Investigating antimicrobial activity of hydroalcoholic extract and essential oil of Tymbra spicata against some pathogenic bacteria

Salar Bakhtiyari¹, Somaieh Sabzali², Arman Rostamzad², Gholam Basati³*

1. Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran
2. Department of Biology, Faculty of Basic Sciences, Ilam University, Ilam, Iran
3. Department of Clinical Biochemistry, Faculty of Allied Medical Sciences, Ilam University of Medical Sciences, Ilam, Iran

*Corresponding author: Tel: +98 841 2227147; fax: +98 841 2227147
Address: Department of Clinical Biochemistry, Faculty of Allied Medical Sciences, Ilam University of Medical Sciences, Banganganjab Avenue, Ilam, Iran
E-mail: basati-gh@medilam.ac.ir
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Abstract

Introduction: Thymbra spicata (T. spicata) is a conventional medicinal herb which is commonly used in the traditional medicine system of Iran. The aim of this study was to assess the antibacterial activity of the essential oil and hydroalcoholic extract of the plant T. spicata on some important bacteria.

Materials and methods: The essential oil and extract of T. spicata were obtained by hydrodistillation and hydroalcoholic methods, respectively. Different concentrations of essential oil and hydroalcoholic extract of the medicinal herb, T. spicata, were evaluated for their antibacterial activities against ten pathogenic bacteria (4 Gram-positive and 6 Gram-negative) by agar disc diffusion and macro Broth dilution method.

Results: All bacteria showed a profound susceptibility to the essential oil and hydroalcoholic extracts of the T. spicata. The essential oil was shown to be more effective against the examined bacteria. The zone of inhibition of the essential oil showed to be maximum against E. faecalis (28 mm) and M. morganii (30 mm). The hydroalcoholic extract of T. spicata, showed the highest antibacterial activity against S. aureus (Gram-positive) and E. coli (Gram-negative) with the zone of inhibitions, 19 mm and 15 mm, respectively. The minimum inhibitory concentration (MIC) values for the essential oil and hydroalcoholic extracts ranged from 7-60μg/mL and 5-19 mg/ml, respectively against the tested bacteria.

Conclusion: This study revealed that the potential using of T. spicata as a source of antibacterial agents could be effectively used for medicinal and pharmaceutical purposes.

Keywords: Tymbra spicata, essential oil, hydroalcoholic extract

Introduction

Herbal remedies used in many types of alternative medicine provide an interesting and still principally unexplored source for the creation and development of potentially new drugs, which might help to overcome the growing problem of the microbial resistance and also the toxicity of the currently available commercial drugs (1). Nowadays, drug resistance to pathogenic microorganisms has become a very serious problem all over the world and hence, there has been an increased interest in investigating natural materials as sources of new antibacterial agents. Antimicrobial activities of various species...
of plants and their derivatives have been consequently reported (2, 3).

*Thymbra spicata* (*T. spicata* from *Lamiaceae* family) is a traditional medicinal plant which is commonly used in the traditional medicine system of Iran. This plant is a perennial herb that tends to grow on dry sunny hillsides and high dry meadows (4). The essential oil found in different parts of *T. spicata* makes it an important antibacterial and antioxidant natural source. It is used in food industry as spice and also utilized for the treatment of asthma and bronchitis in traditional medicine (5). This plant is known as a rich source of flavonoids, terpenoids, and isoprenoids such as thymol and carvacrol (6). Thymol, a naturally-occurring group of biocides, shows strong antimicrobial activity when used alone or accompanied with other biocides such as carvacrol. Furthermore, thymol can reduce bacterial resistance to commonly used antibiotics such as penicillin (7). The antimicrobial effects of thymol, varying from induction of antibiotic susceptibility in drug-resistant pathogens to powerful antioxidant properties, have been shown by several studies (8). It has also been demonstrated that thymol and carvacrol reduce bacterial resistance to antibiotics through a synergistic effect (7).

So far, no report has been published on antimicrobial properties of *T. spicata* extract. Therefore, we aimed to examine and compare the antimicrobial effects of hydroalcoholic extract and essential oil of *T. spicata* on some bacteria by determining the diameter of growth inhibited zone surrounding the discs.

**Materials and methods**

**Plant collection and identification:** The aerial parts of *T. spicata*, used in this study, were collected from hills around Ilam, Iran in May 2011. The taxonomic identity of this plant was authenticated by the voucher specimens deposited at the Department of Horticulture, Faculty of Agriculture, Ilam University.

**Hydroalcoholic extract preparation:** The aerial parts of *T. spicata* were cleaned with distilled water, shade dried at room temperature for 10 days and then ground to a fine powder. 200 gram of the powder was macerated in 1 liter of 50% (v/v) ethanol in distilled water and incubated for 10 days at 25°C. The solution was filtered and its volume was adjusted to 350 mL with the 50% ethanol to give a clear dark brown filtrate (pH 6). The filtrate was afterward dried on the oven at 45°C for two days.

