bactericidal concentrations of ZMEO were MIC=25 μg/mL and MBC=100 μg/mL.</td>
</tr>
<tr>
<td>Saeidi\textsuperscript{35}</td>
<td>Zabol, Kerman</td>
<td>-</td>
<td>Reference strains</td>
<td>-</td>
<td>The MIC and MBC for <em>P. aeruginosa</em> strains were 1,250 μg/mL.</td>
</tr>
<tr>
<td>Varposhti\textsuperscript{36}</td>
<td>Golestan</td>
<td>-</td>
<td>Clinical strains</td>
<td>-</td>
<td>ZMEO completely inhibited biofilm formation at a concentration of 4 μg/mL.</td>
</tr>
</tbody>
</table>

GC-MS: Gas chromatography–mass spectrometry; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; IMP: Imipenem; ZMEO: *Zataria multiflora* essential oil
Effects of Satureja khuzestanica and Zataria multiform against Pseudomonas aeruginosa

Effects of Satureja khuzestanica and Zataria multiform against Pseudomonas aeruginosa

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, the 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.

Acknowledgment

We would like to express our gratitude to our colleagues for their collaboration in the study.

Conflict of Interest: None declared.

References


28 Heidary M, Hashemi A, Goudarzi H,


