Phytochemical evaluation and antibacterial effects of *Medicago sativa*, *Onosma sericeum*, *Parietaria judaica* L., *Phlomis persica* and *Echinophora platyloba* DC. on *Enterococcus faecalis*

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Abstract

**Background:** Since drug resistance has become one of the predominant problems of health worldwide, it is necessary to use new methods to combat drug-resistant bacteria. In this regard, medicinal plants are considered one of the richest sources to produce antibiotics. The aim of this study was to investigate the antibacterial effects as well as total phenolic and flavonoid contents of a number of medicinal plants collected from the Chaharmahal and Bakhtiari provinces of India, in order to investigate their potential use for the production of new antibiotics. **Materials and Methods:** In this experimental study, the maceration method was used to prepare hydroalcoholic extract of *Medicago sativa*, *Onosma sericeum*, *Parietaria judaica* L., *Phlomis persica* and *Echinophora platyloba* DC. The effect of these plants on *Enterococcus faecalis* (ATCC 29212) was investigated. To determine the antibacterial effect of the extracts, broth microdilution in sterile 96-well plate was used according to the McFarland standard (10⁵ CFU/ml). The total phenolic content was assayed by using the Folin-Ciocalteu colorimetric method and expressed in terms of gallic acid equivalent. The total flavonoid content was assayed by aluminum chloride colorimetric method and expressed in terms of rutin equivalent.
Results: Based on the results of this study, the 512, 256, 128, 32 and 32 μg/ml doses were determined to be the minimum inhibitory concentrations (MICs), and the 1024, 1024, 512, 128 and 128 μg/ml doses were derived as the minimum bactericidal concentration (MBCs) of *M. sativa*, *O. Sericeum*, *P. judaica*, *P. persica* and *E. platyloba*, respectively. *E. faecalis* and *P. judaica* contained the highest total phenolic content and flavonoid content, respectively. Conclusion: Given the comparatively higher antibacterial effect of *P. persica* and *E. platyloba*, as well as the presence of phenolic and flavonoid compounds in these plants, it is recommended that these plants be further investigated in feasibility studies for the production of new antibiotics.

Keywords

Drug resistance, Medicinal plants, Minimum bactericidal concentration, Minimum inhibitory concentration

Introduction

Nowadays new bacterial resistance to commonly used chemical drugs has turned into a widespread phenomenon. *Enterococcus faecalis* is one of these bacteria. *E. faecalis* can be a cause of bacteremia, meningitis, septicemia, endocarditis, wound infection, infant infections and urinary tract infections (Kafil and Asgharzadeh, 2014). In addition to vancomycin-resistant enterococci (VRE), this bacterium has been reported to acquire resistance to daptomycin, which has aroused concerns (Werth et al., 2014).

With increasing drug resistance among bacteria, efforts are being made to seek out new therapies. Phytotherapy is one of the most promising therapies for many diseases (Asadi-Samani et al., 2017; Bahmani et al., 2016; Kooti et al., 2016; Moradi et al., 2017; Rahimi-Madiseh et al., 2017; Rahimifard et al., 2017; Rouhi-Boroujeni et al., 2017; Sarrafchi et al., 2016). Indeed, the collection and screening of medicinal plants can be helpful in areas with high potential for growth of medicinal plants (Bahmani et al., 2014). Therefore, the present study was conducted to investigate the antibacterial effects of *Medicago sativa*, *Onosma sericeum*, *Parietaria judaica* L., *Phlomis persica* and *Echinophora platyloba* DC, and to determine their phenolic and flavonoid contents. These plants are grown in the Chaharmahal and Bakhtiari provinces of Iran, which are areas with high potential for the growth of medicinal plants. Understanding their antibacterial effects would address the suitable candidates for phytotherapy and the feasibility for production of new antibiotics.

*M. sativa*, also known as alfalfa and lucerne, comes from the Fabaceae family (Sadowska et al., 2014). *M. sativa* is used as a food additive in the United States,
Russia, North Africa and China because of their high vitamin content (Shi et al., 2014). It produces secondary metabolites, such as coumarins, isoflavones, naphthoquinones, alkaloids and saponins, that have nematocidal, cytotoxic and antimicrobial effects (Sadowska et al., 2014).

*O. sericeum* is a perennial plant that grows naturally in Iran and is a member of the Boraginaceae family (Gharehmatrossian et al., 2016; Naz et al., 2006). In addition to the roots of this herb (which is a dye and used in cosmetics), other properties have been reported for this plant, including antitumor, anti-inflammatory, antipregnancy, antimicrobial, cardiotonic and antiviral effects (Gharehmatrossian et al., 2016).

