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Efficient and selective green extraction of polyphenols from lemon balm

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ABSTRACT

Over the past years polyphenolic compounds have attracted much interest, thanks to the benefits that they provide when used in the formulation of functional foods. They have also been broadly investigated for their ability to act as free radical scavengers and suitable supplements in the prevention of many cardiovascular and neurodegenerative diseases. Lemon balm (*Melissa officinalis* L.) is well known not only for its culinary but also for its curative properties (sedative, spasmolitic, antitumoral, and antioxidant). Recent studies have shown that protocatechuic acid, caffeic acid, and rosmarinic acid (RA) are the most commonly found phenolic compounds in this plant. The present work uses nonconventional methods for the efficient extraction of dried lemon balm aerial parts. Ultrasound- and microwave-assisted extraction protocols were carried out, and their efficiency and selectivity have been compared. Dry extraction was also carried out on a rewetted vegetal material in a ball mill in the presence of β-cyclodextrin. Extraction yields, total phenols, and RA content have all been determined. RA was confirmed as being the main component of the phenolic fractions in all cases, whereas ethanol was the best solvent for both ultrasound- and microwave-assisted extraction procedures.
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1. Introduction

Natural antioxidants have been the focus of ever-growing amounts of interest in recent years because they have been linked to health benefits that reduce the risks of developing chronic diseases, such as cardiovascular disorders and cancer. Moreover, there appears a general perception among consumers that an intake of natural antioxidants is safer than synthetic analogs, meaning that the formulation of functional foods and nutraceutical products has become a hot topic.

Polyphenols possess well-known antioxidant capacity because of their ability to inhibit free radical generation, free radical scavenging activity, and the capability to chelate transition metal ions [1]. This activity, which is thought to be even stronger than that of vitamins C and E for many polyphenols [1,2], has led to increasing attention being paid to plants that are now being investigated both for their secondary metabolite composition and antioxidant activity [3].

Rosmarinic acid (RA) (Fig. 1) is a phenolic compound whose antioxidant activity has been well documented by a large amount of literature data, including in vivo studies, some of which regard their efficacy in reducing diabetes

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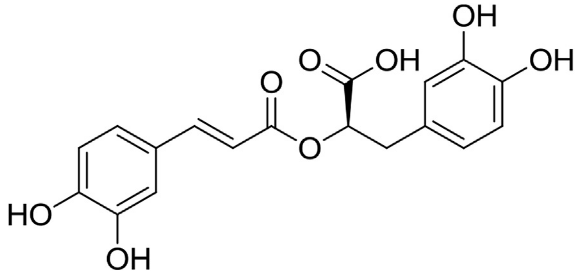

Fig. 1. Rosmarinic acid.

risks [4]. Although rosemary [5] is thought to be the main source of RA, the steady growth of pharmaceutical, cosmetic, and food products that contain this polyphenol has driven the search for other plants that bear high amounts of RA and, at the same time, the study of techniques that can provide rich extracts of this compound.

An alternative source of polyphenols is lemon balm (*Melissa officinalis* L.), an aromatic perennial herb belonging to the mint family, Lamiaceae. Well known for its culinary properties, this plant is used for a variety of cognitive purposes, most of which center around improving cognition and reducing stress and anxiety [6,7]. Recent evidence suggests that lemon balm possesses several other beneficial properties, such as antioxidant, antimicrobial, and antitumor effects [8,9]. Of the constituents present in this herb, it is mainly the phenolic fraction, and principally RA, that possesses the properties mentioned [10,11]. It has recently been highlighted that lemon balm extracts are safe to use as food supplements in healthy individuals [12] and that they can provide relief for heart benign palpitation in humans [13], confirming the interest toward this plant.

