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Chemical variation of leaf essential oil at different stages of plant growth and *in vitro* antibacterial activity of *Thymus vulgaris* Lamiaceae, from Iran

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ABSTRACT

The essential oil components of the leaves were isolated by hydrodistillation from *Thymus vulgaris* (T.) Lamiaceae, at different stages of plant growth. The essential oils from *T.* Lamiaceae leaves were obtained in yields of 0.83–1.39% (w/w). The oils were studied by gas chromatography mass spectrometry (GC/MS) and thirty-six components were identified in the oil. The major components in the leaf oils were: thymol (38.23–63.01%), o-cymene (5.56–15.47%), γ-terpinene (4.43–7.17%), borneol (1.72–6.65%), 4-terpineol (1.24–5.16%) and 1,8-cineole (0.09–1.54%). The results showed that the oil yield and the major constituents' percentage of the leaf were different at different stages of plant growth. The essential oils of *T.* Lamiaceae leaves were tested against five strains of Gram positive bacteria (g^+) and five strains of Gram negative bacteria (g^-). The average minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of essential oils were determined using agar dilution method against the organisms by agar dilution method.

1. Introduction

Even today, the number of plants that have been extensively studied is relatively very few and the vast majority has not been studied at all. Investigations of aromatic and medicinal plants enable finding plants producing effective essential oils that have already found a considerable range of applications (Mohammed and Al-Bayati, 2009). Traditional medicine is widespread globally and it is the almost exclusive source of primary health care for 65% of the world's population (Nezhadali et al., 2010a,b,c). Herbal remedies have long-standing roles in

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treating disease, and in developing countries are widely taken by and administered to women by traditional birth attendants during pregnancy. In the last decade, ethno pharmacological studies showed that herbal remedies have been employed routinely during pregnancy and childbirth (Johns et al., 1990).

The essential oils possess antibacterial, antifungal, antiviral, antioxidant and wide spectrum of pharmacological activities. These properties of essential oils are used in pharmacy and food industry. The essential oils became official drugs in many countries and have been documented in their pharmacopoeias. The essential oils have found the widest use in the treatment of infectious pathologies of the respiratory and gastrointestinal systems, urinary tract as well as at various skin diseases. Various species of the *Thymus* genus were reported to be strongly antibacterial, antifungal and antioxidant activities. These properties depend on the essential oil composition. Thyme oils are listed in pharmacopoeias of Europe, Germany and United Kingdom and used as natural preservatives in the food industry. The volatile components are important in determining the biological activity of *Thymus* species. *T.* is a perennial herb of Lamiaceae family. It has dark green leaves with light mauve-pink flowers in early Summer (Nezhadali et al., 2010a,b,c). Thymol, , which is the principal constituent of thyme oil have been reported to act as antioxidant, antimicrobial agent, antifungal agent treatment for respiratory tract diseases, wound healing, a stomachic carminative, diuretic, urinary disinfectant and vermifuge. The composition and quantity of essential oil from a particular species of thyme plant could be markedly affected by harvesting season, geographical environment and other agronomical factors. The beneficial effects of thyme are well known from ancient times and consumption of its extract is recommended all over the world. It is considered as the main ingredient of many phytopreperations and commonly used as water extracts for its pharmacological activities and thus, have a very important role in phytotherapy. Recently, Thyme has become one of the most important medicinal plants used as a natural additive in poultry and livestock feeding studies. Such studies showed that thyme plant could be considered as an alternative natural growth promoter for poultry instead of antibiotics (Abu-darwish and Abu-dieyeh 2009). The market demand for thyme is rather high, yearly estimates running at about 500 tones in USA and 1000 tones in Europe. Owing to a general popularity of the use of natural substances instead of synthetic compounds, an increase in that demand is predictable. The yield of plant material, the essential oil content and quantitative composition of *Thymus vulgaris* can be influenced by harvest time, ecological and climatically conditions. It has been reported that a fairly tight correlation exists between the soil type and the chemotypic structure of the thyme population growing on it. Where the soil type varies, distinct differences among chemotypes can be found over a few meters. Since the altitude can also be considered as a major factor influencing the physiological and chemical responses of plants, a correlation is attempted between the altitude where aromatic plants occur and their yield in essential oils. However, relatively high values of yield occur in a wide range of altitudes, from 600 to 1900 m (in Greece). Concerning the other chorological groups, it was found that the range of essential oil yield is fairly limited. For Balkan and Eurasiatic elements, in particular, yield does not seem to be related to altitude as in the case of the Mediterranean elements. Knowledge of the factors such as genetic or environmental that influence the essential oil content is insufficient, and the same holds for the role they exerted and now exert in the ecological complex. Region and altitude seems to play a role in the case of oil rich and oil-intermediate aromatic plants, affecting their essential oil content. It does not seem to influence oil poor plants. Drug yield, essential oil content and composition in *T.* plants showed big variation from years to years because of perennial plants.

In this study we investigated chemical variation of the leaves essential oil at different stages of plant growth and *in vitro* antibacterial activity of *T.* Lamiaceae.

2. Material and methods

2.1. GC/MS conditions

The essential oil composition was analyzed using a Shimadzu QP5050 GC/MS with DB-5 capillary column. The analysis program and conditions were as follows: helium at 1.7 mL/min as a carrier gas; the injection volume was 0.1 μL; ionization potential, 70 ev; the initial temperature of the column was kept at 60 °C (an usual temperature in the analysis of herbal plants) for 1 min and programmed to 140 °C at a rate of 3 °C/min and kept constant at 250 °C (column cleaning step) for 3 min.

2.2. Quantification and identification

N-alkenes mixture was performed under the GC/MS temperature condition program to calculate the Kovat's indexes (RI). These observations were further supported by mass spectral data, literature, and NIST computer library which confirmed the identity of each component. The relative percentage of the oil constituents was calculated.

2.3. Essential oil extraction

The plant sample after collected was transferred to the herbarium of Botanical Research Center of Payame Noor University, Mashhad, Iran. The plant was identified using valid references such as botanical Flora Iranica (Rechinger, 1998) and Flora of Iran (Mozaffaryan, 1999). The leaves of *T. vulgaris* Lamiaceae, at different stages of plant growth were collected from Barzo Mountain Shirvan (North Khorasan Province of Iran) at altitude of 1700 m in June–July 2010 (four samples). The collected samples on 6th-June-2010, 13th-June-2010, 3rd-July-2010, and 16th-July-2010 were named S_1, S_2, S_3, and S_4 respectively. The plant leaves were dried at room temperature in a shadow place for 6 days. The leaves of each stage (45 g) were hydrodistillated in a Clevenger-type apparatus for 4 h according to the method recommended in the British Pharmacopoeia. The extracted oil was deoxygenated under nitrogen gas and stored in a sealed vial at low temperature until the analysis time.

2.4. Antimicrobial activity

2.4.1. Disk diffusion assay

The used bacterial strains, which were in this study, were obtained from the Persian Type Culture Collection (PTCC). The