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Antibacterial and Antioxidant Properties of Oregano and Rosemary Essential Oil Distillation By-Products

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Abstract: The objective of this study was to investigate the antibacterial and antioxidant effect of Greek oregano and rosemary by-products from essential oil distillation on pathogenic and spoilage bacteria. The antibacterial effect of raw material of oregano and rosemary before distillation and the post distillation, dried residues, was tested against the following bacteria: *Escherichia coli, Salmonella enterica* subsp. *enterica* ser. Typhimurium, *Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis, Bacillus licheniformis*, and *Bacillus cereus* strains. Results showed that rosemary distillation by-products were able to inhibit the growth of all *Bacillus* (*B. subtilis, B. licheniformis, B. cereus*) strains and *L. monocytogenes*, while oregano affected the growth of *L. monocytogenes* and *S. aureus*, even in the minimum concentration, whereas *B. cereus*, in the maximum concentration, respectively. Total phenolic content in oregano/rosemary raw material and their by-products were approximately similar, however, antioxidant activity were reduced in oregano solid residue, whereas surprisingly increased in rosemary by-products after distillation. These results suggest the potential use of oregano and rosemary distillation by-products as antimicrobial and bioactive agents.

Keywords: oregano; rosemary; distillation residue; antimicrobial; antioxidant; by-products valorization

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1. Introduction

Numerous medicinal and aromatic plants are known for their healing properties since antiquity, and there is an increased research interest nowadays on the bioactive compounds associated to the pharmacological properties of many herbs. In this context, Greek oregano (*Origanum vulgare* subsp. *hirtum* L.) and rosemary (*Rosmarinus officinalis* L.) have been studied for their antioxidant, antibacterial and antifungal properties [1-5]. Oregano essential oil and its main constituents thymol and carvacrol, as well as their precursor monoterpenes γ-terpinene and p-cymene, have been attributed with significant antioxidant properties against lipid oxidation [6], while several reports documented its antibacterial effect against foodborne and food-spoilage bacteria [7-9]. Bioactive effects have been also reported for rosemary essential oil and its extracts [2,4]. More specifically, carnosic acid, carnosol and rosmanol, identified as the main active components in rosemary extracts, are well known for their antioxidant activity [2]. However, there is a negative attribute that limits the use of essential oils for food preservation, due to their odour and the strong effect on food sensory characteristics [10]. Although the essential oils of oregano and rosemary have a significant commercial value and their

pharmacological properties have been well documented [11], the bioactivity and particularly the antibacterial potential of the distillation by-products have not been studied yet.

The essential oil distillation procedure generates large amounts of post distillation – residues, causing thus environmental problems. Therefore, the valorization of the essential oil industry by-products into valuable bioactive constituents, would be an attractive perspective, and it is also in accordance to the bio economy aspects. Taking into consideration the above, the aim of the present study was the assessment in vitro of the antibacterial and antioxidant activities of the post distillation material of *Origanum vulgare* subsp. *hirtum* L. and *Rosmarinus officinalis* L., as potential candidates for their use as antimicrobial and bioactive substances

2. Materials and Methods

2.1. Plant Materials and Preparation of Samples

Aerial parts of *Origanum vulgare* subsp. *hirtum* L. (oregano) and *Rosmarinus officinalis* L. (rosemary), were collected during the flowering season, from cultivated accessions in the Department of Aromatic and Medicinal Plant Hellenic Agricultural Organization – Demeter, Plant Breeding and Genetic Resources Institute, Thessaloniki, Greece).

The plant material was dried under shade until the moisture content reached about 10% (Raw) and then subjected to steam distillation in a pilot scale distillation equipment. After the removal of the essential oil, the remined solid residue were collected immediately and dried as follows: a) sun-dried (SD) for 48 h and b) oven-dried (OD) at 60 °C for 2 h (oregano) or 40 °C for 1 h (rosemary) according to priliminary tests in order to achieve moisture content of wet soild residue below 10%.

Following the drying process, solid materials were separated from stalks and finally were grounded to pass through a 0.5 mm sieve in a Retsch Model ZM1000 mill (Haan, Germany). Grounded samples were stored in plastic bags in a cool, dry, and dark place until analyses.