**Essential oil extraction:** The essential oil of *T. spicata* was prepared based on the method recommended in British pharmacopoeia (9). Briefly, the aerial parts of *T. spicata* were dried in shadow, ground, and powdered in a domestic mixer. The powder was then hydrodistilled using a Clevenger apparatus for 3.5 hours. The resulted yellowish oil was dried over anhydrous sodium sulfate and kept at 4°C in a dark place.

**Bacterial strains:** The essential oil and hydroalcoholic extract of *T. spicata* were screened against six Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonia*, *Enterobacter aerogenes*, and *morganella morganii*) and four Gram-positive bacteria (*Enterococcus faecalis* ATCC 2321, *Bacillus cereus*, *Staphylococcus aureus* ATCC 1885, and *Staphylococcus epidermidis* ATCC 2405). Preparation of inoculums: All bacteria were grown in Brain Heart Infusion liquid medium at 37°C for 8 hours and used as inoculums. The turbidity of the suspensions was adjusted to the McFarland 0.5 turbidity standard.

**Antibacterial assay for extract and essential oil:** Antibacterial assay was performed using the disc diffusion method as previously described (10). Briefly, the total bacterial number for each inoculum was adjusted to 1.5×10⁸ CFU ml⁻¹ with reference to the McFarland turbidimetry,
inoculated on the surface of sterile Muller
Hinton Agar (MHA), and dispersed by a
sterile cotton swab. The plates were then
dried for 2-4 min. Seven concentrations (2,
1, 0.5, 0.250, 0.125, 0.062, and
0.031mg/mL) of hydroalcoholic extract and
seven concentrations (500, 250, 125,
62, 31, 15, and 7 µg/mL) of essential oil of
*T. spicata* were prepared. Subsequently,
the sterile filter paper discs (6 mm
diameter) (11,12) were saturated by adding
50 µL of each concentrations of the
essential oil and extract, and placed on the
surface of inoculated plates. The plates
were incubated at 37°C for approximately
24 hours, and the zones of inhibition
around each disc (mean ± SD of three
replicates) were then measured by
millimeter scale. Sterile paper discs
impregnated only with ethanol served as
negative control. In addition, discs
containing standard concentration of seven
antibiotics, including clindamycin,
streptomycin, kanamycin, gentamicin,
methicillin, ciprofloxacin, and vancomycin
were used as positive controls to compare
their antibacterial activity with the extract
and essential oil.

**Determination of MIC and MBC:**
Minimum inhibitory concentration (MIC)
for the hydroalcoholic extract and essential
oil of *T. spicata* was determined against
the above mentioned bacterial strains using
macro broth dilution assay method (13). In
this assay, the 16-hours cultures were
diluted with sterile physiologic saline
solution to achieve suspensions containing
nearly 1.5×10⁸ CFU mL⁻¹ with reference to
the 0.5 McFarland Standard (14).
Moreover, serial dilutions were prepared
between 15 and 1 mg mL⁻¹ for the extract
and between 500 and 7 µg mL⁻¹ for the
essential oil. Standard bacterial
suspensions were then added to the tubes
containing 1 ml of Muller Hinton Broth
(MHB) and different concentrations of the
extract and essential oil. The tubes were
incubated at 37°C for 24 hours. The
growth of bacteria was determined via
measuring optical density at 600 nm. Gentamicin and ethanol solutions were
tested as positive and negative controls,
respectively. Bacterial growth inhibition
calculated by the following equation (15):
\[
\text{Inhibition} \% = \frac{\left( \text{OD}_c - \text{OD}_i \right)}{\text{OD}_c} \times 100
\]
where \( \text{OD}_c \) is the OD<sub>600</sub> for the negative
control (ethanol solution) and \( \text{OD}_i \) is the
OD<sub>600</sub> for the sample treated with the
extract or essential oil of *T. spicata*.

Minimum bactericidal concentration
(MBC) was determined by culturing one
standard loop of the tubes showing no
apparent growth on MHA and subsequent
incubation at 37°C for 24 hours. The
lowest concentration that inhibited colony
formation on the agar was considered as
the MBC.

**Results**

The antibacterial activity of the
hydroalcoholic extract and essential oil of
*T. spicata* was quantitatively assessed by
measuring the inhibition zone diameter
around each disc, and then compared with
the standard antibiotic discs. The obtained
results are given in Tables 1 and 2.
Our results showed that the extracts and
essential oils of *T. spicata* could
effectively inhibit the growth of both
Gram-positive and Gram-negative. Among
the various concentrations of *T. spicata*
essential oils and hydroalcoholic extract,
the concentrations 500 µg/ml and 76
mg/ml showed the best antibacterial
activity, respectively (Tables 1 and 2). The
obtained results exhibited that the extracts
were more effective on Gram-positive
bacteria than Gram-negative ones.
The MIC and MBC of extract and essential
oil of *T. spicata* varied partly in relation to
the tested bacteria (Tables 3 and 4). The
lowest MIC values for the extract and
essential oil (5 mg/ml and 7µg/ml,
respectively) was observed to be against E.
faecalis and S. epidermidis, respectively
(Table 3).
Table 1. Antibacterial activity of various concentrations of essential oils and hydroalcoholic extracts of *Thymbra spicata* plant against four Gram-positive bacteria as compared with seven traditional antibiotics.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (µg/ml)</th>
<th><em>S. aureus</em></th>
<th><em>E. faecalis</em></th>
<th><em>B. cereus</em></th>
<th><em>S. epidermidis</em></th>
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*Concentrations are in mg/ml.

