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Influence of saponin plants on the volatile fraction of thyme in herbal teas

Christine Tschiggerl, Franz Bucar *

Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitätsplatz 4/1, A-8010-Graz, Austria

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ABSTRACT

Combinations of thyme, cowslip and liquorice roots are often used in the treatment of upper respiratory tract infections. Therefore the volatile fraction of herbal teas prepared from thyme (*Thymus vulgaris*) and the effect of combining with cowslip (*Primula veris*/*P. elatior*) and liquorice roots (*Glycyrrhiza glabra*) on the volatiles were analyzed. Volatile compounds were isolated by hydrodistillation and solid phase extraction and analyzed by GC-MS. Thymol was also quantified by HPLC. The total amount of volatiles as well the thymol content was decreased with increasing proportions of cowslip or liquorice in the infusion extracts whereas the proportion of monoterpene hydrocarbons increased.

1. Introduction

Herbal teas are among the most commonly consumed beverages all over the world. Many herbs such as thyme which beneficial effects are mainly attributed to the volatile constituents are used for the preparation of infusions. The traditional use of *Thymus vulgaris* L.(Lamiaceae) leaves or herbs as tea in the treatment of bronchial catarrh, catarrh of the upper respiratory tract and supportive treatment of pertussis was approved by the former German Commission E. Some further therapeutic indications are stomatitis and halitosis [1]. Besides its application in phytotherapy, also the non-medicinal use of thyme as preservative for food and as an aromatic ingredient for seasoning various dishes is of importance [2,3]. The most characteristic constituents of the herb especially of the leaves are essential oil, flavones, rosmarinic acid, triterpenes and carbohydrates [1,4]. The essential oil composition has been frequently reported. On the basis of the major oil components various chemotypes have been described within *T. vulgaris*. Constituents in *T. vulgaris* essential oil (TEO) also vary depending on origin [5–9]. TEO is known to be beneficial in the internal treatment of different diseases similarly to thyme tea, however, the external use is more common [4]. Antimicrobial activity, antitussive, expectorant, antispasmodic actions and antioxidative effects were recognized as pharmacological actions of TEO [10–14]. Among the constituents of TEO which contribute to its antioxidative and antibacterial activities are the phenols thymol and carvacrol [13,15,16]. Antioxidant properties of thyme infusions have also been reported [17]. Although widely used in European folk medicine as well as for food purposes, the aromatic composition of infusions prepared from thyme has not been investigated so far. In addition, we wanted to explore the influence of mixtures with herbal drugs containing saponins, naturally occurring surfactants and emulsifying agents which might help to solubilize essential oil constituents in aqueous extracts, on the qualitative and quantitative composition of the volatile fraction of thyme infusions.

In this study, the volatiles of thyme infusion extracts were isolated by two different methods, hydrodistillation (TIH) and SPE (TIS). The qualitative and semi-quantitative composition of the essential oil isolated from herbal teas as well as the corresponding genuine thyme essential oil (TEO) was determined. Additionally, variable amounts of cowslip roots

Abbreviations: TEO, *Thymus vulgaris* essential oil; TIH, volatile fraction of thyme infusion obtained by hydro distillation; TIS, volatile fraction of thyme infusion isolated by SPE; GC-MS, gas chromatography—mass spectrometry; SPE, Solid phase extraction; bp, boiling point.

* Corresponding author. Tel.: +43 316 380 5531; fax: +43 316 380 9860.
E-mail addresses: christine.tschiggerl@edu.uni-graz.at (C. Tschiggerl), franz.bucar@uni-graz.at (F. Bucar).

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(roots and rhizomes of *Primula veris* L. and/or *P. elatior* L., Primulaceae) and liquorice root (roots, rhizomes and stolons of *Glycyrrhiza glabra* L., Fabaceae) were combined with fixed amounts of thyme and herbal teas were prepared by infusion. Both, cowslip and liquorice, are often used in various tea mixtures in combination with thyme in the treatment of upper respiratory tract infections such as cough and bronchial catarrh as recommended by the former German Commission E [12]. Previously, the influence of different saponins on the water solubility of compounds like cholesterol, digitoxin, quercetin and aesculin which are poorly soluble in aqueous solutions were investigated [18,19]. Physicochemical properties of saponins were recently reviewed [20]. The synergistic secrectolytic and secretomotoric properties of thyme and primula root have been shown in a clinical study together with the suppression of the release of interleukin-8 in human monocytes [21].

2. Experimental

2.1. Plant material

Commercial samples of *T. vulgaris* leaves (origin: Poland; chemotype: thymol), *Liquiritiae* radix and *Primulae* radix were obtained from a commercial supplier, Mag. Kottas, Vienna (Austria). The materials complied with the monographs of the European Pharmacopoeia (Ph. Eur.) [22]. According to GC-MS analyses of essential oil (see Table 1) the thymol chemotype could be confirmed. Voucher specimens are kept at the Department of Pharmacognosy, Institute of Pharmaceutical Sciences, University of Graz.