2.2. Bacterial Strains and Cultures

The antibacterial activity was tested against the following well known pathogen and spoilage bacteria: *Escherichia coli* ATCC 25922 (American Type Culture Collection, Manassas, VA 20110 USA), *Staphylococcus aureus* ATCC 25923, *Salmonella enterica* subsp. *enterica* ser. Typhimurium DC 193 (provided by the Lab. of Food Microbiology and Biotechnology, Agricultural University of Athens, Greece), *Listeria monocytogenes* Scott A, *Bacillus subtilis* NCIMB 3610 (National Collection of Industrial, Food and Marine Bacteria, NCIMB Ltd., Aberdeen, Scotland, UK), *Bacillus subtilis* NCFB 1069 (National Collection of Food Bacteria, Reading, UK, which incorporated with NCIMB), *Bacillus licheniformis* NCDO 735, (National Collection of Dairy Organisms, which incorporated in NCFB), *Bacillus licheniformis* DSM 13 (DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) and *Bacillus cereus* DSM 31.

Strains were kept in Brain Heart Infusion (BHI) broth plus 25% glycerol at -80 °C, and activated with two successive cultures in BHI broth (Oxoid, Basingstoke, UK) incubated overnight at 30 °C for *Bacillus* species and at 37 °C for the other bacteria.

2.3. Assessement of Antibacterial Activity

The agar dilution method was applied [12]. The raw material (Raw), the sun-dried (SD) and the oven-dried (OD) solid residues were incorporated in Mueller-Hinton (MH) agar (Oxoid, Basingstoke, UK) in various concentrations (5 mg/mL, 10 mg/mL and 20 mg/mL) before sterilization. Following sterilization, the media were distributed in plates and 10 μL of activated culture were spot inoculated on the solidified agar plates in triplicate. After incubation for 5 days at 30 °C for bacilli and 37 °C for the other bacteria, plates were examined for visible bacterial growth [12], recording optimum (+), weak (w)

or no growth (-), compared to the control. To discriminate the mode of action of Raw, SD or OD samples on bacterial cell viability (bacteriostatic or bactericidal action), a part of the inoculated agar surface from plates where no growth occurred after 5 days of incubation, transferred aseptically to Brain Heart Infusion (BHI) broth. Growth in BHI broth after 24-48 h of incubation, indicated bacteriostatic activity, while no growth indicated bactericidal activity [13].

2.4. Bioactive compounds and Antioxidant Activity Assay

Total phenolic contents (TPC) of solid residues were determined using the modified Folin–Ciocalteau's method according to Irakli et al. [14]. Briefly, 0.05 g of dried- solid residue was extracted with 10 mL 70% methanol with the aid of an ultrasonic bath for 15 minutes. After centrifugation at 4,500 rpm for 10 min, the above extraction repeated one more time. An aliquod of mixed supernatants (0.2 mL) was mixed with 0.8 mL of 10% Folin-Ciocalteau reagent and allowed to react for 2 min. Consequently, 2 mL of sodium carbonate (7.5% w/v) solution and 7 mL of distilled water were added to the mixture and the absorbance at 725 nm was measured after incubation for 60 min.at dark place. The results were expressed as mg of gallic acid equivalents per g of sample on a dry weight basis (mg GAE/g dw). All analyses were performed in triplicate.

In order to determine the antioxidant activity of the solid residues' extracts it was employed 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical (ABTS·+) scavenging activity (ABTS assay), according to Irakli et al. [15]. Trolox was used as the standard compound for calibration curve and the results were expressed in mg of Trolox equivalents (TE) per g of sample on dry basis (mg TE/g dw). Analyses were performed in triplicate.

2.5. Statistical Analysis

Values were reported as the mean ± standard deviation of triplicate measurements. All parameters were subjected to one-way analysis of variance (ANOVA), and when ANOVA revealed significant differences between means, a Tukey's test at $p < 0.05$ was used to separate means by using the Minitab 17 (Minitab Inc., State College, PA, USA) software.

3. Results and Discussion

3.1. Antibacterial Effect of Oregano and Rosemary

The antibacterial capacity of the raw material, as well as of the solid residues against pathogenic and spoilage bacteria was clearly verified throughout this study. *Oregano* and *Rosemary* distillation solid residues proved certain antibacterial activity against specific bacteria (Tables 1 and 2). The antibacterial activity of three different concentrations (5, 10 and 20 mg/mL) of Raw, SD and OD samples examined against *E. coli* ATCC 25922, *S. aureus* ATCC 25923, S. Typhimurium DC 193, *L. monocytogenes* Scott A, *B. subtilis* NCIMB 3610, *B. subtilis* NCFB 1069, *B. licheniformis* NCDO 735, *B. licheniformis* DSM 13 and *B. cereus* DSM 31 (Table 1). All strains showed optimum growth in control agar plates. All concentrations of Raw samples inhibited the growth of all bacteria tested, exhibiting mostly bacteriocidal action, except against *E. coli* and *S*. Typhimurium which withstand the lower concentration (5 mg/mL) used. Oregano SD and OD residues presented similar antibacterial activity pattern against the tested strains. Both, inhibited strongly *L. monocytogenes* growth in all concentrations used, while growth of *S. aureus* and *B. cereus* inhibited only in the higher concentration (20 mg/mL), exhibiting bacteriocidal action, whereas the growth of *S. aureus* weakened in the lower concentrations (5 and 10 mg/mL) of SD and OD compared to the control. No antibacterial activity of the oregano residues was noted against *E.coli*, S. Typhimurium and the two *B. subtilis* strains. Limited activity (noted as weak growth) was observed against *B. cereus* and the two *B. licheniformis* strains, only at the maximum concentration (20 mg/mL). The limited antibacterial activity of SD