Table 2. Antibacterial activity of various concentrations of essential oils and hydroalcoholic extracts of *Thymbra spicata* plant against six Gram-negative bacteria as compared with seven traditional antibiotics.

<table>
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<tr>
<th>Substance</th>
<th>Concentration (µg/ml)</th>
<th><em>P. aeruginosa</em></th>
<th><em>E. coli</em></th>
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<th><em>S. typhi</em></th>
<th><em>K. pneumonia</em></th>
<th><em>E. aerogenes</em></th>
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</table>

*Concentrations are in mg/ml.
Accordingly, the highest MIC values for the extract and essential oil (19 mg/ml and 60µl/ml, respectively) was also observed to be against *E. coli*, *S. typhi*, *E. aerogenes* and *S.aureus* (Tables 3 and 4). The extract of *T. spicata* was recognized to be significantly more effective against Gram-positive bacteria (MICs ranging from 2.5 to 9.5 mg/ml) than against Gram-negative ones (MICs> 9.5 mg/ml).

Table 3. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of essential oils and hydroalcoholic extracts of *Thymbra spicata* plant against four Gram-positive bacteria.

<table>
<thead>
<tr>
<th>Bacterium Species</th>
<th>S. aureus</th>
<th>E. faecalis</th>
<th>B. cereus</th>
<th>S. epidermidis</th>
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<td>Hydroalcoholic Extract concentration (mg/ml) MIC</td>
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<td>MBC</td>
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</table>

MIC, Minimum inhibitory concentration; MBC, Minimum bactericidal concentration.

Table 4. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of essential oils and hydroalcoholic extracts of *Thymbra spicata* plant against six Gram-negative bacteria.

<table>
<thead>
<tr>
<th>Bacterium Species</th>
<th>P. aeruginosa</th>
<th>E. coli</th>
<th>M. morganii</th>
<th>S. typhi</th>
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<td>MBC</td>
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</table>

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

**Discussion**

The present study revealed the antibacterial activity of essential oil and hydroalcoholic extract of *T. spicata* plant against some Gram-positive and negative bacteria. Disc diffusion assay demonstrated that the essential oil of *T. spicata* was more effective than the hydroalcoholic extract of the plant. The most important components of the essential oil of *T. spicata* are carvacrol, p-cymene, β-myrcene, γ-terpinene, α-terpinene and trans-caryophyllene that among which, carvacrol was shown to have a strong antibacterial effect (16,17,18). Therefore, the antibacterial activity of the essential oil and hydroalcoholic extract of *T. spicata* may be partly attributed to carvacrol in the current study. Recently, the antibacterial activity of carvacrol and trans-caryophyllene, were examined against *E. coli*, *S. epidermidis*, *S. aureus*, *S. typhimurium*, *K. pneumoniae*, *P. Aeruginosa* and *E. faecalis* (18) and the effectiveness of carvacrol on the bacteria under study, except *P. aeruginosa*, has been demonstrated. In the present study,
the essential oil of *T. spicata* was effective against the all mentioned bacteria including *P. aeruginosa*. The reason of the latter difference is not clear but it may be due to the presence of other compounds in the essential oil of *T. spicata*. In supporting the opinion, Tamara et al. reported that the antimicrobial activity of the essential oil of *T. spicata* comes from the combined effect of its constituted compounds (19). However, further studies are required to discern the issue. In another study by M. Akin and his colleague, the antibacterial activity of the essential oil of *T. spicata* was to be effective only against *B. cereus* and *E. coli* (20, 21). However, MIC was not determined in that study. In the current study, we tested higher concentrations of the essential oil of *T. spicata* compared with M. Akin and his colleague. This may account for the wider range of antibacterial activity that we found in the present study. In the present study, the results for essential oil and hydroalcoholic extracts were partly parallel with each other, although the extract concentrations were three fold higher to be effective against the bacteria under study. The finding may be explained as the effective compounds of carvacrol which is more present in the oil phase, that is, the essential oil of the plant. Therefore, it is reasonable that the essential oil would be more effective against the examined bacteria than the hydroalcoholic extract.

In conclusion, our results demonstrated that essential oil of *T. spicata* have potent antibacterial activity against a relatively typical candidate of Gram-negative and Gram-positive bacteria. Furthermore, the essential of the plant is more active against the bacteria under study than the hydroalcoholic extract.

References


