

Extraction of sweet wormwood (*Artemisia annua* L.) by supercritical carbon dioxide

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In this study, supercritical carbon dioxide was applied for the extraction of *Artemisia annua* L. Moreover, the impact of different parameters of supercritical extraction on total yield was investigated and the obtained yields were compared to the ones obtained by conventional procedures, hydrodistillation and Soxhlet extraction. Supercritical fluid extraction at 40 °C and different pressures (100, 200, and 300 bar) resulted in extraction yields that were in the 2.23-5.18 % range, while the yields at 60 °C and the same pressures were in the range 2.43-3.35 %. The yields obtained by the Soxhlet extraction and hydrodistillation were 10.28 % and 0.10 %, respectively. Although supercritical fluid extraction is inferior to Soxhlet extraction in terms of the recovery of lipophilic components of *A. annua*, the Soxhlet extraction is not the method of choice for obtaining sweet wormwood extracts due to its numerous disadvantages that include the use of toxic solvents, extended extraction time, health safety of the product, the need for further processing, the impossibility of adjusting the selectivity and thus the composition of the product.

Key words: *Artemisia annua* L., supercritical extraction, Soxhlet extraction, hydrodistillation

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1. INTRODUCTION

The genus *Artemisia* belongs to the large family of Asteraceae, encompassing more than 300 species. *Artemisia annua* L., commonly known as sweet wormwood (SW), indigenous to South-east Asia, is an annual herb, which has become cultivated as a medicinal plant in many parts of Asia, Africa, Europe, America, and Australia (Wright, 2005). *Artemisia* spp. are rich in secondary metabolites, which may elucidate their use as bittering agents, perfumes, culinary spices, and hallucinogens (Ferreira et al., 1997; Watson et al., 2002). Apart from *A. annua*, only two other species, *A. apiaceae* and *A. lancea*, have been shown to possess artemisinin (Tan et al., 1998), which is widely used as a single compound anti-malarial drug in combination therapies and approved by The United States Food and Drug Administration (Graziose et al., 2010). In addition, artemisinin, with the help of the endoperoxide group, shows promising anticancer activities when tested *in vitro* and *in vivo* (Efferth et al., 2001; Moore et al., 1995; Singh and Lai, 2001). Artemisinin is a sesquiterpene lactone and its concentration in *A. annua* varies from 0.01 to over 2.0 % in some selected cultivars. Therefore, a great deal of work has been focused on the cultivation and optimization of high artemisinin-yielding cultivars of *A. annua* (Ferreira et al., 1997; Laughlin et al., 2002).

Some studies have reported insecticidal effects of *A. annua* extract including growth retardation, as well as antifeedant and larvicidal effects (Haghighian et al., 2008; Hasheminia et al., 2011; Shekari et al., 2008). Khosravi et al. (2010) showed strong antifeedant activity of this plant extract against *Glyphodes pyralis* larvae and significant effects on nutritional indices and biochemical parameters. In addition to antimalarial activity, extracts obtained from *A. annua* have been used to induce hair growth, to promote longevity, as a food additive, as an anti-inflammatory, as well as a treatment for numerous external ails including hemorrhoids, lice, and ulcers (Hsu, 2006).

Therefore, *A. annua* represents a raw material with great pharmacological potential. Apart from its most investigated compound artemisinin, numerous other classes of phytochemicals have also been isolated from *A. annua* including monoterpenes, sesquiterpenes, triterpenoids, steroids, various lipids, flavonoids, coumarins, and other polyphenolics (Bhakuni et al., 2002). Hence, further investigation of the potential of *A. annua* is necessary.

A very important aspect that dictates the application, market competitiveness, and acceptance of products on the basis of *A. annua* is the form of the product, that is, their production procedure. Well-established conventional methods of extrac-

tion of *A. annua* include the application of non-polar organic solvents under reflux conditions (Lapkin et al., 2006). Considering the unfavorable ecological and health aspects of these production procedures, and the rising aspiration of the industry to implement green technologies and meet stringent environmental regulations, processes that offer an environmentally safe production procedure and quality products are highly required.

One green modern technology that was established as a method of choice for obtaining lipophilic extracts is the supercritical carbon dioxide extraction (SC-CO₂). Due to favorable conditions for achieving supercritical conditions, as well as the low price, availability and safety of application of CO₂, the possibility of selective extraction and fractioning, SC-CO₂ is more present in the industry. The additional advantage is the attainment of extract free from the presence of solvents, hence, purification of extracts is not necessary. Considering the quality of the product, the safety of the process, and market competitiveness, the initial purchase of equipment represents a worthy investment (Nađalin et al., 2014; Sahena et al., 2009; Vladić et al., 2016). In this study, the aim was to compare the efficiency of SC-CO₂ and conventional extraction methods of *A. annua* in terms of extraction yield and to determine optimal pressure and temperature for obtaining the highest yield. The impact of different densities of SC-CO₂ on extraction yield was investigated.