and OD samples possibly explained by the fact that main constituents of oregano essential oil, carvacrol and thymol, known for their strong antimicrobial activity, have been removed during the distillation process, and are not present in the post distillation material [7-9].

Raw rosemary sample inhibited the growth of *L. monocytogenes* and the three *Bacillus* species tested, in all concentrations incorporated in the agar medium, exhibiting mostly bactericidal activity, while no growth inhibition observed against *E. coli, S.* Typhimurium and *S. aureus* (Table 2). Similar trend in antibacterial activity revealed for SD and OD rosemary residues, except that *B. subtilis* strains withstand all concentrations of OD used. Therefore, it would be interesting to elucidate the constitution of the two residues, in order to explain the differences observed in their antibacterial capacity and possibly attribute to specific compounds.

In general, oregano and rosemary were more effective against bacteria tested in their initial form (Raw), while rosemary residues maintained their antibacterial activity after distillation process, against *L. monocytogenes* and *Bacillus* species.

Table 1. Effect of raw dried oregano (Raw) and oregano residues obtained from distillation followed by sun-drying (SD) or oven-drying (OD) treatment, on growth[1] of *E.coli* (Ec), S. Typhimurium (ST), *L. monocytogenes* (Lm), *S. aureus* (Sa), *B. subtilis* (Bs1, Bs2), *B. licheniformis* (Bl1, Bl2) and *B. cereus* (Bc) strains. Where no growth occurred, the mode of action[2] on cell viability is also recorded.

Treatment	Concentration (mg/mL)	Strains								
		Ec	**ST**	**Lm**	**Sa**	**Bs1**	**Bs2**	**Bl1**	**Bl2**	**Bc**
Control	0	+	+	+	+	+	+	+	+	+
Raw	5	+	+	- (c)	- (c)	- (c)	- (c)	- (s)	- (s)	- (s)
	10	- (c)	- (c)	- (c)	- (c)	- (c)	- (c)	- (c)	- (c)	- (c)
	20	- (c)	- (c)	- (c)	- (c)	- (c)	- (c)	- (c)	- (c)	- (c)
SD	5	+	+	- (c)	w	+	+	+	+	+
	10	+	+	- (c)	w	+	+	+	+	+
	20	+	+	- (c)	- (c)	+	+	+	w	- (c)
OD	5	+	+	- (c)	w	+	+	+	+	+
	10	+	+	- (c)	+	+	+	+	+	+
	20	+	+	- (c)	- (c)	+	+	w	w	- (c)

[1] + : optimum growth, -: no growth, w: weak growth. [2] (s): bacteriostatic, (c): bactericidal.

Table 2. Effect of raw dried rosemary (Raw) and rosemary residues obtained from distillation followed by sun-drying (SD) or oven-drying (OD) treatment, on growth[1] of *E.coli* (Ec), S. Typhimurium (ST), *L. monocytogenes* (Lm), *S. aureus* (Sa), *B. subtilis* (Bs1, Bs2), *B. licheniformis* (Bl1, Bl2) and *B. cereus* (Bc) strains. Where no growth occurred, the mode of action[2] on cell viability is also recorded.

Treatment	Concentration (mg/mL)	Strains								
		Ec	**ST**	**Lm**	**Sa**	**Bs1**	**Bs2**	**Bl1**	**Bl2**	**Bc**
Control	0	+	+	+	+	+	+	+	+	+
Raw	5	+	+	- (s)	+	- (s)	- (c)	- (c)	- (c)	- (c)
	10	+	+	- (s)	+	- (c)	- (c)	- (c)	- (c)	- (c)
	20	+	+	- (c)[2]	w	- (c)	- (c)	- (c)	- (c)	- (c)
SD	5	+	+	- (s)	+	+	w	- (c)	- (c)	- (c)
	10	+	+	- (c)	+	- (c)	- (c)	- (c)	- (c)	- (c)
	20	+	+	- (c)	+	- (c)	- (c)	- (c)	- (c)	- (c)
OD	5	+	+	- (c)	+	+	+	- (c)	- (c)	- (c)
	10	+	w	- (c)	+	+	+	- (c)	- (c)	- (c)
	20	+	w	- (c)	+	w	w	- (c)	- (c)	- (c)

[1] + : optimum growth, -: no growth, w: weak growth. [2] (s): bacteriostatic, (c): bactericidal.

