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Assessment of Antioxidant and Antibacterial Potential of Phenolic Extracts from Post-Distillation Solid Residues of Oregano, Rosemary, Sage, Lemon Balm, and Spearmint

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Abstract: Medicinal and aromatic plants (MAPs) are potential sources of natural polyphenols. Solid residues (SRs) from the essential oil (EO) industry are produced in significant volumes and may be used as natural sources of bioactive compounds. Therefore, this work was designed to examine the antioxidant and antibacterial characteristics of phenolic extracts obtained from SRs that have remained after EO distillation. SR extracts of Greek oregano, rosemary, spearmint, lemon balm, and Greek sage were assessed for their total phenolic content (TPC), antioxidant activity, and antimicrobial activity against *Escherichia coli*, *Salmonella* Typhimurium, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus cereus* in the concentration range of 500-3000 mg/L. The rosemary and Greek sage extracts exhibited the strongest antibacterial activities against all the Gram-positive species, while the spearmint and oregano extracts were less effective and only had an effect at the highest concentration used. The lemon balm extract did not show any inhibitory effect; however, it had the highest TPC, showing moderate antioxidant activity, along with spearmint. The oregano extract exhibited the strongest antioxidant activity, followed by Greek sage and rosemary. The experimental findings pointed to the potential use of extracts from post-distillation residues of MAPs as antimicrobials in the food industry, in addition to being rich sources of bioactive compounds.

Keywords: herbs; solid waste; extracts; antioxidant activity; antibacterial effect; biological activity; valorization

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1. Introduction

The rapid changes in the economic, industrial, and technological sectors during the first decades of the 21st century have affected the needs of the global market, which are constantly increasing and reshaping. Specifically, regarding the food industry, food market trends have been shaped around consumer needs for minimally processed, natural, "clean label", and plant-based ingredients and food products. Currently, there is a continuous search for raw materials with biological efficacy in improving health that will be able to replace conventional ones but also have the additional advantage of natural origin and/or "green" production. Among them, essential oils (EOs) are abundant in components with important biological activities, namely antimicrobial, antioxidant, anti-inflammatory, etc. Due to the numerous applications of EOs across a range of industrial sectors (e.g., food, pharmaceutical, cosmetic, agricultural industries), the EO global market is expanding quickly. In fact, EOs have been thoroughly researched as possible antibacterial agents and natural food preservatives in various categories of food products as they possess antimicrobial and antioxidant characteristics [1,2].

EOs can be extracted from various plant materials and/or their parts, e.g., leaves, flowers, seeds, bark, peels (citrus), etc. Medicinal and aromatic plants (MAPs) are a significant group of EO-containing plants. Hydrodistillation and steam distillation are the common methods employed for the extraction of EOs from MAPs. Steam distillation is a conventional technique widely used on an industrial scale to obtain commercial EOs, whereas hydrodistillation is mainly applied in laboratory-scale distillations. However, these methods present several disadvantages in terms of time, energy, and water consumption, as well as losses and thermal degradation of volatile compounds [3]. Therefore, innovative, more eco-friendly "green" methods have been developed, aiming to replace conventional distillation process techniques such as the microwave-assisted distillation method. This method is characterized by short distillation times, low energy consumption, and the protection of thermolabile volatile compounds from degradation compared to conventional techniques [4]. However, the use of the microwave-assisted distillation process to obtain EOs is quite limited as it requires expensive equipment with high operational and maintenance costs.

MAPs comprise numerous bioactive compounds with distinctive antimicrobial and antioxidant properties, namely terpenes, phenolic compounds, terpenoids, and other phytochemicals with reported biological effects. The most abundant phenolic compounds are phenolic monoterpenes (carvacrol and thymol) and diterpenes (carnosol and carnosic acid), phenylpropanoic acids (caffeic acid, rosmarinic acid, and salvianolic acids), and flavonoids (naringenin, eriodyctiol, hesperidin, vicenin-2, luteolin-7-glucuronide, apigenin, luteolin, etc.). The chemical structures of the common phenolic compounds in MAPs are illustrated in Figure 1.

