Rosemary

Subjects: Biochemistry & Molecular Biology
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Rosemary (Rosemarinus officinalis L.) belongs to the Lamiaceae family and is native to the Mediterranean region and part of Asia. It is the most well-known plant with antioxidant activity, and the only one currently approved as natural antioxidant in EU (E-392). The main antioxidant components and the extraction procedures are reviewed in the present work.

Keywords: rosemary ; extraction ; antioxidant

1. Rosemary

Rosemary (*Rosemarinus officinalis* L.) belongs to the Lamiaceae family and is native to the Mediterranean region and part of Asia, but can withstand cool climates and drought. Its name derives from the Latin ros-marinus, meaning “dew of the sea”, because it was believed to survive with no watering, only with the dew coming from the sea. It is the most well-known plant with antioxidant activity and its extract is the only currently approved natural antioxidant in EU (Directive 95/2/EC), assigned the E number E-392 (European Union Directives 2010/67/EU and 2010/69/EU). The antioxidant potency is primarily attributed to the phenolic diterpenes, carnosic acid and carnosol, and secondly to rosmarinic acid (and possibly other hydroxycinnamic acids, like caffeic acid), and minor flavonoid constituents. For this reason, the commercially available formulas of E-392 are standardized according to their content in carnosic acid and carnosol. The same constituents have been associated with several antifungal, antimicrobial, bioplaguicide, anticarcinogenic, anti-inflammatory, and prophylactic effects of rosemary extracts [1][2][3][4][5][6]. Rosemary and some common salvia species are the only herbs that contain carnosic acid and carnosol as major constituents [7]. Other compounds derived through carnosic acid and carnosol degradation like rosmanol, epirosmanol, epirosmanol ethyl ether, rosmadial, and methylcarnosate may be also present in the extracts [8][9]. The presence of triterpenoid acids, i.e., ursolic and oleanolic has been also reported [10]. The main flavonoids of the plant are apigenin, luteolin and other flavones, found mostly as glucosides [9][11][12][13].

Carnosic acid and carnosol are compounds of medium polarity and therefore are effectively extracted with acetone or ethanol [13][14][15][16][17]. Other non-polar solvents like hexane and butanone proved also effective [16][17]. The extraction of carnosic acid in a shaking bath was enhanced with temperature (25–50 °C) and time (30–180 min), while butanone was more effective than ethanol, due to lower polarity [16]. The presence of water in mixtures with organic solvents decreases the extraction yield [13]. Confirming this observation, the fresh plant material presented lower carnosic acid extraction yield than the dried material due to the presence of water that combined with ethanol, which was used as solvent, resulting in a more polar solvent [16][18]. Additionally, carnosic acid is oxidized to carnosol and derivatives during extraction in the presence of water [19]. Comparing ethanol and methanol as solvents, it was found that the less polar ethanol is effective for the extraction of carnosic acid, while methanol for rosmarinic acid [18]. Water is an excellent solvent for rosmarinic acid, while increasing the organic solvents concentration in water decreases its extraction yield [13].

De AR Oliveira et al. [14] examined acetone, methanol, ethanol, and their mixtures with water for the quantitative recovery of rosmarinic acid, carnosol, and carnosic acid, and observed that ethanol 59–70% or acetone 80% gave similar results, while methanol 50% presented lower carnosic acid recovery due to its transformation to carnosol. Consequently, they used a central composite design to optimize the conditions for the simultaneous extraction of the three compounds with ethanol–water mixtures. Optimum conditions were defined as 70% ethanol in water, at a solid to liquid ration of 1:5, and extraction time 55 min, to recover 90% of the antioxidants, while achieving a high purity of the extract. Additionally, ethanol concentrations varying between 30% and 96% were tested in maceration experiments and 50% ethanol in water showed the highest phenolic yield and antioxidant activity [20]. Ethanol–water mixtures are considered green solvents and, therefore have been used by other researchers [21][22]. Psarrou et al. [13] examined ethanol or acetone mixtures with water and observed the highest total phenolic content (TPC) recovery, antiradical activity, and extraction selectivity with either ethanol 60% or acetone 60%. Mixtures of organic solvents with water are more effective than pure water because they can extract more quantitatively non-polar, e.g., phenolic diterpenes and flavonoid aglycones, plus polar compounds

(phenolic acids and flavonoid glycosides). Furthermore, they examined the extraction kinetics and observed a fast initial extraction stage, followed by a much slower one, both of them following the unsteady state diffusion law. The increase of temperature (22–60 °C) enhanced swelling of the raw material, solubilization and diffusion of the solutes, thereby, and increased the extraction rate, but decreased selectivity as more non-flavonoid compounds were simultaneously extracted. Total terpenoids recovery increased with temperature but a high portion of carnosic acid was transformed to carnosol at 60 °C [13].

