Short Communication

Antibacterial Activity of Anti-Aphthous Spray and Oral Drop: Two Thymus Commercial Products

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Article Info
Article History:
Received: 9 October 2016
Accepted: 4 January 2017
ePublished: 30 June 2017

Keywords:
- Antibacterial effect
- Thymus
- Staphylococcus aureus
- Streptococcus pyogenes
- Escherichia coli
- Pseudomonas aeruginosa

Abstract

Background: Today, traditional medicine is developed globally as an important source for health care of the world's population. The current study describes the antibacterial activity of thymus commercial products against both Gram-positive including Staphylococcus aureus, Streptococcus pyogenes and Gram-negative bacteria including Escherichia coli, Pseudomonas aeruginosa.

Methods: Two commercial products of thymus with standard expiration date (and in three different batch numbers) including anti-aphthous spray and oral drop were purchased from the pharmacies of Tabriz city. Minimum Inhibitory Concentration (MIC) and disk diffusion are used to investigate the antibacterial efficiency of the mentioned products.

Results: The results of disk diffusion method showed zones of growth inhibition against S. aureus and S. pyogenes for the investigated products. Based on MICs, thymus oral drop had inhibitory effects against S. aureus, S. pyogenes while anti-aphthous spray showed inhibitory effects against S. aureus, S. pyogenes and P. aeruginosa. The findings also indicated that the thymus anti-aphthous spray had more inhibitory effects than thymus oral drop.

Conclusion: This study showed that thymus can be used as an optimistic antibacterial agent against the selected microorganisms.

Introduction

Medicinal plants have been known during human history. These plants make many chemical compounds and according to reports, 12,000 compounds have been isolated from them so far. These compounds may present biological functions, including antifungal and antibacterial activities. Herbal drugs may possess helpful pharmacology effects and the same potential as conventional drugs. Therefore, the growth in utilization of medicinal plants and also the development of attentiveness has improved the skill of pharmacists and chemists in order to reduce current problems such as side effects of conventional drugs.1-4

Like other medicinal plants, aromatic plants have been applied for their medicinal properties for centuries. The genus thymus contains about 350 species of herbaceous plants and is native to temperate regions in Europe, North Africa and Asia.1 Thymus species have medicinal and cosmetics applications.3 The antifungal and antibacterial activity of thymus is reported by several investigators.2,4 Oussalah et al reported antimicrobial activity of thymus vulgaris (with a MIC ≤ 0.1% (v/v)) for some pathogenic bacteria (including Escherichia coli, Listeria monocytogenes, Salmonella paratyphi and Staphylococcus aureus).5 Maksimović et al confirmed that essential oil of thymus possesses notable in vitro antimicrobial activity against S. aureus, Enterococcus faecalis, Pseudomonas aeruginosa, E. coli.1 Marino et al assessed the antimicrobial activity of thyme essential oils against nine strains of Gram-negative bacteria and six strains of Gram-positive bacteria. Their results revealed that all the thyme essential oils had a remarkable bacteriostatic effect against the tested microorganisms. Their results also showed that the antimicrobial activity was more evident against the Gram-positive bacteria and E. coli was the most sensitive species even in the lowest concentration of oil.6 The earlier investigates have exposed that most features of thymus medicinal uses are associated to the numerous levels of thymol.

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carvacrol, and phenolic derivatives with strong and wide-spectrum antimicrobial activity.\(^1\)\(^7\)

The aim of this study was to investigate the in vitro efficiency of some thymus commercial products against selected pathogens including both Gram-positive (S. aureus, S. pyogenes) and Gram-negative bacteria (E. coli, P. aeruginosa).

Material and Methods

**Media and chemicals**

All media including Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB) were obtained from Merck Company (Darmstadt, Germany). Thymus commercial products (anti-aphthous spray and oral drop) were purchased from Tabriz, Iran.

**Microorganisms**

The microorganisms used in this study (Staphylococcus aureus PTCC 1112, Streptococcus pyogenes PTCC 1447, Escherichia coli PTCC 1338, Pseudomonas aeruginosa PTCC 1074) were obtained from Iran's Biotechnology Institute of Scientific and Technical Research (Tehran, Iran).

**Inoculum preparation**

The bacteria were activated according to supplier protocols to prepare the bacteria’s stock cultures. Then, a single colony from the stock cultures was transferred into Mueller Hinton Broth. After overnight incubation (at 37 °C), cells were collected by centrifugation at 3000 rpm. Cells were washed and re-suspended with a sterile physiologic saline solution to prepare the bacteria inoculum equal to 10^8 CFU.mL^-1.

**Sample preparation of commercial products and Determination of MICs**

MIC determination was performed by broth macro dilution method according CLSI protocol. Ten tubes contain 1 ml sterile buffer were used as serial dilution tubes. Then, 1 ml of samples (anti-aphthous spray and oral drop each from three different batches) was added in the first tube and twofold serial dilutions were prepared using sterile buffer. Then, 100 µl of bacterial inocula and 900 µl of Mueller-hinton broth were transferred into the tubes and all tubes were incubated at 35 °C for 24 h.

After 24 h incubation of dilution tubes, MICs of the products was determined for most sensitive bacterial species. To this end, the first tube of the series with no sign of visible growth after streak culture to the agar plates and incubation was considered as the MICs.\(^7\) This process has been done three times.

