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# Selective recovery of rosmarinic and carnosic acids from rosemary leaves under ultrasound- and microwave-assisted extraction procedures

Maria Bellumori [a, b], Marzia Innocenti [a, b], Arianna Binello [c], Luisa Boffa [c], Nadia Mulinacci [a, b, **], Giancarlo Cravotto [c, *]

[a] Department of NEUROFARBA, Division of Pharmaceutical and Nutraceutical Sciences, Via U. Schiff, 6, 50019, Sesto F.no, Florence, Italy
[b] CeRA (Multidisciplinary Centre of Research on Food Sciences), Italy
[c] Dipartimento di Scienza e Tecnologia del Farmaco, University of Turin, Via P. Giuria 9, 10125, Turin, Italy

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ABSTRACT

Ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) are currently amongst the foremost green techniques for accelerating extraction processes. Several methods for the efficient recovery of the phenolic compounds from rosemary leaves have so far been proposed, but little data are available on the use of UAE and MAE. The aim of this work is to investigate the efficiency and selectivity of these techniques in recovering fractions of specific phenolic compounds from dried rosemary leaves using solvents that are suitable for food applications. UAE has been carried out by means of a probe system equipped with a titanium horn working at 19.5 kHz (140 W). MAE has been performed in a closed multimode reactor under $N_2$ (20 bar) at 100 °C. All obtained extracts were dissolved in a defined solvent volume and the solutions were directly analyzed using a combination of the HPLC-DAD-MS and TOF techniques. MAE and UAE in ethanol and acetone dramatically increased phenol yield (more than three times) as compared to more traditional solid–liquid extraction processes. In terms of selectivity, remarkably high rosmarinic acid content (6.8% of the dried extract) was obtained in ethanol under ultrasound (US). Even more impressive is the selectivity of UAE in *n*-hexane which gave the highest carnosic acid content, up to 13% of the dried extract. In conclusion, non-conventional energy sources and, in particular, high-intensity US have proven themselves to be rapid, efficient, and selective techniques for rosemary leaf extraction and provide fractions with high rosmarinic and carnosic acid contents.


## 1. Introduction

Rosemary (*Rosmarinus officinalis* L.) is a very rich source of bioactive phenols that are primarily responsible for the plant's high bioactivity, leading to its use in gastronomy and traditional medicine for centuries [1]. Rosemary extracts are used in a broad range of applications, including food preservation [2], nutraceuticals, phytomedicines [3], and cosmetics [4]. Consequently, the scientific community has shown great interest in attaining these bioactive extracts by means of efficient and ecological processes. *R. officinalis* L. (Labiatae), extensively found in Western Mediterranean countries, is well known for its many uses in the kitchen and for its pharmacological properties. Essential oil is obtained from the leaves of the plant which are also used

* Corresponding author.
** Corresponding author.
*E-mail addresses:* nadia.mulinacci@unifi.it (N. Mulinacci), giancarlo.cravotto@unito.it (G. Cravotto).

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to prepare phenolic extracts that are natural remedies for a number of common diseases [5]. Besides strong antioxidant activity, hydroalcoholic extracts are recognized to have choleretic, cholagogue, hepatoprotective, antitumor, and antiviral properties [6]. The biological activity attributed to these extracts is closely related to their phenolic fractions with rosmarinic and carnosic acids being the main constituents, together with several minor flavonoids.

Designing more efficient extraction processes, which can address the dual requirements of process intensification and energy savings, is currently becoming an increasingly important research topic. Safety, sustainability, environmental, and economic factors are all forcing industries to turn to non-conventional technologies and greener protocols [7].

UAE can be considered as an ecological process as it helps to greatly accelerate the extraction process and reduce energy consumption. Extraction enhancement using UAE has been attributed to the propagation of ultrasound (US) pressure waves, and the resulting cavitation phenomena. The method is clean and, thanks to low bulk temperatures and rapid execution, it helps to prevent thermal degradation phenomena; it usually leaves no residue in the extract and uses no moving mechanical parts. It also offers advantages in productivity, yield, and selectivity; it improves processing time, and enhances quality while reducing chemical and physical hazards [8]. Despite there being few reports in the scientific literature, industrial applications have been made available since the 1990s with batch reactors, from 100 up to 500 L, mainly used in the preparation of extracts for the phyto-pharmaceutical, cosmetic, and liqueur industries.