2. Result

The main research results about the effect of extraction solvent and procedure are summarized in Table 1. Apart from the conventional solvent extraction (CSE), novel extraction methods, and, among them, ultrasound assisted extraction (UAE), has been examined by many researchers. UAE decreased extraction time and lead to more effective extraction, at lower temperature with less dependence on solvent [15][16]. In particular, it was found to markedly increase the efficiency of ethanol to extract carnosic acid and to enhance the antioxidant activity of the extract [16][18]. Both the extraction rate and the TPC yield increased by UAE compared to conventional solid liquid extraction performed under the same conditions, and the difference was more pronounced when ethanol 60% in water was used as a solvent instead of acetone 60% [13]. The fact can be explained by the lower penetration and solubilization ability of ethanol that is enhanced by UAE. Ultrasound intensifies mass transfer, due to collapse of cavitation bubbles near the cell walls that causes partial destruction of the cell walls and production of an ultrasonic jet, which may act as a micropump that can force solvent into the cell and dissolve the solutes [16]. Thus, UAE resulted in a meaningful shortening of processing time at about 10–12 min [13][23][24].

Table 1. Solvents and methods reported in literature for the extraction of phenolic compounds from rosemary.

Solvent	Method	Measured Parameters	Main Results	Reference
Butanone Ethyl acetate Ethanol (solid/liquid 1/10, *w/v*)	CSE (25–50 °C, 0.25–3 h) UAE (probe 20 kHz) UAE (bath 40 kHz)	CA	CA yield increased with temperature. UAE probe or bath gave similar results and decreased extraction time (0.25 h compared to 3 h at 50 °C by CSE to obtain 15 mg CA/g dry plant)	[16]
Ethanol Methanol (solid/liquid 1/20, *w/v*)	CSE UAE (probe 20 kHz) UAE (bath 40 kHz) 25–50 °C, 0.25–2.0 h	CA RA DPPH	Ethanol gave higher yield of CA and methanol of RA and antiradical activity. UAE leads to more effective extraction, at lower temperature with less dependence on solvent Scale up (125 L) with ethanol resulted in 22 and 1.6 mg/g dry plant for CA and RA, respectively.	[18]
Hexane Acetone Ethanol Water (solid/liquid 1/10, *w/v*)	UAE (probe 20 kHz, 10 min) MAE (under N_2, 100 °C, 10 min) UAE: Single or successive extractions	HPLC	UAE with ethanol or acetone gave the highest terpenoids yield. Highest TPC was obtained with UAE or MAE with ethanol (35 and 36 mg/g dry plant, respectively). UAE with hexane showed a high selectivity in CA extraction, and with acetone low CA oxidation	[17]
Ethanol Methanol Acetone Water mixtures	CSE (ethanol in water 44.8–95.2%, solid/liquid 1/4.6–1/21.4, *m/v*, time 4.8–55.2 min)	CA COH RA	Ethanol 59% or 70% and acetone 80% gave the best results for all three compounds. Optimum conditions: ethanol 70%, solid/liquid 1/5, extraction time 55 min to obtain highest yield and antioxidant concentration in the extract	[14]
Ethanol in water (0–96%) Acetone in water (0–100%) (solid/liquid 1/20, *w/v*)	CSE UAE Pretreatment: deoiling by water-steam distillation, milling, maceration	TPC HPLC DPPH	60% ethanol or acetone showed the highest TPC yield and concentration in the extract. Highest RA yield was obtained with water gave, flavonoids with 60% acetone, and terpenes with 80% acetone UAE enhanced TPC extraction and antiradical capacity of the extract, especially with ethanol 60%. Grinding increased the extraction rate.	[13]

