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Rosemary Essential Oil Extraction and Residue Valorization by Means of Polyphenol Recovery †

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Abstract: The increasing demand for natural bioactive ingredients extracted from Aromatic and Medicinal Plants (AMPs) has produced disposal problems associated with residual solid waste. One of the main sectors interested in the exploitation of AMPs is the Essential Oils (EOs) industry. Despite EOs being the main commodity in the EOs industry, they only represent a small part of AMPs, generally less than 5% (*w*/*w*). This results in the production of a remarkable quantity of biomass that has no apparent commercial value and is therefore underestimated and underutilized by the EOs industry. Among AMPs, *Rosmarinus officinalis* L., commonly known as rosemary and belonging to the *Lamiaceae* family, is an aromatic plant endemic to the coastal area of the Mediterranean region but spread worldwide. Rosemary can be cultivated or grow wild as an ornamental evergreen shrub. Their leaves are usually used fresh or dried to flavor foods, mostly in traditional Mediterranean gastronomy, and recently, rosemary extracts were approved for use as food additives in Europe. The antioxidant activity of rosemary leaves has been acknowledged and is ascribed to EOs and polyphenolic compounds. To the best of our knowledge, the optimization of polyphenol recovery from rosemary residues after EO extraction has not yet been investigated. Hence, for the present study, EO extraction from rosemary leaves was performed using the hydro-distillation method, and the antioxidant (EC_{50}) and sun-protective (measured in SPF) activities were evaluated. The polyphenolic fraction was extracted from the rosemary residue under specific experimental variables. In particular, tests were conducted at different extraction times (15 min, 30 min, and 60 min), temperatures (25 °C, 40 °C, 50 °C, 60 °C, and 70 °C), and ethanol concentrations (50%, 60%, 70%, and 80%). In this study, an EO yield of 1.57% was obtained, and the EO had an EC_{50} value of 240.39 µL/mL and a SPF of 2.55. The maximum amount of polyphenols extracted from the rosemary residue was 24.14 mg GAE/g DW, achieved by using an 80% ethanolic solution at 70 °C for 60 min. This preliminary study reveals how the exploitation and consequential valorization of AMP solid waste may represent new answers for circular economy strategies adopted by European countries.

Keywords: rosemary; essential oil; waste valorization; polyphenols; green extraction; by-products

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1. Introduction

The annual production of essential oils (EOs) has surpassed 70,000 tons per annum, and it is estimated that about 65% of global production is supported by developing countries [1,2]. Furthermore, the USA (40%), Western Europe (30%), and Japan (7%) are the main consumers of EOs, with a continuously increasing demand for natural products for use in different human activities [2]. In fact, in the global market, EOs are extensively used in the fragrances and cosmetics sector (e.g., in perfumes, skin creams, body lotions, soaps, shampoos, make-up products), as well as in food products and beverages (e.g., in herbs, spices, and additives) and in the medicinal field (e.g., in the pharmaceutical industry, aromatherapy, dentistry, and medicinal supplements).

Some of the EOs and their constituents are applied as alternatives to the synthetic compounds broadly used in the chemical industry. In fact, natural substances are safer and more sustainable than chemical ones, as the latter have the following drawbacks: their possible connection to toxicity problems, the use of organic solvents, and the release of carbon dioxide and other greenhouse gases during their production [3,4].

On the other hand, the growing demand for EOs extracted from Aromatic and Medicinal Plants (AMPs) causes a key problem linked with the management of residual wastes from the distillation process. Considering that the primary use of AMPs revolves around the production of EOs—which rarely yields more than 0.5–5% *w*/*w* of dry biomass, and in the processing of EOs, often only a single herb part is employed—this means that most biomass remains discarded and therefore becomes waste [5]. It is estimated that, annually, about 200,000 tons of solid residues are generated worldwide due to the extraction of EOs from AMPs [5].

In this context, the transition from a linear to a circular management of AMPs residues may drive the development of new strategies to produce high-added value biomolecules via valorizing agricultural and industrial wastes and reducing the volume of residues to be treated. The use of this model could lead to rapid reductions in costs and energy consumption, aligning with the aims of the European Union's Circular Economy Action Plan [6].