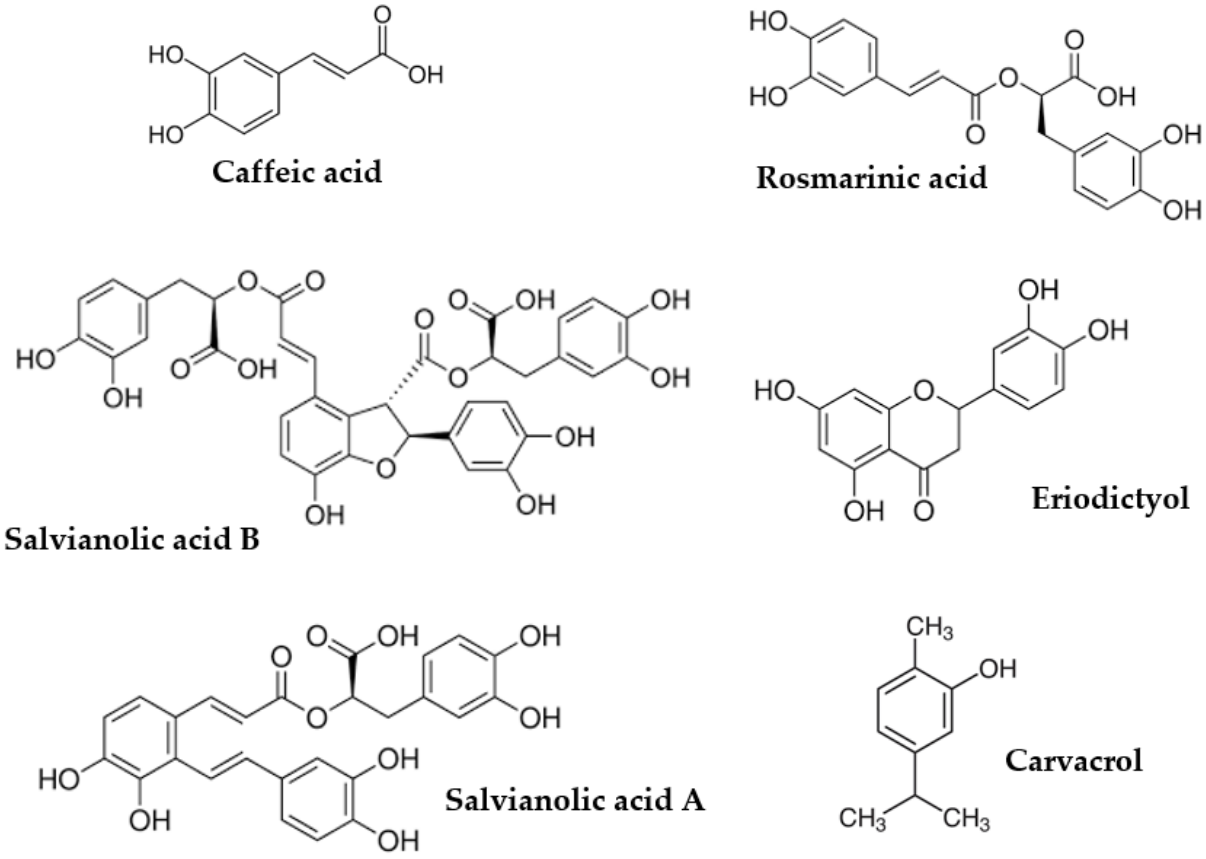

Figure 1. *Cont.*

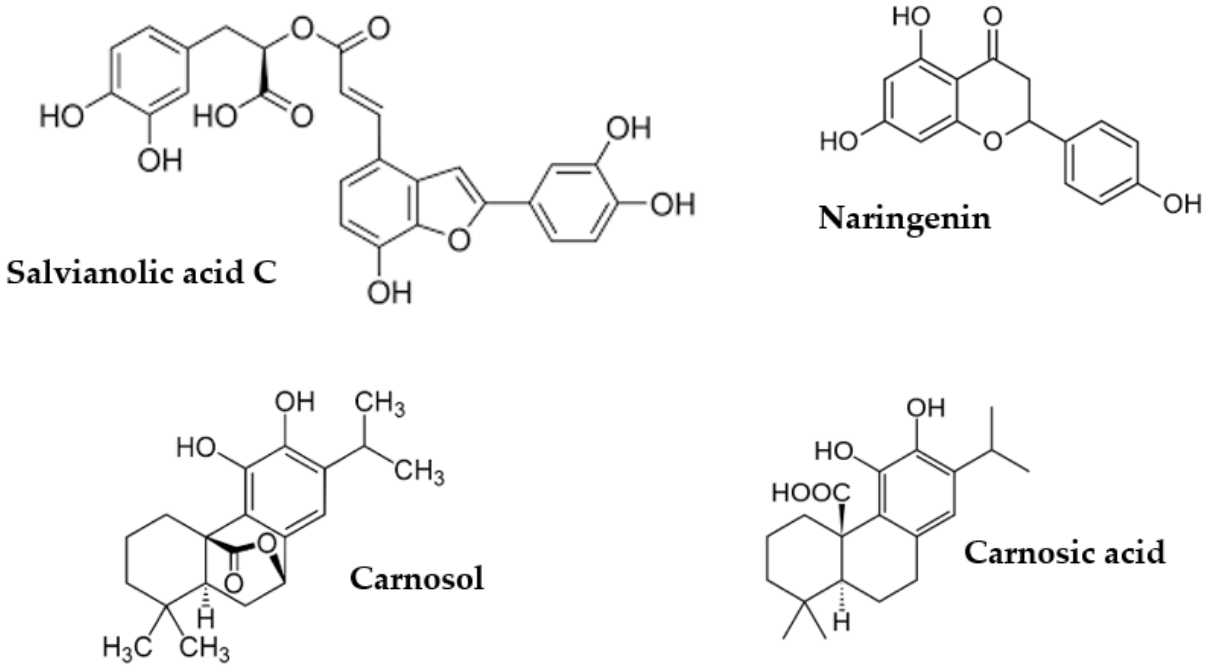

Figure 1. Chemical structures of typical phenolic compounds existing in medicinal and aromatic plants.

The Lamiaceae family includes commercially important species whose EOs have been largely studied. Among them, Greek oregano (*Origanum vulgare* subsp. *hirtum*), rosemary (*Rosmarinus officinalis*), Greek sage (*Salvia fruticosa*), lemon balm (*Melissa officinalis*), and spearmint (*Mentha spicata*), which are investigated in the present study, are common plants of the Mediterranean flora. Greek oregano is an endemic plant to the Mediterranean region, rich in EOs, with the main constituents being carvacrol and thymol, followed by p-cymene and ɣ-terpinene. It also contains tannins and phenolic acids (chlorogenic and rosmarinic), as well as flavonoids, namely naringenin, apigenin, luteolin, and quercetin [5]. Different EO compositions may exist due to different oregano subspecies, chemotypes, plant origins, distillation methods, etc. Oregano EOs and aqueous extracts have strong antioxidant, antimicrobial, anti-inflammatory, and antiproliferative properties associated with the presence of specific bioactive compounds [5].

Rosemary, primarily indigenous to Asia and the Mediterranean region, is rich in EOs, containing mainly 1,8-cineol, α-pinene, camphor, borneol, verbenone, and α-terpineol. The majority of the non-volatile phenolic compounds found in its extracts include chlorogenic acid, rosmarinic acid, and the diterpenes carnosic acid and carnosol [6–8]. Numerous studies have reported the biological activities of rosemary, including antioxidant, anti-inflammatory, antidiabetic, antibacterial, and cognitive-enhancing properties [9].

Spearmint, which is native to the Mediterranean region and southern temperate Asia, is used in herbal infusions and as a flavoring agent in several food preparations and health care products. Carvone, spearmint EO's most prevalent volatile component, is responsible for its distinctive flavor and aroma. Other EO constituents include limonene, pulegone, linalool, 1,8-cineole, piperitone, menthone, and isomenthone [10,11]. Additionally, several bioactive components, among them flavonoids, phenolic acids, triterpenoids, and steroids, have been reported in spearmint extracts.

