Short communication

Volatile oil composition and antimicrobial activity of two *Thymus* species

Mojtaba Hosseini Behbahani\(^a\),\(^c\), Younes Ghasemi\(^b\),\(^*\), Mohammad Javad Khoshnoud\(^d\), Pouya Faridi\(^e\),\(^*\), Gholamali Moradli\(^c\), Nima Montazeri Najafabady\(^a\)

\(^a\) Department of Pharmaceutical Biotechnology, Faculty of Pharmacy and Pharmaceutical Sciences Research, Shiraz University of Medical Sciences, Shiraz, P.O. Box 71345 1583, Iran  
\(^b\) Prof., Department of Pharmaceutical Biotechnology, Faculty of Pharmacy and Pharmaceutical Sciences Research, Shiraz University of Medical Sciences, Shiraz, P.O. Box 71345 1583, Iran  
\(^c\) Department of Horticulture, School of Horticulture, Islamic Azad University (Saveh Branch), Saveh, Iran  
\(^d\) Department of Toxicology and Pharmacology, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran  
\(^e\) Department of Traditional Pharmacy, Faculty of Pharmacy and Pharmaceutical Sciences Research, Shiraz University of Medical Sciences, Shiraz, Iran

**ABSTRACT**

Background: Medicinal plants have potential for using as antimicrobial agents against pathogens. In genus *Thymus* (Lamiaceae), phenolic compounds with terpene origin are responsible of these effects. **Objective:** Evaluated essential oil constituents and antimicrobial activity of *Thymus daenensis* compared with *Thymus vulgaris*.

Materials and methods: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger* were used for antimicrobial activity assay with using disc diffusion technique. Volatiles oils compositions were analyzed by GC/MS.

Results: *Escherichia coli* was resistant to essential oils. Essential oil obtained from *T. daenensis* showed better antimicrobial activity than with *T. vulgaris*. Thymol (84.45%) and carvacrol (46.62%) were the main components of *T. daenensis* and *T. vulgaris* respectively.

Conclusion: The results showed that *T. daenensis* was more effective and could be an alternative for *T. vulgaris*. In addition, these essential oils could be used as natural antimicrobial agents.

**1. Introduction**

Antimicrobial effects especially against infectious pathogens are one of potentials of medicinal plants. *Thymus* genus (Lamiaceae) has with about 215 species.\(^1\) In genus *Thymus*, phenolic compounds with terpene source are responsible for these effects. Most of their properties are due to essential oils.\(^2\) Volatile oils possess antimicrobial, antitussive, digestive, expectorant and carminative activities.\(^3\) *Thymus daenensis* which is endemic of Iran\(^4\) has antimicrobial properties\(^2\) and its beneficiary for asthma and bronchitis has been proved.\(^6\) *Thymus vulgaris* is a well-known species of *Thymus* genus. The oil extracted of *T. vulgaris* is used as food preservative, anti-worm,\(^7,8\) antiseptic and antispasmodic.\(^9\) In traditional medicine, juices of flowers have been generally used for relieve headache, dysmenorrhea, and digestive disorders in some countries.\(^10\) Aerial parts of plants containing glands which are rich in essential oil\(^11,12\) Antimicrobial and antiseptic effects of genus *Thymus* have been studied by many researchers, although fewer studies carried out on *T. daenensis*. Present study indicated comparison of antimicrobial activity and essential oil compounds of *T. daenensis* and *T. vulgaris.*

**2. Materials and methods**

**2.1. Plant materials**

*T. daenensis* and *T. vulgaris* were collected from Daran (East of Esfahan province, Iran) and Abadeh (Northwest of Fars province, Iran) respectively in early of June 2011 in flowering stage. *T. daenensis* identity was confirmed and voucher specimen was deposited at the Forest and Rangel research center of Iran with No. 13353 and voucher specimen of *T. vulgaris* deposited in Shiraz Faculty of Pharmacy herbarium with No. pm153.