The intensification of the extraction process using highly efficient green techniques is currently a very attractive topic [14,15], as the community aims to produce extracts that are richer in bioactive compounds to be used in the formulation of functional foods and nutraceutical products. It is also generally accepted that phenolic compounds can suffer from thermal degradation, meaning that extended exposure to high temperatures, as occurs in some conventional solvent extraction methods, should be avoided.

Supercritical carbon dioxide extraction, ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) are efficient, unconventional techniques used for lemon balm treatment [10,16–18]. In particular, UAE and MAE are green technologies that could dramatically reduce extraction times and solvent volumes and at the same time increasing product yields and protecting the extract from thermal degradation [19–23].

Ince et al. [24] have recently compared the microwave (MW) and ultrasound (US) effects of phenolic extraction from lemon balm and found that MW extraction is the most advantageous in terms of yield and extraction time. Moreover, Nicolai et al. [25] have showcased a rapid and effective US-assisted process that is able to preserve the antioxidant activity of ethanolic *M. officinalis* L. extracts.

The stability, solubility, and bioavailability of natural bioactive compounds can be increased by cyclodextrins (CDs), polysaccharides that are well known for their ability to form inclusion complexes with several kinds of molecules [26]. This property has also been exploited to selectively extract bioactive compounds from plants [27]. The complexation of poorly water-soluble bioactive molecules in CDs can be achieved through solventless grinding in a ball mill, thus enhancing oligosaccharide and guest molecule interactions [28–30]. Mechanochemistry is, in fact, an enabling interdisciplinary research field that can present advantages in terms of extraction time, simplicity, and waste reduction [31]. These features could be exploited for the selective green extraction of natural compounds via the cogrinding of vegetal matrices with CDs in the solid state.

To use lemon balm extracts with high polyphenolic acid contents as natural additives in functional foods, extraction procedures that selectively enrich the product in the RA content would be useful [32–34], as they can limit or completely avoid the need for subsequent purification processes.

The present work evaluates the efficiency and selectivity of UAE and MAE dried lemon balm aerial part extraction processes by testing a variety of solvents. Pure ethanol, 70% ethanol, and pure water were chosen for MAE, whereas two sequences of solvents with increasing polarity were used for UAE: *n*-hexane (Hex), ethanol (EtOH), water (sequence 1); Hex, acetone (Ace), EtOH, water (sequence 2). We also investigated the dry extraction of a rewetted vegetal material in a ball mill in the presence of β-cyclodextrin (β-CD). Extraction yields, total phenols, and RA content were determined in the final extracts of each sample using an high-performance liquid chromatography-diode array detector (HPLC-DAD) method with the help of RA as the sole external standard.

2. Results and discussion

This work evaluates the efficacy of US and MW in the rapid and selective recovery of phenolic compounds from *M. officinalis* L. leaves. An extraction time of only 10 min and solvents with different polarities (Hex, Ace, and EtOH) were used in accordance with our previous experience with rosemary leaves [35]. Longer extraction times (20 and 30 min) did not improve extraction yields. Extraction efficiency was tested across a series of single-extraction step (MAE) and sequential procedures (UAE), carried out on the same sample batch. Yields, in terms of percentage of dried extract (DE) weight obtained over dried leaves (DL) weight for each sample, are reported in Table 1. Single step MAE, both in EtOH and EtOH/water 7:3 v/v, gave yields near 16%, whereas MW irradiation in pure water afforded the highest extraction yield (31.3%). The two US-assisted sequences started with apolar solvents and finished with water. Hex (UAE 1a and 2a) gave yields near 1%, acetone (UAE 2b) 1.83%, whereas higher yields (near 3.5%) were reached with EtOH (UAE 1b and 2c). The last step with water (UAE 1c and 2d), suitable for coextracting several polar components, showed the highest yields that ranged from 16% to 22%. The global extraction yield values for both the US-assisted sequences (26.5% and 22.7%, respectively, for UAE 1 and 2) fell between those of single step MAE in EtOH and water (around 16% and 31%, respectively), whereas ball mill extraction gave a lower

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