Solvent	Method	Measured Parameters	Main Results	Reference
Ethanol water (solid/liquid 1/6, *w/v*)	CSE (40 °C, 4 h) UAE (probe) MAE Pretreatment: deoiling by solvent free MAE, milling	Yield TPC CA RA DPPH	CA not detected in water extracts. Higher yields of TPC, RA and lower EC_{50} in water extracts. UAE and MAE decreased extraction time. De-oiling and milling increased yield, TPC and RA content in the extract.	[15]
Ethanol in water Water	CSE (solid/liquid 1/10–1/20, *m/v*, 27–70 °C, 30–300 min UAE probe (solid/liquid 1/20, *m/v*, 40–90% ethanol in water, 40 °C, 60–200 W, 3–13 min)	Yield TPC DPPH	30 min by CSE or 11 min for UAE were sufficient to obtain the maximum TPC and antiradical efficiency. Combination of CSE (step 1) and UAE (step 2) did not improve results. 56% ethanol presented best results in either CSE or UAE	[24]
Ethanol in water 70%, 90% Water + Tween 20 (solid/liquid 1/15, *w/v*)	UAE bath Maceration (90% ethanol, room temperature, 48 h) Percolation	TPC RA UA OA DPPH	The highest yield of UA (15.8 mg/g) was obtained by UAE with 90% ethanol, 60 °C, 10 min; RA (15.4 mg/g) by UAE with 70% ethanol, 50 °C, 30 min, or water (at pH 9); and OA (12.2 mg/g) by maceration. Highest TPC was obtained by water extraction.	[10]
Ethanol in water 90% (solid/liquid 1/20, *w/v*)	Heat reflux extraction (78 °C, 0.5 or 5 h) Maceration (40 °C, 0.5 h) UAE bath/reactor/probe (40 °C, 0.5 h) MAE under reflux (78 °C, 0.5 h) under N_2 pressure under vapor pressure	RA CA UA COH	Heat reflux extraction for 0.5 h resulted in extraction yield of 19%, compared to 10% obtained by maceration. UAE with probe showed similar yield to heat reflux extraction but higher recovery of CA and UA. In MAE, extraction and RA yields increased with temperature but CA and UA yield decreased. Pressure does not enhance extraction.	[22]
Ethanol in water 30–96% (solid/liquid 1/5, *w/v*)	Maceration (3 days with occasional shaking) Percolation	TPC DPPH	Highest TPC obtained with 50%, no significant differences in antiradical activity Percolation gave higher TPC yield but lower antiradical activity.	[20]
Water Methanol:water (60:40) Acetone:water (60:40) Ethyl acetate:water (60:30) (solid/liquid 1/40, *w/v*, 1/20 in MAE)	MAE (4 min, under N_2) Heat reflux extraction (90 °C, 2 h, under N_2)	TPC HPLC	MAE gave comparable TPC yield to conventional extraction at shorter time Acetone in water presented highest TPC yield in MAE. Water presented the highest TPC in heat reflux extraction followed by methanol, acetone and ethyl acetate in water mixtures. Content of individual phenolics was similar in either method	[25]
Methanol:water 50:50–100:0 Ethanol:water (70:30) Acetone:water (70:30) Ethyl acetate:water (70:30) (solid/liquid 1/5, *w/v*)	MAE (2 × 1–2 × 15 min) UAE bath (2 × 5 min) Soxhlet (1–5 h)	TPC flavonoids, anthocyanins	MAE gave comparable TPC yield with the optimum obtained in Soxhlet extraction (3 h), and 2-fold higher than UAE. Maximum TPC with methanol:water, 70:30, flavonoids with ethanol:water, 70:30, anthocyanins ethanol:water, 70:30 + 1% HCl, for 2 × 5 min.	[26]
Methanol in water 32–88%	Maceration (1/50, *w/v*, 80% methanol, room temperature, overnight) ASE (66–200 °C, 103 atm)	TPC, HPLC FRAP	Optimum conditions through RSM: 56% methanol, 129 °C. TPC (101.7 mg/g dry herb) and antioxidant recovery at optimum ASE conditions were higher than those obtained by solid/liquid extraction.	[23]
Ethanol Water (solid/liquid 1/10, *w/v*) CO_2 CO_2 + 7% ethanol	ASE (50–200 °C, 100 bar, 20 min) SFE (40 °C, 100–400 bar, 300 min) WEPO	TPC DPPH HPLC	ASE with water gave the highest yield and antioxidant activity of the extract. TPC, yield and antiradical activity increased with temperature and water was more efficient than ethanol. The extract obtained by SFE with CO_2 + 7% ethanol had good TPC and antiradical activity but low yield.	[27]

Solvent	Method	Measured Parameters	Main Results	Reference
Ethanol Water (solid/liquid 1/10, *w/v*) CO_2 CO_2 + 6.6% ethanol	ASE (150 °C with ethanol, 100 or 200 °C with water, 100 bar, 20 min) SFE (40 °C, 150, 400 bar)	HPLC	SFE extracted compounds of low polarity. RA was extracted by ASE with either solvent, while most flavonoid glycosides were extracted only by ASE with water	[11]
Ionic liquids in water (solid/liquid 1/20, *w/v*)	UAE (bath 100–250 W, 0.5 h, after 2 h soaking)	CA RA	The extraction efficiency was comparable to 80% ethanol used in UAE (0.5 h), solvent extraction (24 h) or CSE (24 h).	[28]