The *Lamiaceae* family is probably one of the most important in the context of EO production since the species in this family play a vital role in the health and well-being of people [7]. This botanical family consists of approximately 236 genera and 7200 species native to the Mediterranean basin, among which oregano, sage, rosemary, and thyme are the main ones from a commercial point of view [8].

In particular, rosemary (*Rosmarinus officinalis* L.) an ornamental and aromatic shrub that can be grown wild or cultivated, is one of the best known herbs, and it has been used since ancient times [9]. It has traditionally been used as a medicinal herb because it possesses many beneficial features, such as anti-inflammatory, analgesic, astringent, antimicrobial, anti-rheumatic, carminative, antifungal, and antioxidant properties [7,9]. Rosemary leaves are used as a spice in many food products and dishes, often in the form of a ground powder. The antioxidant activity of these leaves are well known, and many recent studies have demonstrated that their biological properties are mainly attributable to the bioactive compounds present in rosemary EO and in polyphenolic extracts [7,9,10]. From a chemical point of view, rosemary EO contains about 90–95% of monoterpenes and monoterpenes derivatives and a lower quantity of sesquiterpenes (2–5%). Its foremost compounds are 1,8-cineole, α-pinene, limonene, verbenone, camphor, borneol, and camphene, as reported in many studies [11–13]. Its chemical composition depends not only on the plant species but also on age, variety, part utilized, origin, climate, soil, stocking time, preparation [7,9,12]. The polyphenolic compounds in rosemary are also renowned and mainly include phenolic diterpenes (e.g., carnosol, carnosic acid, rosmanol, epirosmanol, and isorosmanol) and phenolic acids such as rosmarinic and caffeic acids [10,14,15].

The European Food Safety Authority (EFSA) has been evaluating rosemary extract as a food additive since 2008 because of its numerous compounds with significant biological functions [16]. The European Commission, with the implementation of Directive 2010/67/EU, approved the use of rosemary extracts as new food additives, assigning them the label E392 [17]. Nowadays, in the European Union, rosemary extracts are added to food products and beverages at levels of up to 400 mg/kg, considering the sum of carnosic acid and carnosol, the most powerful antioxidants contained in rosemary extracts [18].

To the best of our knowledge, polyphenol recovery from the rosemary residue of EO extraction has never been explored. For this reason, for the present study, rosemary EO was extracted from rosemary leaves by using hydro-distillation. Following this, EO extraction yield, antioxidant activity, and sun protection factor (SPF) were evaluated. The rosemary residue after EO distillation was studied for its polyphenolic compounds, and some experimental parameters were varied to optimize the protocol extraction. Specifically,

tests were conducted at different extraction times (15 min, 30 min, and 60 min), temperatures (25 °C, 40 °C, 50 °C, 60 °C, and 70 °C), and ethanol concentrations (50%, 60%, 70%, and 80%) were tested.

2. Materials and Methods

2.1. Reagents and Standards

Folin-CiocalteuFolin–Ciocalteu reagent, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and gallic acid were supplied by Sigma-Aldrich (St. Louis, MO, USA). Analytical-grade ethanol and methanol were bought from Carlo Erba Reagents (Milan, Italy).

2.2. Plant Sampling and EO Extraction

Wild rosemary (*Rosmarinus officinalis* L.) was harvested in a field where it grows spontaneously in Agerola (Latitude: 40°38′19″32 N; Longitude: 14°32′22″92 E), Naples province (Italy). The plant material was transported into the laboratory, where the fresh leaves were removed from the branches and stored at 4 °C until EO extraction.

The extraction of rosemary EO was performed according to the European Pharmacopeia method 2005.2812 [19] via hydro-distillation in a Clevenger-type apparatus.

Briefly, 70 g of fresh rosemary leaves (slightly blended) and 350 mL of distilled water (ratio 1:5 w/v) were placed in a 1 L spherical flask. The balloon was connected to the Clevenger apparatus and was placed in a thermostatic bath at 100 °C for 3 h. After extraction, the rosemary EO was collected in a glass vial, dried under anhydrous sulphate, and stored in the dark at 4 °C until further analyses.