*P. judaica* (Urticaceae) grows abundantly in urban areas of the Mediterranean region. *P. judaica* is a perennial herb, with individual plants consisting of many shoots emerging from a common rootstock (Fotiou et al., 2011; Mozaffarian, 2015). *P. persica* is from the family Lamiaceae. The genus Phlomis consists of 100 species worldwide, with 17 species in Iran. This herb in phytotherapy is used to treat topical wounds and respiratory diseases. Some other properties including analgesic, anti-diarrheatic, hemorrhagic ulcer-treating, antimalarial, anti-inflammatory, antimicrobial and immunosuppressive effects have also been reported for this plant (Sarkhail et al., 2006). Moreover, some sources have reported tonic, diarrhea and free radical-inhibitory effects (Hussain et al., 2010).

The Echinophora genus consists of 10 species of which *Echinophora orientalis*, *Echinophora sibthorpiiana*, *Echinophora cinerea* and *E. platyloba* are present in Iran (Shahneh et al., 2013). *E. platyloba* is used more often as a food additive in Iran (Avijgan et al., 2010). This plant has been reported to exhibit antifungal, antioxidant and antibacterial properties (Sharafati-chaleshtori et al., 2012).

**Materials-Methods**

**Collecting plants**

The plants were collected from different regions of the Chaharmahal and Bakhtiari provinces (such as Shahrekord, Teshniz, and Saman) between March 2016 and September 2016. The plants were identified as the plants of interest by a botanist (Dr. Shirmardi) at the Research Center of the Agricultural Jihad Organization of Chaharmahal and Bakhtiari province.

**Extraction**

Extraction was conducted by maceration of *Medicago sativa*, *Onosma sericeum*, *Prietaria judaicae* L., *Phlomis persica* shoots, and from aerial parts of *Echinophora platyloba* D.C; these were done in triplicates (for 72h each time).
this method, water and butyric acid-free bitter ethanol at 30/70 ratio were used. The resulting extract was filtered using filter paper and evaporated under next-to-vacuum pressure and 40°C by a rotary evaporator to concentrate. The resulting solution was stored at -20°C until later use.

Preparing different dilutions of extract

The extracts were prepared using dimethyl sulfoxide (DMSO) and distilled water. Different dilutions (4, 8, 16, 32, 64, 128, 256, 512 and 1024 μg/ml) of the extracts were prepared using Mueller-Hinton broth agar. The maximum concentration of DMSO was 0.2% in the final concentration.

Preparing standard bacterial strains

*Enterococcus faecalis* (ATCC 29212) was purchased as lyophilized from Iranian Research Organization for Science and Technology.

Preparing microbial suspension

To prepare a microbial suspension equivalent to 0.5 McFarland standard (10^5 CFU/ml), a 24-hour culture of the bacteria was performed on blood agar, and then a suspension with 0.5 McFarland turbidity was prepared in normal saline.

Determining minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)

The antibacterial effects of the extracts were determined by broth microdilution in a sterile 96-well plate with reference to 0.5 McFarland standard (10^5 CFU/ml). In this method, the first well was considered “culture medium + extract” (negative control), and the second well was considered “culture medium + bacterium” (positive control). After adding the culture medium at 95μl and the extracts at 100μl to microplate wells and diluting them, we incubated the samples at 37°C for 24h. The concentration of the last (most diluted) well without turbidity was considered MIC (Andrews, 2001). To determine MBC, we subsequently performed a culture of the samples of each tube at 10μl on Mueller-Hinton agar and left them at 37°C to incubate for 24h. The lowest concentrations of the extract in which the bacteria could not grow were considered MBCs. The tests were performed to determine the MICs and MBCs, and were conducted in triplicates (National Committee for Clinical Laboratory Standards, 2001).

Determining total phenolic and flavonoid content

Total phenolic content was measured by Folin-Ciocalteu colorimetric assay and expressed in terms of gallic acid equivalent. Total flavonoid content was measured by aluminium chloride colorimetric method and expressed in terms of rutin equivalent (Dulf et al., 2016; Karimi and Moradi, 2015; Singleton et al., 1999).
Results

M. sativa extract could inhibit E. faecalis at 512 μg/ml. At 1024 μg/ml, M. sativa extract could destroy the bacteria. O. sericeum displayed more potent bacteriostatic activity against E. faecalis than M. sativa; the bactericidal activity of O. sericeum was the same as M. sativa. P. judaica showed MIC and MBC of 128 and 512 μg/ml, respectively. Moreover, 32 μg/ml of P. persica and E. platyloba inhibited E. faecalis, while 128 μg/ml of P. persica and E. platyloba eliminated E. faecalis.