In recent years, MAE has also been the subject of significant research across numerous fields, but especially that of medicinal plants as its unique heating mechanism, moderate capital cost, and good performance under atmospheric conditions provide a variety of benefits [9]. The main advantage of MAE resides in the performance of its heating source. In addition to the base closed (sealed-vessel above atmospheric pressure) and open MAE systems [10], many modifications have been introduced to enhance performance over the last decade. Recent technological advances have led to dramatic improvements in analyte recovery and MAE reproducibility, making it an irreplaceable plant extraction tool.

Numerous extraction methods for the efficient recovery of phenols from rosemary leaves have so far been proposed [11]; however, little data on the use of UAE and MAE are currently available. The effect of various solvents and US on the extraction of carnosic acid from rosemary has, however, been investigated [12]; ethanol was significantly less effective than ethyl acetate and butanone in a conventional stirred extraction while US improved the relative performance of ethanol. High-intensity US may therefore reduce solvent dependence [13] and facilitate the use of alternative solvents with more attractive economic, environmental, health, and safety benefits.

Although microwaves have mostly been used in rosemary steam distillation [14], methanol/water, acetone/water, ethyl acetate/water, and ethanol/water mixtures have been proposed for total polyphenol recovery [15,16]. Dielectric heating has also been proposed as a means to dry rosemary leaves; this method minimizes the decrease in quality by providing rapid and effective heat distribution throughout the material [17]. MAE has more recently been compared with Soxhlet and US extraction methods; however, the paper only discussed total phenol content and not the different phenolic classes that are typically present in rosemary [18]. A comparison of ten different rosmarinic and carnosic acid extraction processes, which only use a 9:1 v/v ethanol/water mixture, has recently been published [19]. Intensified extraction processes at various extraction temperatures gave similar yields to conventional processes (heat reflux extraction and maceration). Carnosic acid was efficiently enhanced by UAE whereas MAE was more suitable for rosmarinic acid recovery.

The aim of this work is to investigate US potency in the rapid and selective recovery of the phenolic compounds in dried rosemary leaves, a material not suitable for recovering volatile terpenes, but that is still rich in powerful antioxidant compounds. Simultaneously, the efficacy of MW extraction with ethanol and water was preliminarily evaluated and compared with the US results. A very short process time (10 min) was applied to the recovery of the phenolic fractions from rosemary leaves. Furthermore, the aim of improving extraction yields and process selectivity was tackled using sequential UAE procedures and *n*-hexane, ethanol, acetone, and water as the only extraction solvents. Extraction yields and final dried extract (DE) quality were determined by HPLC/DAD, which measured dried extract weight over dried leaf weight (w/w%) and the phenolic content of the dried sample.

## 2. Results and discussion

### 2.1. Extraction yield and time

This work investigates US potency for the rapid and selective recovery of phenolic compounds from rosemary leaves. MW efficacy in this extraction has also been preliminarily evaluated. An extraction time of only 10 min was used to shorten the process and only solvents that are suitable for food applications were used. Extraction efficiency was evaluated across a series of single-extraction steps (UAE and MAE) and in some sequential procedures (only UAE) that were carried out on the same dried leaf batch. Table 1 compares yields, in terms of DE weight over dried leaves (DL) weight for each sample. As can be seen, the UAE and MAE single extraction steps showed similar results (values close to 18–21% w/w) in water and ethanol.

These values are in agreement with those recently obtained by Jacotet-Navarro et al. [19] who applied UAE and MAE to rosemary leaves after hydrodistillation; their work was carried out with an ethanol/water 9:1 v/v mixture, 30 min of extraction and a solid/liquid ratio of 20. The authors obtained final yields ranging from 13% to 18% which increased (up to 25%) at the higher extraction temperature of 150 °C. Our findings suggest that it is possible to obtain comparable DE yields after only 10 min extraction time and a lower solid/liquid ratio of 10. More lipophilic solvents, such as hexane and acetone, gave lower yields as is also the

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