Greek sage is a significant medicinal plant endemic to the Eastern Mediterranean basin [12], and it is a source of phenolic compounds, such as flavonoids, phenolic acids, and tannins, as well as terpenoids, which have strong antioxidant capacities [13]. More than 75 constituents, including 1,8-cineole, α-pinene, camphene, β-pinene, camphor, α-terpineol, thujone, etc., have been identified in *Salvia fruticosa* EO [14–16]. These compounds are responsible for the EO's bactericidal and fungistatic properties against *Bacillus*, *Staphylococcus*, *Salmonella*, *Listeria*, *Proteus*, *Pseudomonas*, *Penicillium*, *Candida*, and *Aspergillus* strains [16].

Lemon balm is also a perennial herb native to the Mediterranean basin, central Asia, and Iran. Its EO is rich in neral, geranial, citronellol, citronellal, isogeraniol, neryl acetate, geraniol acetate, β-caryophyllene, and β-caryophyllene oxide, while important phenolic compounds have been detected in lemon balm extracts, such as luteolin, quercetin, rosmarinic acid, rhamnocitrin, caffeic acid, and protocatechuic acid [17].

However, after distillation and EO recovery, as they are non-volatile and less readily degradable by heat treatment, the majority of the phenolic components of the raw material remain in the solid plant residue [18]. In addition, since the EO yield ranges between 0.5 and 8% *w*/*w* of the dry biomass, it is obvious that a huge amount of biomass is generated as a by-product. As reported by Olofsson and Börjesson [19], any biological substance that is not purposefully generated throughout the course of production is known as residual biomass. This means that residual biomass is produced as a by-product, which may or may not be waste. Although the concept of valorizing such by-products for the recovery of important bioactive compounds is not novel, the interest of researchers in this topic has been quickly increasing in the last few years. Environmental concerns have led to the adoption of new ways of processing and end-of-life options for raw materials, aiming to reduce waste volume. In this respect, technological developments have also enabled the implementation of novel technologies for this purpose.

Many studies in the literature have investigated the extraction of phenolic compounds from post-distillation residual biomass (e.g., [20–24]), whereas the topic has also been discussed in some very interesting reviews [25,26]. In this context, most of the studies mainly focus on the extraction process optimization or extract characterization regarding the total phenolic content and antioxidant activity. On the other hand, few studies have examined the antibacterial effects of post-distillation residual biomass or those of the extracts derived from these materials [27–31]. In fact, to the best of the authors' knowledge, no data are available regarding the post-distillation extracts' antibacterial activity of the four out of five studied Lamiaceae plant materials, rosemary being the exception [27,29]. In this context, the current study's objective was to evaluate the antibacterial and antioxidant potential of phenolic extracts from the distillation solid wastes of Greek oregano, rosemary, Greek sage, lemon balm, and spearmint against *E. coli*, *S. Typhimurium*, *L. monocytogenes*, *S. aureus*, two *B. subtilis*, two *B. licheniformis*, and one *B. cereus* strain, aiming to further valorize them as novel natural antioxidants and antimicrobial agents.

2. Materials and Methods

2.1. Plant Materials

The plant materials, consisting of the aerial parts of *Origanum vulgare* subsp. *hirtum* L. (Greek oregano), *Rosmarinus officinalis* L. (rosemary), *Mentha spicata* (spearmint), *Melissa officinalis* L. (lemon balm), and *Salvia fruticosa* Miller (Greek sage), were collected during the flowering season in 2022 from the Hellenic Agricultural Organization "Dimitra" (Institute of Plant Breeding and Genetic Resources, Thermi, Thessaloniki, Greece; coordinates: 40°33′ N, 23°01′ E) cultivated accessions and were subjected to a 2 h steam distillation process in a pilot-scale essential oil distillation unit. Following distillation, the wet solid residue of each plant material was sun-dried for 48 h. After being dried to around 10% moisture content, the material was ground (<0.5 mm) in a laboratory mill (Retsch, Model ZM1000, Haan, Germany) and kept at 4 °C until the analysis was performed.

2.2. Chemicals and Reagents

Sigma-Aldrich (Steinheim, Germany) supplied the analytical reagents 2,2-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. Extrasynthese (Genay Cedex, France) supplied the analytical standards for rosmarinic acid (RMA), gallic acid (GA), and catechin (CAT), while Carbosynth (Berkshire, UK) supplied the standards for carnosol (CARO), carnosic acid (CARA), and salvianolic acid B. For the chromatographic analysis and extraction, only HPLC- or LC-MS-grade solvents were employed.

