Optimization of carvacrol, rosmarinic, oleanolic and ursolic acid extraction from oregano herbs (Origanum onites L., Origanum vulgare spp. hirtum and Origanum vulgare L.)

Juste Baranauskaitė¹, Valdas Jakštas², Liudas Ivanauskas³, Dalia M. Kopustinskiene⁴, Gailutė Drakšienė⁵, Ruta Masteička⁶ and Jurga Bernatoniene⁷*

¹Department of Drug Technology and Social Pharmacy, Lithuanian University of Health Sciences, Eivenių str. 4, LT-50161, Kaunas, Lithuania; ²Department of Pharmacognosy, Lithuanian University of Health Sciences, Kaunas, Lithuania; ³Department of Drug Chemistry, Lithuanian University of Health Sciences, Kaunas, Lithuania; ⁴Department of Pharmaceutics, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

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The aim of our study was to increase the extraction efficiency of carvacrol, rosmarinic, oleanolic and ursolic acid from the different species of oregano herbs (Origanum onites L., Origanum vulgare spp. hirtum and Origanum vulgare L.). Various extraction methods (ultrasound-assisted, heat-reflux, continuous stirring, maceration, percolation) and extraction conditions (different solvent, material:solvent ratio, extraction temperature, extraction time) were used, and the active substances were determined by HPLC. The lowest content of carvacrol, rosmarinic, oleanolic and ursolic acid was obtained by percolation. During heat-reflux extraction, the content of active substances depended on the solvent used: ethanol/non-aqueous solvent (glycerol or propylene glycol) mixture was more effective compared with ethanol alone. The results showed that for each species of oregano the most optimal extraction method should be selected to maximize the content of biologically active substances in the extracts.

Keywords: Origanum; carvacrol; ursolic acid; oleanolic acid; rosmarinic acid; HPLC; extraction

1. Introduction

The genus Origanum (Lamiaceae) is rich in phenolic compounds with powerful antibacterial and antifungal properties (Govaris et al. 2010). Oregano essential oils including carvacrol (CA) have been shown to possess antioxidant, antibacterial, antifungal, diaphoretic, carminative,
antispasmodic and analgetic activities (De Falco et al. 2013). Antiviral, antibacterial, anti-inflammatory, antioxidant activities of oregano (Petersen & Simmonds 2003; Radusienė et al. 2008) are associated with its constituents rosmarinic acid (RA), ursolic acid (UA) and oleanolic acid (OA). The impact of extraction methods and different oregano species on the extraction yields of RA, UA, OA and CA has not been determined yet to our knowledge. Therefore, we evaluated them in this study using three species of oregano herb: Origanum onites L., Origanum vulgare spp. hirtum and Origanum vulgare L.

2. Results and discussion
The highest amount of RA from *O. vulgare* spp. *hirtum* was obtained by heat-reflux extraction. This method was by $1.2 \pm 0.02$ times more effective (Supplementary Table 1) than the traditional ultrasound-assisted extraction (UAE) (Jäger et al. 2009). The extracted maximal RA amount from *O. vulgare* L. by heat-reflux and from *O. onites* L. by continuous stirring was respectively by $3.85 \pm 0.03$ and $2.2 \pm 0.01$ times lower ($p < 0.05$) compared with *O. vulgare* spp. *hirtum* species. Radusienė et al. (2008) showed that RA content in *O. vulgare* L. extract prepared by 72 h maceration with 96% of ethanol as solvent ranged within 0.99–9.65 mg/g dry weight: 1.11–7.42 mg/g from leaves and 0.53–0.77 mg/g from stems. This content was 4–9 times lower compared with the RA content identified in our extracts from *O. vulgare* L. herb. UA and OA are pentacyclic triterpenoids of similar chemical structure (Zhou et al. 2007). The highest amounts of these compounds were extracted from *O. vulgare* spp. *hirtum* by 48 h maceration (Supplementary Table 1). The highest extracted UA amount from *O. onites* L. using UAE and from *O. vulgare* L. using heat-reflux method was respectively by $1.9 \pm 0.02$ and $6.6 \pm 0.11$ times lower ($p < 0.05$) compared with the corresponding extracts from *O. vulgare* spp. *hirtum* (Supplementary Table 1). In the study of Jäger et al. (2009), only OA traces were determined, while UA content was 2.8 mg/g dry matter in *O. vulgare* L. extracts. In our study, we identified lower amounts of UA and OA in *O. vulgare* L. herb extract (Supplementary Table 1), possibly due to the differences in the plant growing conditions, chemical composition of herbal preparations or extraction methods.