ASE: accelerated solvent extraction, CA: carnosic acid, COH: carnosol, CSE: conventional solvent extraction, DPPH: 1,1-diphenyl-2-picrylhydrazyl radical, FRAP: ferric reducing antioxidant power, MAE: microwave assisted extraction, OA: oleanolic acid, RA: rosmarinic acid, SFE: supercritical fluid extraction, TPC: total phenolic content, UA: ursolic acid, UAE: ultrasound assisted extraction, WEPO: pressurized water extraction with particle on-line formation.

Bellumori et al. [17] examined UAE with different solvents in single or successive extraction steps. Ethanol and acetone gave the highest TPC yield, while water the lowest due to its inability to extract terpenoids, although it was the most effective for the recovery of rosmarinic acid and flavonoids. Additionally, sonication of water results in the formation of highly reactive hydroxyl radicals, which may participate to degradation reactions. The highest terpenoid recovery was obtained with acetone, accompanied with very limited oxidation of carnosic acid. Hexane presented low overall yield but a very high selectivity in terpenoids extraction. Thus, the authors concluded that UAE can be very favorably compared with the CSE in acetone that is used to prepare commercial rosemary antioxidants [17]. The investigation of the optimal conditions for the extraction of rosmarinic acid, ursolic acid, and oleanolic acid from rosemary leaves by UAE or maceration (90% ethanol, 48 h) indicated UAE with 70% ethanol the most efficient for rosmarinic acid recovery, UAE with 90% ethanol for ursolic acid, and maceration for oleanolic acid. Maceration showed also the highest TPC yield and antioxidant activity [10]. UAE performed with a probe presented higher extraction yield and carnosic acid and ursolic acid recovery, compared to a bath, possibly due to a better ultrasonic power delivery [22]. The results obtained at 40 °C for 30 min were comparable or slightly better than those obtained by conventional extraction at 78 °C for 30 min, except for rosmarinic acid that presented lower yield [22]. It is generally recommended to use reactors with 20 kHz as operating frequency in the case of UAE with a probe because, at lower frequencies of irradiation (e.g., 20 kHz), the physical effects of ultrasound-induced cavitation phenomena, i.e., liquid circulation currents and turbulence that are the controlling factors in extraction, are dominant [21][16][17][22][29].

Microwave assisted extraction (MAE) has been also examined [17][22][25][26]. MAE performed with water resulted in lower TPC yield than a conventional heat reflux extraction, while this was not observed when water mixtures with acetone, methanol, or ethyl acetate were used [25]. Water has a high dielectric constant but a low dissipation factor, compared to the other solvents. Thus, the rate of microwave energy absorbance is higher than the rate of heat dissipation, resulting in overheating and possibly destruction of some of the phenolic compounds [25]. Mixtures of methanol or acetone with water (70:30) presented the highest TPC yield [25][26], while mixture of ethanol with water (70:30) proved the most efficient for flavonoids [17][26], and, when acidified with 1% HCl, for anthocyanins [26]. The increase of temperature (78–150 °C) in MAE with 90% ethanol increased the extraction yield and rosmarinic acid recovery but decreased carnosic acid and ursolic acid recovery. Additionally, the use of vapor or N_2 pressure was examined but did not enhance extraction yield [22].

Solid free microwave extraction (SFME) has been used mainly for the recovery of EO [30][31][32]. The principle of the method is the internal heating of the in-situ water of the plant by microwaves, which leads to rupture of the glands and oleiferous receptacles. The released EOs and bioactive compounds are evaporated with the in situ water of the plant material. If SFME is performed under pressure, at high temperature (around 180 °C), the polarity and viscosity of the water decrease and it can dissolve, and consequently, extract less polar compounds like flavonoid aglycons that are not soluble at atmospheric temperature and pressure [32].