2.3. Ultrasound-Assisted Extraction (UAE) of Phenolics from Post-Distillation SRs

Samples of dried and ground SRs (0.01 g) were extracted with 20 mL of 50% ethanol (v/v) for 2 min at 30 °C using an ultrasonic probe (model HD 4100, Sonoplus, Berlin, Germany), working with a frequency of 20 kHz and adopting an amplitude of 50%, a pulse length of 2 s, and an interval of 0.5 s. The extract was then centrifuged at 10,000× g for 10 min at 4 °C and filtered through Whatman filter paper no. 1, and the ethanol was removed using a rotary evaporator (Heidolph Instruments GmbH & Co. KG, Kelheim, Germany). The remaining liquid was lyophilized (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 48 h. The extract was then stored under freezing conditions (<−20 °C) until use.

2.4. Bacterial Strains and Cultures

The following well-known pathogens and spoilage microorganisms were used to test the antibacterial activity: *Escherichia coli* ATCC 25922 (Ec) (American Type Culture Collection, Manassas, VA, USA), *Staphylococcus aureus* ATCC 25923 (St), *Bacillus subtilis* NCFB 1069 (Bs1) (National Collection of Food Bacteria, Reading, UK—incorporated with NCIMB), *Bacillus subtilis* NCIMB 3610 (Bs2) (National Collection of Industrial, Food and Marine Bacteria, NCIMB Ltd., Aberdeen, Scotland, UK), *Bacillus licheniformis* NCDO 735 (Bl2) (National Collection of Dary Organisms, which incorporated in NCFB), *Salmonella enterica* subsp. *enterica* ser. Typhimurium DSM 17058 (St) (DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany), *Listeria monocytogenes* DSM 15675 (Lm), *Bacillus licheniformis* DSM 13 (Bl1), and *Bacillus cereus* DSM 31 (Bc). For convenience, the names of bacteria thereafter are abbreviated with their initials, as given above in parenthesis.

The strains were preserved in TSB (Tryptone Soya Broth Oxoid, Basingstoke, UK) containing 25% glycerol at −80 °C, then activated with two subsequent cultures in TSB, and incubated overnight at 30 °C for *Bacillus* species and at 37 °C for the other bacteria before the trials.

2.5. Assessement of Antibacterial Activity

The antibacterial properties of the phenolic extracts were assessed with the broth dilution method [32] in flat-bottomed 96-well microtiter plates (Corning, NY, USA). Each extract diluted in an aqueous solution was incorporated into TSB (Tryptone Soya Broth Oxoid, Basingstoke, UK), forming the stock solution (10,000 mg/L). Specifically, 180 μL of the broth containing various concentrations (500, 750, 1500, and 3000 mg/L) of the aqueous extracts were distributed in each well of the sterile polystyrene microtiter plate. All rows of the wells were inoculated with a volume of 20 μL of the activated bacterial culture (approximately 10^6 CFU/mL). For each tested bacterium, inoculations were performed in triplicate (three columns) for each extract concentration. TSB with extracts of each concentration without inoculum as well as inoculated TSB without any extract (optimum growth) served as controls. The optical density was monitored at 620 nm (at a temperature of 30 °C) in a BIOTEK TS 800 (BioTek® Instruments, Inc., Winooski, VT, USA) microplate reader before incubation (0 h) and after incubation for 48 h at 30 °C for bacilli and 37 °C for the other bacteria. A representative image of the 96-well microtiter plates of the SR oregano extract at 0 h and 48 h is illustrated in Figure S1 (Supplementary data). The plate was agitated for 10 sec before each measurement. Absorbance measurements were used to calculate the percent (%) inhibition of bacterial growth due to the presence of an extract in the growth medium. In particular, the following relationship was used to compute the percentage of inhibition:

$$\% \text{ inhibition of growth} = [(A_{620\,nm} \text{ of control} - A_{620\,nm} \text{ of sample})/A_{620\,nm} \text{ of control}] \times 100$$