**2.2. Essential oil extraction**

Essential oils were obtained from aerial, air shad dried parts, after powdered and subjected to hydro-distillation using Clevenger system during 5 h, according to the method recommended in British Pharmacopoeia. The oils were dehydrated by sodium sulfate and stored in refrigerator in dark vials.\(^13\)

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\(^*\) Corresponding authors.  
E-mail addresses: ghasemi@sums.ac.ir (Y. Ghasemi), faridip@sums.ac.ir (P. Faridi).
2.3. Gas chromatography/mass spectrometry conditions

A Hewlett–Packard GC/MS 6890 system with an HP-5M capillary column (phenyl methyl siloxan, 25 m × 0.25 mm i.d., Hewlett Packard part No.190915.433, USA) was used for the identification of volatile compounds. GC oven temperature was kept constant at the rate of 3 °C/min. Mass spectra (Hewlett Packard 5973, USA) were taken at 70 eV. The injector temperature was 250 °C; mass range was between 30 and 600 m/z. Compounds identify was accordance on comparison of their RT and mass spectra with Willey (275) and Adams libraries spectra.14

2.4. Standard bacteria and fungi

Standard germs (ATCC) were used for investigation of antimicrobial activities. The germs were prepared from center of microbial collection of Iran and stock cultures were stored in refrigerator. Staphylococcus aureus (1112), Bacillus subtilis (1023), Escherichia coli (1397), Candida albicans (5027) and Aspergillus niger (5162) were the microorganisms.

2.5. Culture media

Muller Hinton agar media (Merck, Darmstadt, Germany) was used as a medium for antifungal assay. Agar diffu

2.6. Antimicrobial assay

Paper disk diffusion method was used for investigating inhibition zones of bacterial and fungal growth.16 50 ml of culture media was applied for each plate and autoclaved at 121 °C for 15 min. Solutions containing 1.5 × 10−4 of each microbes per ml equivalent were prepared to 0.6 and 0.5 standard of McFarland’s tube from germs strain respectively and were read by spectrophotometer. Sterile media inoculated, and then loaded disk with essential oil was placed in the center of the plates by sterile forceps (one disk in each plate). Ampicillin and gentamicin were used for Gram-positive and Gram-negative as a positive control. For fungi, polymixin was applied (300 unites per disc). Concentrations used were 5, 4, 3 and 2 μl of essential oils. After that plates were incubated at 37 °C during 18–24 h. Diameters of non-growth zones were measured using a caliper.

3. Results and discussion

Analyzing the spectra of GC/MS, retention indices and mass spectra of objects detected and compared with standard compounds and references; six components identified in T. daenensis and 25 components identified in T. vulgaris which are accounts for 98.82% and 88.45% of total essential oil respectively (Table 1). Thymol (84.41%) and β-caryophyllene (4.48%) were the main compounds of T. daenensis and oxygen containing monoterpenes were major group of terpene compounds (Table 1). There is just one sesquiterpenes component which is β-caryophyllene (4.482%). Some other reports show that identified compounds were 24 which main components were thymol (74.61%), p-cymene (4.6%), γ-terpinene (4.48%), carvacrol methyl ether (4.27%)9 and 26 compounds which thymol (16.4%), carvacrol (52.3%)18 were the main components. Inhibitory effects of T. daenensis are shown in Table 2 and indicated significant activity against Gram-positive and Gram-negative bacteria and also fungal. E. coli had most resistance among the selected microbes and the best result was observed in treatment with A. niger. Main compounds of T. vulgaris were carvacrol (46.62%), γ-terpinene (9.69%), pentacosane (6.36%) and oxygen containing monoterpenes were major groups of terpene compounds (Table 1). Essential oil of T. vulgaris was containing 0.64% unknown and 1.81% non-terpene compounds. Main compounds in other reports were thymol (79.15%), carvacrol (4.63%), p-cymene (3.27%)9 or thymol (39.9%), p-cymene (19.5%), γ-terpinene (15.4%) and borneol (5.3%).9 Inhibitory effects of T. vulgaris are shown in Table 2. Result indicated antimicrobial activity against all of microorganisms but effects in case of E. coli was less than positive control. Both oils had the highest effect on B. subtilis among the selected bacteria.

4. Conclusion

Both of these essential oils have antimicrobial activity against all of cases but T. daenensis has stronger effects in compare with T. vulgaris especially in the case of E. coli. Most effects were on A. niger.
while positive control (polymixin, 300 unit per disc) indicated no activity against fungal. Considering high amount of thymol and according to our results, *T. daenensis* is a good alternative in antimicrobial cases for *T. vulgaris* and has potential for future works in this field.

**Conflicts of interest**

All authors have none to declare.

**References**