The highest CA content was obtained from *O. onites* L. by 24 h maceration and from *O. vulgare* spp. *hirtum* – by UAE and continuous stirring extraction (Supplementary Table 1). The latter method was also efficient for CA isolation from *O. vulgare* L., however, in these conditions the CA amount depended on the oregano species: it was by $3.48 \pm 0.02$ and $1.1 \pm 0.05$ times lower ($p < 0.05$) in *O. vulgare* L. compared with *O. onite* L. and *O. vulgare* spp. *hirtum*, respectively. Başer (2008) identified carvacrol as the main compound responsible for oregano biological activities. Therefore, it could be essential to optimize the maximal CA extraction yield.

The present study revealed that different types of extraction methods had a big influence on the extraction yield of compounds. The effects of extraction parameters, i.e., extraction time, temperature, agitation speed and solvent to solid ratio were very important for the diffusion of biologically active compounds from the raw material to the extracts. Heat-reflux extraction and continuous stirring extraction gave significantly higher values, while percolation, maceration and UAE extraction yielded lower values. Based on the extraction time, heat-reflux extraction was the fastest extraction method. Based on the extraction efficiency, it was similar to continuous stirring extraction. In addition, the disadvantages of the extraction by percolation were low extraction efficiency and long extraction time.

Further, we tested the influence of non-aqueous solvent on the amounts of OA, UA, RA and CA extracted from oregano herbs. Zacchigna et al. (2014) determined that polyethylene glycol and UA conjugates could increase UA content in plant-derived extracts. Our results (Supplementary Table S2) showed that ethanol/propylene glycol (10–30%) solvent mixture as
well as ethanol/glycerol (1–20%) solvent mixture increased only the RA and CA extraction yield by 1.4 ± 0.3 and 1.2 ± 0.1 times (p < 0.05) compared with the control extract. Ethanol/glycerol (10%) solvent mixture increased CA extraction yield by 1.1 ± 0.08 times (p < 0.05) compared with ethanol/propylene glycol solvent mixture (Supplementary Figure S1). A binary solvent system is more efficient compared with a mono-solvent system in the extraction of phenolic compounds in regard to their relative polarity (Dent et al. 2013). Thus, the observed effects could be related to the polarity of the investigated compounds (RA > CA > UA > OA).

3. Conclusions

CA, RA, OA and UA extraction from oregano herbs depended not only on the oregano species (O. vulgare L., O. onites L. ir O. vulgare spp. hirtum), but also on the extraction method used for the study. The lowest amounts of CA, RA, OA and UA were extracted by percolation. Ethanol/non-aqueous solvent (glycerol or propylene glycol) mixture instead of ethanol alone resulted in significantly higher extraction yields of CA, RA and UA during heat-reflux extraction from O. onites L., therefore, it could be important for increasing the content of biologically active substances in the herbal preparations.

Supplementary material

Experimental materials relating to this article are available online.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Başer KH. 2008. Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. Curr Pharm Design. 14:3106–3119.
De Falco E, Mancini E, Roscigno G, Mignola E, Tagliatela-Scafati O, Senatore F. 2013. Chemical composition and biological activity of essential oils of Origanum vulgare L. subsp. vulgare L. under different growth conditions. Molecules. 18:14948–14960.
Dent M, Dragovic-Uzelac V, Penic M, Brncic M, Bosiljkov T, Levaj B. 2013. The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in Dalmatian wild sage (Salvia officinalis L.) extracts. Food Technol Biotechnol. 51:84–91.
Govaris A, Solomakos N, Pexara A, Chatzopoulou PS. 2010. The antimicrobial effect of oregano essential oil, nisin and their combination against Salmonella enteritidis in minced sheep meat during refrigerated storage. Int J Food Microbiol. 137:175–180.
Jäger S, Trojan H, Kropp T, Laszczyk MN, Scheffler A. 2009. Pentacyclic triterpene distribution in various plants - rich sources for a new group of multi-potent plants extracts. Molecules. 14:2016–2031.
Petersen M, Simmonds MS. 2003. Rosmarinic acid. Phytochemistry. 62:121–125.
Radusiene J, Ivanaukas L, Janulis V, Jakstas V. 2008. Composition and variability of phenolic compounds in Origanum vulgare from Lithuania. Biologija. 54:45–49.
Zacchigna M, Cateni F, Drioli S, Procida G, Altieri T. 2014. PEG-ursolic acid conjugate: synthesis and in vitro release studies. Sci Pharm. 82:411–421.
Zhou C, Chen K, Sun C, Chen Q, Zhang W, Li X. 2007. Determination of oleanolic acid, ursolic acid and amygdalin in the flower of Eriobotrya japonica Lindl. by HPLC. Biomed Chromatogr. 21:755–761.