2.6. Determination of Total Phenolic (TPC) and Flavonoid Content (TFC)

The Folin–Ciocalteu spectrophotometric technique, slightly modified, was used to determine the TPC of the SR phenolic extracts [33]. Briefly, 0.8 mL of 1:10 diluted Folin–Ciocalteu reagent and 0.2 mL of phenolic extracts (0.005 g of dried extract was dissolved in 4 mL of 50% ethanol) were combined. Two minutes later, two milliliters of sodium carbonate (75 g/L) was added, and distilled water was used to adjust the final volume to ten milliliters. After 1 h of incubation at room temperature, the absorbance was obtained at 725 nm, and the results were reported as mg GAE/g extract. The TFC was determined using the colorimetric test with aluminum chloride according to the protocol of Bao et al. [34]. The test involved the mixing of 300 µL of phenolic extract with 225 µL of sodium nitrite (50 g/L), then the addition of 225 µL of 10% aluminum chloride hexahydrate (100 g/L), and finally 750 µL of NaOH (2 N). The absorption was measured at 510 nm after 20 min of incubation. The results for the TFC were expressed as mg CATE/g extract.

2.7. Determination of Antioxidant Activity of Phenolic Extracts

2.7.1. ABTS Radical Scavenging Assay

The scavenging ability of phenolic extracts against ABTS radical cations was assessed according to Re et al. [35]. The test involved the mixing of 3.9 mL of the ABTS+ solution with 100 µL of phenolic extract, and after 4 min, the absorbance was measured at 734 nm in comparison to a control. Trolox equivalents (TEs) per gram of dry weight (mg TE/g) were used to express the ABTS results.

2.7.2. DPPH Radical Scavenging Assay

With a few minor adjustments, the scavenging activity of the phenolic extracts based on the DPPH test was assessed in accordance with Yen et al. [36]. The method involved mixing 2.85 mL of newly prepared 0.1 mM DPPH in methanol with 100 µL of phenolic extracts, and the decrease in absorbance was recorded at 516 nm after 5 min of reaction. The DPPH data were reported as mg TE/g.

2.7.3. Ferric Reducing Antioxidant Power (FRAP) Assay

Based on the Benzie and Strain [37] method, 3 mL of FRAP solution and 100 µL of phenolic extract were mixed together at 37 °C to determine the FRAP activity of the extracts. Exactly 4 min later, the absorbance at 593 nm was recorded in comparison to a control, and the FRAP results were reported as mg TE/g.

2.8. HPLC-DAD-MS Quantification of Phenolics from Solid Residues Extracts

The identification of the phenolic compounds in post-distillation SR extracts as well as the quantification of the main phenolic compounds (rosmarinic acid, RMA; carnosol, CARO; and carnosic acid, CARA) were carried out according to the protocol described by Irakli et al. [24], using a Shimadzu Nexera HPLC system (Kyoto, Japan), equipped with a diode array detector (DAD) and a single-quadrupole mass spectrometer combined with an electrospray ionization (ESI) interface. Phenolic compounds were separated on a Poroshell 120 EC-C_{18} column (4.6 × 150 mm, 4 µm) thermostated at 35 °C with a flow rate of 0.5 mL/min and an injection volume of 10 µL. The mobile phase consisted of 0.1% aqueous formic acid (*v*/*v*) (solvent A) and acetonitrile (solvent B), adopting the following gradient program: 0 min, 15% B; 5 min, 25% B; 10 min, 35% B; 28 min, 60% B; 28.01 min, 60% B; 35 min, 100% B; 35.01 min, 15% B; 42 min 15% B. The DAD acquisition ranged from 190 to 400 nm, while the mass spectrometer recorded in a negative ionization mode. The interface and curved desolvation line (CDL) voltages were +4.5 kV and 20 V, respectively, while the temperatures of the block heater and CDL were adjusted at 200 °C and 250 °C, respectively. The flow rates of nebulizing gas and drying gas were 1.5 L/min and 15 L/min, respectively.