**Disc diffusion method**

The filter paper discs with identical diameter were autoclaved and impregnated by 30 µl of solution of the antimicrobial products. The disks were placed on the nutrient agar plates and incubated at 37 °C for 24 h. The inhibition zone diameters were read for samples and recorded.\(^7\)

**Results**

**MIC results**

Based on the obtained results for MIC determination, both thymus commercial products exhibited inhibitory effects on selected microorganisms. Thymus oral drop exhibited inhibitory effects against S. aureus, S. pyogenes whereas anti-aphthous spray showed inhibitory effects against S. aureus, S. pyogenes and P. aeruginosa as well. The results also showed that the thymus anti-aphthous spray had more inhibitory effects compared to thymus oral drop. The results of MIC tests against four selected microorganisms are shown in Table 1 and the pertaining plates pictures are shown in Figures 1 to 4.

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**Figure 1.** Streak cultures with thymus Anti-aphthous Spray sample on S. aureus PTCC 1112 (Second batch).

**Figure 2.** Streak cultures with thymus Anti-aphthous Spray sample on S. pyogenes PTCC 1447 (Second batch).

**Figure 3.** Streak cultures with thymus Anti-aphthous Spray sample on E. Coli PTCC 1338 (Second batch).

**Figure 4.** Streak cultures with thymus Anti-aphthous Spray sample on P. Aeruginosa PTCC 1074 (Second batch).
Table 1. The results of MICs against four selected microorganisms.

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-aphthous Spray</td>
<td>1/4</td>
<td>1/2</td>
<td>1/2</td>
<td>1/4</td>
<td>1/2</td>
<td>1/2</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>1/2</td>
<td>1/2</td>
<td>ND</td>
</tr>
<tr>
<td>Oral Drop</td>
<td>1/4</td>
<td>1/4</td>
<td>1/4</td>
<td>1/4</td>
<td>1/2</td>
<td>1/2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*ND: Not Detected

Table 2. The results of disk diffusion method against four selected microorganisms.

<table>
<thead>
<tr>
<th>Mean zones of growth inhibition (mm) ± SD</th>
<th>S. aureus</th>
<th>S. pyogenes</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-aphthous spray</td>
<td>15±3</td>
<td>14±4</td>
<td>ND</td>
<td>10±2</td>
</tr>
<tr>
<td>Oral drop</td>
<td>11±3</td>
<td>10±2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Standard Amikacin disk</td>
<td>19±3</td>
<td>18±2</td>
<td>12 ±2</td>
<td>19±3</td>
</tr>
</tbody>
</table>

*ND: Not Detected

**Disk diffusion method**

The results of disk diffusion method showed “zones of growth inhibition” only for *S. pyogenes* and no “zones of growth inhibition” for other bacteria. The findings also indicated that “zones of growth inhibition” for thymus oral drop was greater than thymus anti-aphthous spray. The results of disk diffusion method against four selected microorganisms are shown in Table 2.

**Discussion**

The previous researches have shown that most aspects of thymus medicinal applications are related to the various levels of thymol and/or carvacrol, phenolic derivatives with strong and wide-spectrum antimicrobial activity. Based on MIC and MBC results, thymus oral drop exhibited inhibitory effects against Gram negative bacteria (*P. aeruginosa* and *E. coli*) whereas anti-aphthous spray showed inhibitory effects against both Gram positive and Gram negative bacteria (*S. aureus, S. pyogenes, P. aeruginosa*). The results also showed that the thymus anti-aphthous spray had more inhibitory effects compared to thymus oral drop. The producer company did not provide information about the excipients incorporated into the formulations and it can be probably said that in the case of using the same concentration of thymus extract in both products, the solvents and excipients in the anti-aphthous spray may potentiate the antimicrobial activity of the thymus extract compared to that of oral drop. On the other hand, MIC values in the case of first batch are higher than the remaining two batches for anti-apthous spray and oral drop. Martin et al tested and compared the antioxidant and antibacterial properties for decoction, infusion and hydroalcoholic extract of thymus. Their results showed that all samples had efficiency against Gram-positive (*S. aureus and S. epidermidis*) and Gram-negative (*E. coli, P. aeruginosa and E. aerogenes*) bacteria. They concluded that the use of thyme infusion and decoction (by both internal and external use) is safe without adverse reactions. The observed batch to batch variations in MIC and MBC results can be attributed to the differences between manufacturing processes and the variations during construction or formulation of these products or lack of reproducibility of them. If batch effects go undetected, the technologies developed for clinical outcomes (using data) may produce results that are more variable than expected. Then, development of precise and repeatable manufacturing processes with reproducible pharmaceutical products is necessary that can decrease batch to batch variations. Also the assay method is of great importance in evaluation of antimicrobial activity. The results of disk diffusion method showed “zones of growth inhibition” only for *S. aureus and S. pyogenes* and no “zones of growth inhibition” for other bacteria in the case of both products. Maksimović et al reported antimicrobial efficiency for essential oil of thymus against *S. aureus, E. faecalis, P. aeruginosa, E. coli*. They reported maximum activity for *S. aureus* and *E. coli*, moderate activity for *P. aeruginosa* and one of the tested strains of *K. pneumoniae (MIC = 200 μl/ml)*, and *E. faecalis* expressed a higher degree of resistance.

**Conclusion**

The present study is focused on the antimicrobial activity of two commercial products of thymus. The efficacy of these products can develop a new approach for using of the medical herbs in the incorporation into the drug formulations in order to treatment of infectious diseases. This strategy may consider more important in the case of the infectious diseases that have developed resistance to antibiotics. So therefore, herbal drugs may hold helpful pharmacology and the same potential as
conventional drugs. However, plant material comes with a variety of compounds that may have undesired effects that should be efficiently processed.

Conflict of interests
The authors claim that there is no conflict of interest.

References