The yield (Y) of rosemary EO was calculated according to Equation (1):

$$Y(\%) = \frac{V_{EO}}{m_s} \times 100 \quad (1)$$

where V_{EO} is the EO volume (reported in mL), and m_s is the weight mass of rosemary (expressed in g).

2.3. Polyphenols Extraction and Quantification

After EO extraction, the residual leaves were recovered, frozen at −20 °C, and lyophilized. Subsequently, 250 mg of this biomass were utilized to evaluate polyphenol content by adding 5 mL of ethanol extraction solution (ratio 1:20 w/v). Polyphenolic compounds extractions were carried out, varying three different parameters: extraction time (15 min, 30 min, and 60 min), temperature (25 °C, 40 °C, 50 °C, 60 °C, and 70 °C), and ethanol concentration (50%, 60%, 70%, and 80%). Ultrasound-assisted extractions (UAEs) were carried out, applying a sonication power of 120 W with a frequency of 40 Hz. All the extracts were recovered via centrifugation at 13,000× g at 4 °C for 10 min and dried using a rotary evaporator.

Polyphenols were determined by means of the spectrophotometrical Folin–Ciocalteu method according to Singleton and Rossi [20]. Briefly, 150 µL of each rosemary extract was added to 750 µL of Folin–Ciocalteu reagent and 600 µL of Na_2CO_3 at 7.5% (w/v). After 2 h of incubation in the dark, absorbance was determined at 765 nm. Gallic acid was used as standard, and the results were reported as mg of gallic acid equivalents (GAE) per g of DW biomass. All extracts were analyzed in triplicate (n = 3).

2.4. Antioxidant Activity Assay

The antioxidant activities of the rosemary EO and polyphenolic extracts were assessed in vitro through the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay [21]. In particular, 1.35 mL of 60 µM DPPH methanolic solution was blended with different samples amounts. The reduction in absorbance was continuously recorded at 517 nm. The

radical scavenging activity percentage (%RSA) of DPPH discoloration was obtained using the following the formula:

$$\%RSA = \frac{(A_{DPPH} - A_s)}{A_{DPPH}} \times 100 \quad (2)$$

where A_{DPPH} is the absorbance of the DPPH solution, and A_S is the absorbance of the solution when the sample was added. The EC_{50}, the extract concentration required to achieve 50% of radical DPPH inhibition, was calculated by graphing the RSA percentage vs. the concentrations. The results were expressed as mg/mL, as reported by Vella et al. [22].

2.5. Sun Protection Factor Determination

The sun protection factor (SPF) was determined in vitro by measuring the percentage of transmittance in the range of 290–320 nm, considering the known erythemal factors at each wavelength, as reported in Equation (3):

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs \quad (3)$$

where CF = correction factor (=10), EE (λ) = erythemal effect spectrum, I (λ) = solar intensity spectrum, and Abs = absorbance of samples. The EE (λ) × I (λ) values, determined according to the method described by Sayre et al. [23], were previously reported by Vella et al. [24].

For the determination of the SPF values, EO solution was prepared in ethanol (0.1% *v*/*v*). The absorbance of the sample was spectrophotometrically measured at intervals of 5 nm in the range of 290–320 nm [24].

2.6. Statistical Analyses

Means, standard deviations (SDs), calibration curves, and linear regression analyses (R^2) were carried out using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA).

3. Results and Discussion

3.1. Rosemary EO

Initially, the study was focused on extracting EO from rosemary leaves via hydrodistillation using a Clevenger-type device. Due to the need for a temperature below 100 °C, the distillation process has become the most common method for extracting EOs from plant material. Two phases are produced at the end of distillation, the upper organic one. In this way, the EO obtained is protected from the surrounding water phase, which acts as a barrier to prevent it from overheating.

The resulting rosemary EO yield was 1.57%, which is higher than reported in some other studies in the literature. Boutekedjiret et al. [25] reported a yield of 0.44%, while Conde-Hernández et al. [26] and Bousbia et al. [27] recorded a yield of 0.35%. Our results are in agreement with Flamini et al. [28], Angioni et al. [29], and Jamshidi et al. [11], who reported comparable total yields of *R. officinalis* EO of 1.44%, 2.13%, and 2.60%, respectively. These little differences could be attributed to plant age, variety, and the environmental conditions of the country of origin (e.g., climate, soil, altitude, water availability) [7,9,11,12].