2. MATERIALS AND METHODS

2.1. Plant material

The aerial parts of *A. annua* L. grown on the experimental field in Bački Petrovac, within the Institute for Organic Production and Biodiversity, Novi Sad were used in this study. The dried *A. annua* was grounded in a domestic blender and the mean particle size (0.35 mm) of herbal material was determined using vibration sieve sets (CISA, Cedacteria, Spain). Moisture content of the herbal material was 7.86 %.

2.2. Chemicals

Commercial CO₂ (purity of 99.9 % w/w) (Messer, Novi Sad, Serbia) was used for laboratory supercritical fluid extraction. All other chemicals were of analytical reagent grade.

2.3. Hydrodistillation

The content of essential oil in SW was determined by the Clevenger apparatus following the official method of hydrodistillation prescribed by Ph. Jug. IV (1984).

2.4. Soxhlet extraction

Soxhlet extraction of SW was conducted with methylene chloride as a solvent (solid to liquid ratio 1:15 (g/mL)). The extractions lasted until the plant material was completely exhausted (8 h). After the extraction was complete, the solvent was removed from the extract by evaporation on a rotary vacuum evaporator to dryness, and the extraction yield (% w/w) was determined.

2.5. Supercritical carbon dioxide extraction (SC-CO₂)

The laboratory-scale high pressure extraction system (HPEP, NOVA, Swiss, Effertikon, Switzerland) was used for the extraction process. The SC-CO₂ extraction of SW was conducted at pressures 100, 200, and 300 bar at two temperatures, 40 and 60 °C. Other parameters were kept constant: extraction time 3 h and CO₂ flow rate 0.194 kg/h. The separator conditions maintained constant at 15 bar and 23 °C.

Table 1. Extraction yield of SC-CO₂ extraction of sweet wormwood

Pressure [bar]	Temperature [°C]	Density [kg/m ³]	Yield [%]
100	40	628.7	2.23
200	40	839.9	3.94
300	40	910.0	5.18
100	60	290.0	2.43
200	60	723.8	3.06
300	60	830.0	3.35

3. RESULTS AND DISCUSSION

3.1. Conventional extraction methods

The yield of essential oil generally varies between 0.3 and 0.4 % (v/w) (Holm et al., 1997; Woerdenbag et al., 1993). The hydrodistillation of SW provided an essential oil content of 0.1 %. The volatile components of SW are mainly attributable to essential oils with the content being 0.2-0.25 % (WHO, 2006). Khangholi and Rezaeinodehi (2008) investigated the effect of drying temperature on SW essential oil content and composition. The results demonstrated that higher drying temperatures decreased the essential oil content from 1.12 % at room temperature to 0.50 % and 0.37 % at temperatures 55 °C and 65 °C, respectively. Using methylene chloride as a solvent in Soxhlet extraction of SW, provided a yield of lipophilic components of 10.28 %. The cause of the significant difference in yields achieved by hydrodistillation and Soxhlet extraction lies in the fact that hydrodistillation extracts only volatile compounds, that is essential oil. On the other hand, the Soxhlet extraction with organic solvents extracts not only volatile compounds, but also all other lipophilic compounds such as waxes, pigments, and albuminous materials (Danh et al., 2013). Mohammadian et al. (2016) used methanol as a solvent in Soxhlet extraction of SW aerial parts (20 h) and obtained a yield of 11.69 %. Eteng et al. (2013) subjected SW leaves to Soxhlet extraction at 17-20 °C for 48 h using 98 % ethanol and obtained a yield of 14 %. Additionally, the Soxhlet extraction of SW leaves with hexane yielded 7.28 % (Quispe-Condori et al., 2005). The differences in contents could be attributed to the application of different solvents and different geographical origins of the plant, the plant's development stage, climatic conditions, drying and storage methods, and particle size.

3.2. Influence of process parameters of SC-CO₂ on extraction yield

SC-CO₂ was applied to obtain SW extracts at 100, 200, and 300 bar and temperatures of 40 and 60 °C, i.e. densities of CO₂ in the range 290-910 kg/m³. After 3 h of SC-CO₂ extraction at 40 °C and different pressures (100, 200, and 300 bar) extraction yields were in the range 2.23-5.18 % (Table 1 and Figure 1). The lowest yield was obtained at 100 bar, while the application of 300 bar provided a 2.3-fold higher yield. With the increase of pressure at a constant temperature, an increase in the extraction yield was observed, which is in agreement with the statement that with increasing carbon dioxide density, its solvating power also increases (Jerković et al., 2017; Uddin et al., 2015).

