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Research paper

Integrated extraction process to obtain bioactive extracts of *Artemisia annua* L. leaves using supercritical CO_2, ethanol and water

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ABSTRACT

Artemisia annua L. is an herb used in traditional Chinese medicine due to presence of biocompounds with biomedical and pharmaceutical applications. In this work, an integrated process for extraction of bioactive compounds present in *A. annua* leaves was performed. The process comprised two-step extractions: in the first step supercritical carbon dioxide ($scCO_2$) was used as a solvent, and from the solid residue of the supercritical extraction other extracts were obtained (second stage) using ethanol or water as solvents. Single-step extractions using ethanol or water as solvents were also performed for comparison. In all extracts the variables analyzed were overall extraction yield, the content and yield of total phenolic, total flavonoids and artemisinin, as well as antimalarial activity. The supercritical (SC) and ethanolic (E) extracts obtained in a single step showed the highest yields of artemisinin and were very active against *Plasmodium falciparum*, with IC_{50} values less than 0.1 $\mu g\,mL^{-1}$. On the other hand, the aqueous (SCA) and ethanolic (SCE) extracts from the second extraction step were free of artemisinin, but these extracts contained roughly 90 mg of phenolic compounds per gram of extract, including a high overall yield in the aqueous extract. The volatile fraction (SC-V) obtained from the supercritical extraction consisted mainly of camphor. Therefore, the two-step extraction in two steps proved to be advantageous because the residue of supercritical extraction could be used for obtaining aqueous or ethanolic extracts containing phenolic compounds.

1. Introduction

Artemisia annua L. is an annual herb used in traditional Chinese medicine for treating fever and malaria (Tzeng et al., 2007). Moreover, *A. annua* extracts exhibit a variety of biomedical and pharmaceutical applications and their presence is associated with antimicrobial, antioxidant and anti-inflammatory activities (Kim et al., 2015; Skowyra et al., 2014; Tajehmiri et al., 2014).

The chemical composition of *A. annua* consists of volatile and non-volatile constituents. The main non-volatile ingredients include sesquiterpenoids, flavonoids and coumarins. Among the sesquiterpenoids is artemisinin (Fig. 1) (Balint, 2001 Schwikkard and van Heerden, 2002), which represents the major bioactive compound present in leaves and flowers of *A. annua* (between 0.01% and 0.8% dry weight) (Van Agtmael et al., 1999) because it is responsible for the antimalarial activity of this specie.

Malaria is one of the major impact diseases in poor populations worldwide. According to the latest estimates of World Health Organization (WHO), there have been 214 million cases and 438,000 deaths up until September 2015 (WHO, 2015). Malaria is an infection caused by unicellular parasites (genus *Plasmodium*) that enter the blood via the Anopheles mosquito bite. WHO declaring artemisinin as a component of ACT (artemisinin based combination therapies) for malaria treatment (WHO, 2006). The high cost of the chemical synthesis of artemisinin on a large scale (Covello, 2008), in addition to the fact that *A. annua* leaves are the only natural source of this molecule, makes the study and improvement of artemisinin extraction processes from *A. annua* very interesting (Schwikkard and van Heerden, 2002).

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Table 1
Artemisinin in extracts of *A. annua* leaves obtained by different extraction methods.

Extraction type	Solvent	Conditions	Artemisinin recovery (mg/g leaves)	Reference
Sohxlet	hexane	–	9.7	(Lin et al., 2006)
	hexane	–	7.7	(Quispe-Condori et al., 2005)
	n-hexane, petroleum ether	60–80 °C	6.0–6.2	(Misra et al., 2013)
	water, methanol, ethyl acetate, *n*-hexane and ethanol	2–20 h	1–12	(Tzeng et al., 2007)
Supercritical extraction	CO_2	300 bar and 50 °C	7	(Quispe-Condori et al., 2005)
	CO_2	150 bar and 30 °C	6.2	(Quispe-Condori et al., 2005)
	CO_2	17.3–31.1 MPa/40–60 °C	2.1–6.7	(Lin et al., 2006)
	CO_2	100 bar and 40 °C	~9.5	(Della Porta et al., 2004)
	CO_2 + ethanol 16.25%	17.3–31.3 psig/40–60 °C	7.8–11.5	(Tzeng et al., 2007)
	CO_2 + ethanol (1, 3 and 5%)	150 bar, 50 °C	~8	(Kohler et al., 1997)
	CO_2 + EtOH (20%)	4500 psig and 50 °C	6.7	(Lin et al., 2006)
	CO_2 + *n*-hexane (16.25 wt.%)	7.0–20.8 MPa/30–50 °C	1.4–8.8	(Lin et al., 2006)
	CO_2 + metanol (1, 3, 5 and 10%)	150 bar, 50 °C	~6	(Kohler et al., 1997)
	CO_2 + toluene (1, 3, 5 and 10%)	150 bar, 50 °C	~6.5	(Kohler et al., 1997)
	CO_2 + metanol-water (1, 3 and 5%)	150 bar, 50 °C	~7	(Kohler et al., 1997)
Ultrasound-assisted extraction (UAE)	hexane	25–45 °C/15–120 min	not reported	(Briars and Paniwnyk, 2013)
	ether petroleum	30–60 °C/120–300 W	4.2–7.4	(Zhang et al., 2014)
Microwave-assisted extraction (MAE)	Cyclohexane, *n*-hexane, petroleum ether, ethyl acetate, chloroform, acetone, methanol and acetonitrile	160 W and 60 s	1.8–6.6	(Misra et al., 2013)

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