In this study, the in vitro SPF measurement was applied as a rapid and suitable test to screen the potential ingredients for use as natural additives in foods and cosmetics. The higher the SPF value is, the more protection offered by the biomolecules against UV light. In particular, the EOs used in foods or in cosmetic formulations have the ability to absorb UV radiation, preventing and reducing skin damage and other health problems related to the formation of free radicals caused by sun exposure [24].

The wavelength values obtained and the related SPF calculation are reported in Table 1.

Table 1. Wavelength and sun protection factor (SPF) values.

Wavelength (nm)	Absorbance (Abs)
290	0.1619 ± 0.022
295	0.1902 ± 0.013
300	0.2186 ± 0.010
305	0.2412 ± 0.017
310	0.2821 ± 0.024
315	0.3754 ± 0.035
320	0.6075 ± 0.029
SPF	2.55

In this study, the SPF value of rosemary EO was 2.55. This value was determined based on the chemical components of the EOs, which can depend on the growing conditions and harvest time of the plants [7,9,11,12]. Modified values can be recorded for EOs extracted from diverse cultivars of rosemary, or even in the same variety grown in various geographic places. Despite the great variability in the SPF values, this assay was important, as it helped to preliminarily assess the potential use of this EO in foods and cosmetics to provide oxidative protection and protection from the sun.

3.2. Polyphenol Extraction from Rosemary EO Residue

The residue remaining after rosemary EO distillation was extracted using the UAE method under different ethanol concentrations (50%, 60%, 70%, and 80%). Furthermore, the extraction times (15 min, 30 min, and 60 min) and temperatures (25 °C, 40 °C, 50 °C, 60 °C, and 70 °C) were tuned in order to identify the best polyphenolic yields. This procedure is recommended and accepted as a green approach by the food industry [15].

The results derived from the extraction of polyphenols from the rosemary residue under different conditions are shown in Table 2.

Table 2. Amounts of polyphenols (mg GAE/g DW ± SD) extracted from rosemary residue after EO distillation.

Extraction Parameters	25 °C	40 °C	50 °C	60 °C	70 °C
50%–15 min	10.14 ± 0.38	15.04 ± 0.51	18.56 ± 0.53	18.01 ± 0.46	18.12 ± 0.60
60%–15 min	15.37 ± 0.62	16.31 ± 0.46	19.22 ± 0.54	20.18 ± 0.65	21.19 ± 0.52
70%–15 min	17.62 ± 0.81	20.96 ± 0.47	20.32 ± 0.48	20.59 ± 0.52	21.21 ± 0.50
80%–15 min	15.14 ± 0.82	19.67 ± 0.40	21.11 ± 0.57	21.01 ± 0.28	21.60 ± 0.54
50%–30 min	15.14 ± 0.32	15.78 ± 0.65	18.69 ± 0.51	18.35 ± 0.57	17.75 ± 0.51
60%–30 min	16.31 ± 0.62	19.65 ± 0.86	18.81 ± 0.38	18.05 ± 0.63	21.27 ± 0.77
70%–30 min	18.90 ± 0.61	21.24 ± 0.68	21.46 ± 0.69	20.83 ± 0.81	22.11 ± 0.58
80%–30 min	19.02 ± 0.63	22.89 ± 0.42	20.74 ± 0.62	22.15 ± 0.48	23.34 ± 0.71
50%–60 min	13.12 ± 0.61	17.64 ± 0.69	19.73 ± 0.65	19.12 ± 0.61	18.31 ± 0.55
60%–60 min	16.83 ± 0.49	18.17 ± 0.56	20.75 ± 0.58	21.99 ± 0.64	21.29 ± 0.42
70%–60 min	18.63 ± 0.58	20.96 ± 0.42	21.39 ± 0.57	22.22 ± 0.42	21.50 ± 0.65
80%–60 min	19.47 ± 0.57	19.99 ± 0.62	21.62 ± 0.64	22.30 ± 0.40	24.14 ± 0.54

Conventional extraction procedures, which typically involve the use of different solvents and temperatures, have some drawbacks, including the high temperature requirements and their time-consuming nature, and they often lead to low extraction yields. Therefore, it is recommended to employ other assisted extraction methods, such as those that involve the utilization of sonication. It has been reported that ultrasounds increase the extraction efficiency of active compounds from plants, as a consequence of the noteworthy disruption of cell walls and also for enhancement of mass transfer induced by cavitation bubble collapse in the solvent [30]. Moreover, the mechanical effect of ultrasound waves

facilities the penetration of the solvent into the matrix and enhances the contact surface between the solid and liquid phases [15,30–33].