Table 1. The minimum inhibitory concentrations of the hydroalcoholic extracts of the studied plants for Enterococcus faecalis

| Plants                      | Concentration (μg/ml) | 4  | 8  | 16 | 32 | 64 | 128 | 256 | 512 | Control |
|-----------------------------|-----------------------|----|----|----|----|----|-----|-----|-----|---------|
| Medicago sativa*           |                       | -  | -  | -  | -  | -  | -   | -   | +   | +       |
| Onosma sericeum*           |                       | -  | -  | -  | -  | -  | -   | +   | +   | +       |
| Parietaria judaica*        |                       | -  | -  | -  | -  | -  | +   | +   | +   | +       |
| Phlomis persica*           |                       | -  | -  | -  | +  | +  | +   | +   | +   | +       |
| Echinophora platyloba D.C**|                       | -  | -  | +  | +  | +  | +   | +   | +   | +       |

+: Lack of microorganism growth in culture medium and the antimicrobial activity of the hydroalcoholic extracts of plants; -: Microorganism growth in culture medium and lack of antimicrobial activity of the hydroalcoholic extracts of plants; * extraction of shoot; ** extraction of aerial parts

Table 2. The minimum bactericidal concentrations of the hydroalcoholic extracts of the studied plants for Enterococcus faecalis

| Plants                      | Concentration (μg/ml) | 4  | 8  | 16 | 32 | 64 | 128 | 256 | 512 | 1024 | Control |
|-----------------------------|-----------------------|----|----|----|----|----|-----|-----|-----|------|---------|
| Medicago sativa*           |                       | -  | -  | -  | -  | -  | -   | -   | +   | +    | +       |
| Onosma sericeum*           |                       | -  | -  | -  | -  | -  | -   | -   | +   | +    | +       |
| Parietaria judaica*        |                       | -  | -  | -  | -  | -  | -   | -   | +   | +    | +       |
| Phlomis persica*           |                       | -  | -  | -  | -  | +  | +   | +   | +   | +    | +       |
| Echinophora platyloba D.C**|                       | -  | -  | -  | -  | +  | +   | +   | +   | +    | +       |

+: Lack of microorganism growth in culture medium and the antimicrobial activity of the hydroalcoholic extracts of plants; -: Microorganism growth in culture medium and lack of antimicrobial activity of the hydroalcoholic extracts of plants; * extraction of shoot; ** extraction of aerial parts
The least potent bactericidal effects among the studied plants was seen by *M. sativa* and *O. sericeum*. Lastly, *P. persica* and *E. platyloba* showed the most potent antibacterial effects on *E. faecalis* (Tables 1 and 2).

According to Table 3, all plants contained phenols and flavonoids. *P. judaica* had the highest total phenolic and flavonoid content. The plant with the least flavonoid content was *E. platyloba* (Table 3).

### Table 3. Total phenolic and flavonoid content of the studied plants

| Plants                      | Source (organ) | Total phenolic content (mg GAE/100g DW) | Flavonoid content (mg RU/100 g DW) |
|-----------------------------|----------------|----------------------------------------|------------------------------------|
| *Medicago sativa*           | Shoot          | 49.80                                  | 16.87                              |
| *Onosma sericeum*           | Shoot          | 54.52                                  | 13.81                              |
| *Parietaria judaica* L.     | Shoot          | 67.85                                  | 35.20                              |
| *Phlomis persica*           | Shoot          | 55.51                                  | 24.39                              |
| *Echinophora platyloba D.C* | Arial parts    | 43.98                                  | 6.53                               |

### Discussion

This present study was conducted to investigate the antibacterial effects as well as the total phenolic and flavonoid contents of a number of medicinal plants collected from the Chaharmahal and Bakhtiari provinces of Iran. The aim was to evaluate which plants could be considered as suitable alternatives for development of antibiotics to fight treatment-resistant bacteria.

Our findings showed the antibacterial effects of *M. sativa* on *E. faecalis*, with MIC of approximately 512 μg/ml and MBC of 1024 μg/ml for this plant. Chavan *et al.* conducted a study to investigate the antibacterial properties of *M. sativa*. The methanol extract of this plant created inhibition zones of 23, 22 and 23 mm in diameter for *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus aureus*, respectively. The authors also reported an MIC of 37 μg/ml for *E. coli*, 12.03 mg/ml for *P. aeruginosa* and 111 μg/ml for *S. aureus* (Chavan *et al.*, 2015). The antibacterial effect of this plant could be due to their phenolic compounds. Notably, our study and the study of Chavan *et al.* demonstrate the optimal antibacterial effects of *M. sativa*.