3.2. Antioxidant Effect of Oregano and Rosemary

The content of total phenols (TPC) in oregano solid residues ranged from 89.9 mg GAE/g dw for the SD residue to 90.5 mg GAE/g dw for the OD residue, statistically ($p \leq 0.05$) similar to the raw material (93.8 mg GAE/g dw) (Fig. 1). On the contrary, the SD and OD treated oregano solid residues exhibited lower antioxidant activity using ABTS test than the Raw sample. This could be attributed to the antioxidant effect of its essential oil components [1-3].

On the other hand, the TPC in rosemary solid residues ranged from 58.0 mg GAE/g dw in the case of OD residue to 60.1 mg GAE/g dw for the SD residue that was statistically ($p \leq 0.05$) similar to the Raw material (59.0 mg GAE/g dw). However, antioxidant activity of SD and OD rosemary residues evaluating using ABTS assay was statistically ($p \leq 0.05$) higher than Raw material (Fig. 1). This may explain the retained antibacterial avtivity of rosmary residues. Therefore, it would be interesting to elucidate the constitution of the two residues, in order to explain the differences observed in their antibacterial capacity and possibly attribute to specific compounds.

In general, in the case or Raw materials, oregano had approximately 2.5-fold higher antioxidant activity than rosemary, whereas in the respective solid residues, the ratio was on average 1.7.

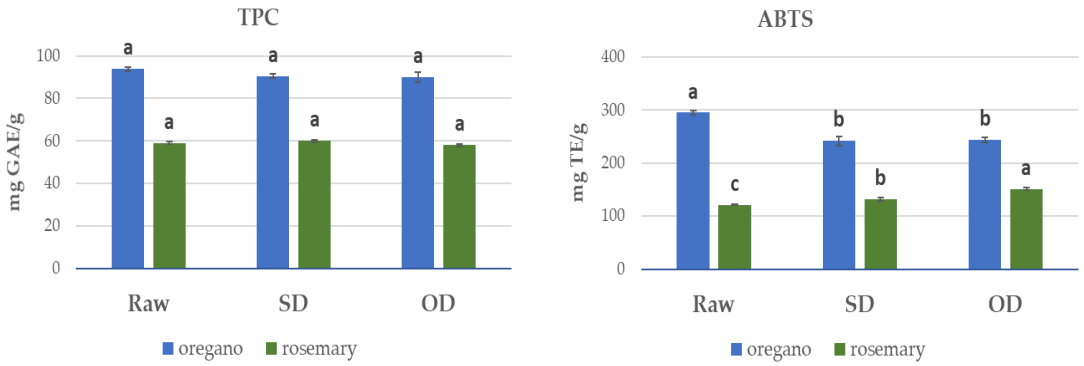

Figure 1. Main effects plot for total phenolic content (TPC, mg GAE/g) and ABTS radical scavenging activity (mg TE/g); of raw and solid residues after steam distillation of oregano and rosemary.

5. Conclusions

This is the first published work about the antibacterial properties of Greek oregano and rosemary residues derived from essential oil distillation, to our knowledge. The method of incorporation of solid residues into the medium for testing the antibacterial capacity is simple, quick and cheap. Results showed that oregano was more effective against all bacteria tested in its initial form (Raw), and after distillation the inhibition occurred only against *L. monocytogenes* and *S. aureus*, while rosemary residues maintained their antibacterial activity even after distillation process, against all the three *Bacillus* species (*B. subtilis*, *B. licheniformis*, *B. cereus*) and *L. monocytogenes*. In general, *L. monocytogenes* proved the most susceptible strain and gram-negative bacteria *E. coli* and S. Typhimurium the most resistant. Gram-negative bacteria, in general, are more resistant to the action of various antibacterial substances since they have an outer membrane consisted of lipopolysaccharides that restrict diffusion of hydrophobic compounds [16]. These results suggest a potential use of the solid residue from the essential oil distillation of oregano and rosemary as antimicrobial substrate. The reduced flavour of residues after distillation in comparison to the respective essential oil is considered an advantage towards using the residues to food products. Additionally, the antioxidant assessments showed that both solid residues could be used as a bioactive material, although rosemary residue was more promising antioxidant matrice than oregano. However, further studies

should take place, in order to determine the components that are responsible for the antibacterial and antioidant activity of the essential oil residues.

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Conflicts of Interest: The authors declare no conflict of interest.

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