Taking into account all of the data, increasing ethanol concentration (from 50% to 80%), extraction temperature (from 25 °C to 70 °C), and time (from 15 min to 60 min) led to an increase in polyphenol content. The maximum amount of polyphenols was found to be 24.14 mg GAE/g DW, and this result was achieved using the following parameters: 80% ethanolic solution at 70 °C for 60 min.

The utilization of a solvent containing both water and ethanol is reported to facilitate polyphenol extraction because water causes the plant material to swell up and ethanol can penetrate more easily to disrupt the bonds between the bioactive compounds and plant matrix [32,33]. The extraction of polyphenols improved with increasing temperature due to an increase in phenolic solubility. As reported in the literature, the diffusion rate, the mass transfer, as well as the reduction in solvent viscosity and surface tension, is enhanced [15,34]. Moreover, the extraction rate of polyphenols is greatly influenced by extraction time. The results of polyphenol extraction are generally more fruitful when a longer extraction time is used, but degradation can occur at high temperatures (over 70 °C).

3.3. Antioxidant Activity

The growing interest in bioactive compounds for use in the food and cosmetic markets, in line with the emerging demands of new applications, could be explored by means of routine biological activity tests.

For this study, the antioxidant activity of the best polyphenolic extract and of the rosemary EO was evaluated using a direct method based on radical scavenging capacity. Assays that use linoleate or ABTS cation radicals are known for their turbidity and interference with hydrophobic samples. Almela et al. [35] recommended the use of the free radical DPPH assay for this reason. This assay is based on the ability of a bioactive compound to reduce and stabilize the DPPH radical, which acts as a hydrogen donor.

As stated above, for this study, the antioxidant activity of the rosemary EO and of the best polyphenolic extract (PE; 80% ethanolic solution at 70 °C for 60 min) from the rosemary residue was evaluated, and the results are reported in Table 3.

Table 3. Antioxidant activity (expressed as EC_{50}) of the best polyphenolic extract (PE) and of the rosemary essential oil (EO).

	EC_{50} (μg/mL)
PE	143.90
EO	240.39

The results of the activities were expressed as EC_{50}, defined as the concentration of the EO needed to scavenge 50% of the DPPH present in the test solution.

We observed an EC_{50} value of 240.39 μL/mL for the EO, which is greater than that reported by Almela et al. [35]. Mostly, the difference in the results is due to plant age, variety, and environmental conditions [7,9,11,12]. The sample PE showed an EC_{50} of 143.90 μg/mL, a value lower than that reported by Almela et al. [35], suggesting that the PE extract has a higher antioxidant activity.

This important outcome demonstrates that by-products of EO distillation can be considered low-cost and interesting candidates to obtain natural biomolecules, indicating the potential suitability of rosemary waste products as an alternative to synthetic antioxidants.

4. Conclusions

The utilization and recycling of AMP biomass waste after the distillation of EOs represent new and interesting processes that can be exploited to improve progress towards circular economies. Consumers, due to the reduced side effects of polyphenols compared with their synthetic counterparts, generally recognize them as valuable antioxidants that

can be employed in the food sector as natural preservatives. In fact, they could be used to improve the shelf life of foodstuffs, or in the cosmetic industry as antiaging agents, in order to prevent natural oxidation and deterioration.

In this context, this preliminary research on rosemary residues from the EO industry could help to significantly improve the knowledge regarding the extraction of bioactive phytochemicals, thus valorizing a by-product discarded from the distillation process. Further chemical investigations will be conducted in order to completely identify the patterns of relevant polyphenols.

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