Another plant investigated in our study was *O. sericeum*; its antimicrobial effect on *E. faecalis* was demonstrated- with MIC and MBC of 256 μg/ml and 1024 μg/ml.
A study conducted by Naz et al., to examine the antimicrobial effects of another species from the Onosma genus (Onosma hispidum), showed that this plant could inhibit the growth of various bacteria and could have an antibacterial effect on *E. faecalis* (Naz et al., 2006). The study by Vukic et al. on *Onosma visianii* showed that main components of this plant extract were 5,8-O-dimethyl isobutyrlyshikonin, acetylshikonin, α-methylbutyrylshikonin, β-hydroxyisovalerylshikonin, isobutyrlyshikonin, deoxyshikonin, and 5,8-O-dimethyl deoxyshikonin. Indeed, *O. sericeum* plant had a potent antibacterial effect not only on *Enterococcus faecalis* but also *Bacillus megaterium*, *Micrococcus luteus*, *Staphylococcus epidermidis* and *Stenotrophomonas maltophilia* (Vukic et al., 2017). The results of the current study and other studies, thus, show that the species of the Onosma genus can have antibacterial effects on *E. faecalis*, which can be due to the presence of phenolic compounds in their aerial parts.

*P. judaica*, which was observed to have the highest phenolic and flavonoid content among the studied plants, also exhibited robust antibacterial effects on *E. faecalis*. Fares et al. who investigated the antibacterial effects of extracts of wild plants in Palestine showed that *P. judaica* had antibacterial effect on *Streptococcus pneumoniae*; the aqueous and ethanol extracts of this plant had an MIC of 3.125 and 100 mg/ml, respectively (Fares et al., 2013). Our present study and other studies have demonstrated the antibacterial effects of this plant. The antibacterial effects of this species, therefore, is not an unexpected observation due to the presence of phenolic and flavonoid compounds. Moreover, it is not a surprising observation due to the fact that extracts from this plant can be isolated for its antibacterial compounds.

*P. persica* and *E. platyloba* in our study were found to exert the most potent antibacterial effect on *E. faecalis* when compared to the other studied plants. A phytochemical study showed the presence of relatively equal amounts of phenolic content in both plants. Amor et al. studied the antibacterial effects of *Phlomis crinita*. They reported that the essential oil of its flower had an inhibitory effect on *S. aureus*, *E. faecalis* and *Salmonella typhimurium* with the MIC ranging between 39 μg/ml and 625 μg/ml (Amor et al., 2008). Sarkhail et al. (2006) showed that *P. persica* shoot extract had chrysoseryl-7-O-β-D-glucoside, verbascoside and two other glycosidic flavonoids to which the antibacterial effects of this plant can be attributed (Sarkhail et al., 2006). Besides that, Ranjbar and Babaiei (2016) studied the antimicrobial properties of *E. platyloba* on different Salmonella species and concluded that this plant, at 150 mg/ml, exerted antifungal effect on *Salmonella enteritidis* and *Salmonella typhi* and, at 250 mg/ml, on *Salmonella choleraesuis* (Ranjbar and Babaiei, 2016). Entezari et al. (2009) investigated the antibacterial effects of *E. platyloba*, and reported that the methanol extract of this plant had an inhibitory effect on *S. aureus* and *P. aeruginosa*. Thus, at high doses, the *E. platyloba* plant could completely inhibit the growth of these bacteria. An investigation of the chemical compounds of the essential oil of *E. platyloba* also showed that monoterpens and sesquiterpenes are the major compounds of this plant (Rahimi-Nasrabadi et al., 2010). As it has been clearly shown in this study and other studies, *E. platyloba* is
a plant that can be an appropriate choice to be further studied for feasibility as an antibacterial agent; it has potent antibacterial effects on different bacterial species as well as phenolic and flavonoid compounds.

**Conclusion**

All five plants were collected from the Chaharmahal and Bakhtiari provinces, had relatively equal amounts of phenolic compounds, and showed antibacterial effects on *E. faecalis*. It is recommended that these plants be further investigated in feasibility studies for the production of new antibiotics.

**Abbreviations**

DMSO: Dimethyl sulfoxide  
DW: Dry weight  
GAE: Gallic acid equivalent  
MIC: Minimum inhibitory concentration  
MBC: Minimum bactericidal concentration  
RU: Rutin

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**Author Contribution**

All authors equally contributed on all experiments; designed the study; wrote the manuscript; approved the manuscript for publication.
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