Another approach used for the extraction of antioxidants is the accelerated solvent extraction (ASE) that is also defined as pressurized liquid extraction (PLE), and in case water is used as the solvent, pressurized water or pressurized hot water extraction (PWE, PHWE), or subcritical water extraction (SWE). Similar to UAE and MAE, ASE has several environmental and economic advantages compared to CSE. It is a fast extraction technique, requiring lower amounts of solvents, while non-toxic solvents like ethanol or water can be effectively used. In particular, when applying ASE with water, the polarity of water decreases as temperature increases while it remains at the liquid stage, thus it approaches the properties of organic

solvents [27]. Ethanol proved a good solvent for the recovery of carnosic acid and carnosol by ASE, while rosmarinic acid was equally recovered by either ethanol, or water, and more polar acids (caffeic, chlorogenic) and flavonoid glycosides by water [11][27]. High temperatures, 150–200 °C, which may be used in ASE, cause degradation of rosmarinic acid [23][27]. Rosmarinic acid may be cleaved to its monomer, caffeic acid, which increased as temperature increased [23]. Additionally, increasing temperature caused an increase in gallic acid, while carnosic acid and carnosol were not affected, and consequently antioxidant capacity was favored. Nevertheless, as temperature increased, melanoidins were formed through Maillard reactions, which may lead to harmful products, thereby ASE at 150–200 °C was not recommended [23].

Additionally, supercritical fluid extraction (SFE) has been examined by some researchers. SFE with neat CO_2 provides very low yield that can be improved with the addition of a modifier such as ethanol [11][33][34]. In fact, CO_2, as a non-polar solvent, can recover only carnosic acid, carnosol, and other carnosic acid derivatives, even at 400 atm, while the addition of 7% or 10% ethanol was necessary for the extraction of minor amounts of other phenolic compounds [11][27][34]. Zabot et al. [35] proposed a sequential extraction of the EO and the phenolic compounds by using supercritical CO_2 and PWE in the same equipment. Water is a polar solvent, thus suitable for rosmarinic acid extraction that was recovered at the beginning of PWE. As temperature increased above 100 °C, the polarity of water was reduced and the less polar compounds, i.e., carnosic acid, carnosol, rosmanol, and methyl carnosate were obtained [33][35].

Another research team proposed a pressurized hot water extraction combined with particle formation on line (WEPO) to obtain dry antioxidant powder from rosemary [27][36]. The extraction is performed at 200 °C and 80 atm, the extract is continuously transformed to an aerosol by the use of a supercritical CO_2 nebulization system, and the aerosol is instantaneously dried by a hot N_2 current [36]. After 40 min of extraction a powder yield of 34%, dry basis, was obtained with good DPPH radical scavenging properties, while no details about the phenolic profile are provided by the authors. The procedure was favorable in terms of environmental impact, compared to PHWE (200 °C, 103 atm, 20 min) and SFE (40 °C, 150 atm, 300 min, ethanol as modifier) giving powder with similar antioxidant capacity. Additionally, ionic liquids have been examined, as novel, green solvents [37] but separation of the antioxidant compounds from the extraction liquor needs further research.

The plant material is dried (usually at room temperature) before the extraction so as to avoid microbial spoilage during storage and facilitate transportation. Mulinacci et al. [19] observed that drying caused a significant loss of flavonoids and rosmarinic acid, while total terpenoids were not affected. Additionally, freeze drying caused significant losses [19][38]. Freezing, on the other hand, caused a high loss of rosmarinic acid, possibly due to phenoloxidase activity [19]. On the contrary, grinding of the raw material to smaller particle size, facilitated mass transfer phenomena, and consequently, enhanced extraction [13]. The geographical region, and possibly the soil type, and altitude have an effect on the profile and concentration of the phenolic compounds [12][39][40]. The harvesting period has a significant effect on the phenolic content that presents a maximum on flowering period (e.g., May and November), and on flavonoid content that follows the same trend [38]. Furthermore, the highest concentration of carnosic acid and rosmarinic acid present a reverse trend, the former showing a maximum in summer and the latter in winter [39]. However, results for phenolic compounds seasonal variations from plants of different regions and countries do not agree and seem to depend, among others, to variations in temperature and rainfall [37][40][41].

Rosemary extracts have been proposed and used as bioactive, antioxidant additives in food, cosmetics, packaging, etc. [42][43][44][45][46]. Their worldwide market is expected to present an annual growth rate of roughly 3.7% over the next five years, and will reach 260 million US$ in 2024 from 210 million US$ in 2019 [47]. For industrial uses dried extracts have several advantages, e.g., they are easier to handle, transport and store, and to be used in solid formulations like tablets and capsules. Dried extracts have been obtained through spray drying of an ethanol:water (80:20) extract at an inlet temperature of 140 °C. Although the dried products lost some of their polyphenols, they presented appreciable antioxidant activity [48]. Other investigators reported much lower inlet temperature (80 °C) as optimum [49]. Efforts for encapsulation in maltodextrin, through spay drying, presented promising results, too [28].

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