2.2. Chemicals

All reagents and solvents used were of analytical or HPLC grade. Solvents and materials were purchased from the following suppliers: *n*-hexane (Fluka, Buchs, Switzerland); methanol, trifluoroacetic acid (Merck, Darmstadt, Germany); methylene chloride, sodium sulphate, acetic acid, ammonia (Roth, Karlsruhe, Germany); acetonitrile (Sigma Aldrich, Steinheim, Germany), water was purified by nanopure filtration prior to HPLC use (Millipore, Billerica, Massachusetts, USA). Authentic standards for GC-MS analyses were obtained from different suppliers: Fluka, Aldrich, Roth and Dragoco (Holzminden, Germany). The *n*-alkanes C8–C26 for the determination of the linear retention index were from Sigma, USA. Isolute C18 (EC) columns 1 g (15 ml) from Biotage (Uppsala, Sweden) were used as SPE cartridges.

2.3. Gas chromatography–mass spectrometry

The composition of the essential oil and the volatile compounds of the infusion extracts were determined by GC-MS. Each sample was analyzed ternary. Analyses were performed using an Agilent 7890A GC system coupled with an Agilent 5975C MSD operating at 70 eV, ion source temperature 230 °C, interface temperature 280 °C. A split injection (split ratio, 80:1) at 240 °C injector temperature was utilized. Injection volumes were 1 μl. A fused silica capillary column of 5%phenyl–95%methylpolysiloxane (HP-5MS, 30 m × 250 μm × 0.25 μm, Agilent J & W, USA) was used. The temperature program was as follows: 2 min at 45 °C, then to 250 °C at 4 °C/min, finally held at 250 °C for 2 min. The carrier gas was helium 5.6 at a flow rate 0.9 ml/min. Data acquisition was performed with Agilent GC/MSD ChemStation Version E.02.00 for the mass scan range 40–300 u.

Compounds were identified by retention indices [23] and by comparing their mass spectra with spectral data libraries [23,24] and a laboratory own data base. Furthermore, for some compounds pure standard substances were available.

2.4. Essential oil hydrodistillation

Hydrodistillation procedures were done according to the European Pharmacopoeia (Ph. Eur.) [22]. 30 g of *T. vulgaris* leaves were hydrodistilled for 2 hours. Similarly, the infusions (3000 ml) of thyme and its combination with cowslip and liquorice roots were also hydrodistilled for 2 hours immediately after preparation to avoid loss of volatiles. Five separate analyses were performed of each experiment. The essential oil samples were dried over anhydrous Na_2SO_4, stored in dark glass bottles at −20 °C until analysis. The oil samples were diluted with *n*-hexane (1:30) prior to GC-MS analysis.

2.5. Preparation of thyme infusions

Infusions were prepared according to literature [1]. Boiling distilled water (3000 ml) was poured onto thyme leaves (40 g), and the infusion was left to brew for 10 min. Then it was filtered and rinsed three times with distilled water and brought to a final volume of exactly 3000 ml. These high amounts of infusion were necessary to determine the quantitative essential oil volume with the Clevenger apparatus. Furthermore, thyme tea (40 g/3000 ml) was also prepared with various amounts of cowslip roots (10 g, 40 g, 120 g) and liquorice roots (10 g, 40 g, 120 g) and hydrodistilled.

For SPE and HPLC analyses of thymol, infusion extracts with 150 ml boiled water and 2 g thyme leaves and addition of various amounts of cowslip roots (0.5 g, 2 g, 6 g) and liquorice roots (0.5 g, 2 g, 6 g) were prepared in the same way as for hydrodistillation. SPE experiments were done in triplicate, HPLC experiments were duplicated.

2.6. Solid phase extraction (SPE) of the infusion

The method was adapted from literature [25]. A 1 g C18 (EC) solid phase extraction cartridge was conditioned twice with 8 ml methylene chloride and twice with 8 ml methanol. Thereafter, 8 ml of distilled water were passed through the cartridge twice and not allowed to dry before the filtered infusion (150 ml) was loaded onto the cartridge with a flow of 1–2 ml/min. The compounds retained on the SPE column were eluted into a 5 ml graduated flask with exactly 5 ml of methylene chloride for qualitative and semi-quantitative analyses. For quantitative analyses the compounds were eluted into a 10 ml volumetric flask with exactly 10 ml of methylene chloride, which contained the internal standard, linalyl acetate (200 μg/ml). Pure methylene chloride was added to a final volume of 5 ml and 10 ml, respectively. Three to five separate determinations were performed. These samples were stored in glass bottles at −20 °C until they were used for GC-MS analysis.