Extraction yields obtained at 60 °C temperature and pressures 100, 200, and 300 bar were in the range 2.43-3.35 % (Table 1 and Figure 2). The lowest yield was obtained at 100 bar, while a 1.4-fold higher yield was obtained at 300 bar. By comparing the extraction yields at constant temperature and different pressures, it can be concluded that the highest yield was achieved

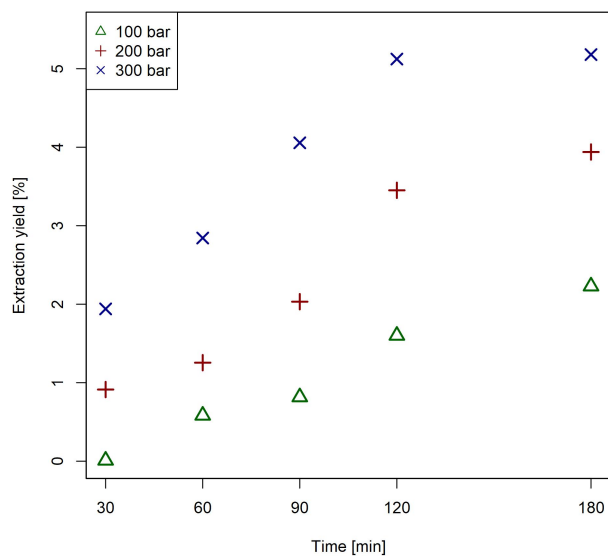


Fig. 1. Extraction kinetics of SC-CO₂ extraction of sweet wormwood at 100, 200, 300 bar and at 40 °C

at 300 bar and 40 °C. This data is important from the economic aspect as well because by using a lower temperature and investing a lower amount of energy compared to the extraction at 60 °C, a higher yield is provided. According to [Quispe-Condori et al. \(2005\)](#), maximum artemisinin global yield is achieved at 300 bar and 50 °C temperature. [Li et al. \(2017\)](#) showed that the extraction time and pressure had the highest positive effect on oil extraction, while the effects of CO₂ flow rate and temperature were less important. The extraction yield of 11.17 % was achieved at 250 bar, 50 °C, 25 g/min, and 2 h extraction time ([Li et al., 2017](#)). However, the range of investigated CO₂ flow was 10-30 g/min, which is a significantly higher CO₂ flow than the one applied in this study. Therefore, a significantly higher total yield than the yield obtained in this study could be caused by the higher CO₂ flow rate, as well as the difference in particle size.

Considering the yield obtained by the reference Soxhlet method, the SC-CO₂ extracted 50.38 % of the lipophilic fraction. Therefore it is necessary to further optimize the SC-CO₂ extraction to obtain higher yields. One approach for obtaining higher yields and better efficiency can be the inclusion of different pretreatments of the raw material such as increasing the moisture of the material, exposing the material to high pressure or ultrasound waves, or adding modifiers ([Tzeng et al., 2007](#); [Vidović et al., 2014](#)). [Tzeng et al. \(2007\)](#) showed that SC-CO₂ modified with 2 h ethanol was superior to 16 h Soxhlet hexane extractions in producing artemisinin. In addition, the amount of the extracts increased with the increase in the density of SC-CO₂.

Kinetics of SC-CO₂ of SW delivered two extraction periods, rapid and slow. During the rapid period, components that are easily soluble in SC-CO₂ are extracted. This period is characterized by a coefficient of rapid extraction which represents the amount of material obtained by rapid extraction. This coefficient is directly proportional to the degree of destruction of the plant material used for extraction. In the second extraction period, which is much slower, components with higher boiling points and higher molecular weights, such as fatty oils, waxes, and pigments are extracted. Mass transfer in the slow extraction period is done by slow diffusion ([Sovová, 2012](#)).

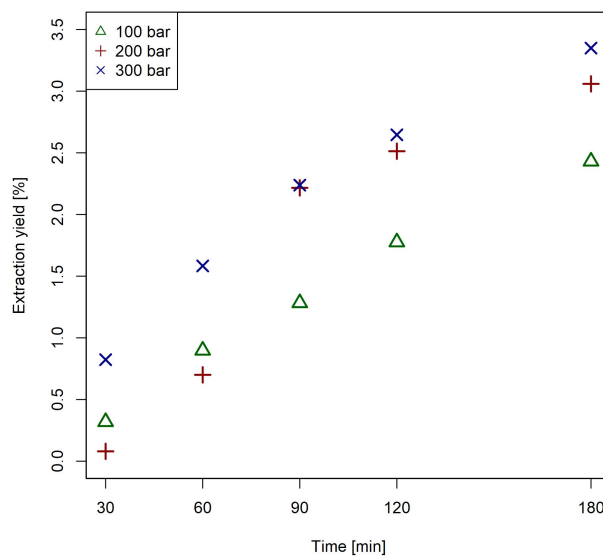


Fig. 2. Extraction kinetics of SC-CO₂ extraction of sweet wormwood at 100, 200, 300 bar and at 60 °C

CONCLUSION

Increasing environmental awareness of the concept of green production processes has imposed the need to implement environmentally friendly innovative processes in production plants. Therefore, SC-CO₂ can be classified as a promising alternative technology, which provides a quality product, with full integration into the concept of clean and environmentally friendly processes, thus overcoming one of the main disadvantages of this technology - high investment costs. The achieved results of this study demonstrate that SC-CO₂ did not result in the complete exploitation of the raw material and maximal extraction of lipophilic compounds. The referential Soxhlet method of extraction achieved an approximately two times higher yield. Therefore, further investigation is necessary to exploit the material more efficiently, as well as the chemical analysis of the obtained extracts.

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