For identification, mass acquisitions were performed in a full scan mode in the range of 100–1000 *m*/*z* using Lab Solutions LC-MS software version 5.97.1, (Shimadzu, Kyoto, Japan). By comparing the samples' retention periods, UV absorbance spectra, and mass spectra of

unknown peaks with those of reliable standards or published data, the primary phenolic chemicals were identified in the samples. For quantification, a selective ion monitoring (SIM) mode was performed using the calibration curves of the relevant standard solutions. Salvianolic acid B's calibration curve served as the basis for quantification in the case of the isomers of the acid. The results of the analyses were expressed as mg per g of extract, and the analyses were carried out in triplicate.

2.9. Statistical Analysis

The experimental results were expressed as the means ± standard deviation of three measurements. SPSS Statistics, version 25 (IBM SPSS Inc., Chicago, IL, USA), was used to analyze the data. Duncan's multiple range test was used to determine whether there were any differences between the means for various extracts using a one-way analysis of variance (ANOVA); differences at $p < 0.05$ were regarded as significant.

3. Results and Discussion

3.1. Antibacterial Effect of Solid Residues Extracts in the Microplate Assay

The antimicrobial activity of phenolic extracts derived after UAE with 50% ethanol from the SRs remaining after the EO distillation of five aromatic plant species (i.e., rosemary, Greek sage, Greek oregano, spearmint, and lemon balm) was screened against selected foodborne pathogens and spoilage bacteria. The antibacterial activity of the SR extracts at four different concentrations (500, 750, 1500, and 3000 mg/L) was examined against *E. coli* ATCC 25922 (Ec), *S. aureus* ATCC 25923 (Sa), *S. Typhimurium* DSM 17058 (St), *L. monocytogenes* DSM 15675 (Lm), *B. subtilis* NCIMB 3610 (Bs1), *B. subtilis* NCFB 1069 (Bs2), *B. licheniformis* DSM 13 (Bl1), *B. licheniformis* NCDO 735 (Bl2), and *B. cereus* DSM 31 (Bc). In the control inoculation wells (without the inclusion of extract), every strain grew to its optimum potential.

The antibacterial capacity of the studied SR phenolic extracts against pathogenic and spoilage microorganisms was confirmed during this study. Bacteria belonging to the genus *Bacillus* were selected to be tested, with the intention of the extracts being utilized in bread products, where bacilli are a group responsible for food spoilage, causing ropiness in bakery products [38]. As can be seen (Figures 2–6), different SR extracts showed varying efficiencies against the tested strains. Overall, the *S. aureus*, *L. monocytogenes*, and bacilli strains (all Gram-positive) proved to be the most sensitive to all the extracts investigated, although at different concentration levels. The lowest growth inhibition effect was detected for *S. Typhimurium* and *E. coli* (both Gram-negative), as Gram-negative bacteria have an outer membrane consisting of lipopolysaccharides that restrict the diffusion of hydrophobic compounds [39].

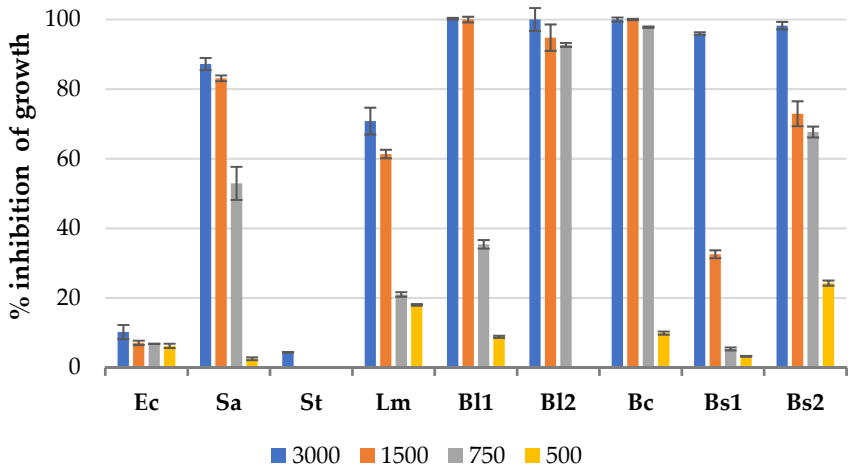

Figure 2. Antimicrobial activity (% inhibition of growth at 48 h) of rosemary SR extracts against pathogenic and spoilage bacteria using different concentration levels (mg/L) of the extracts.

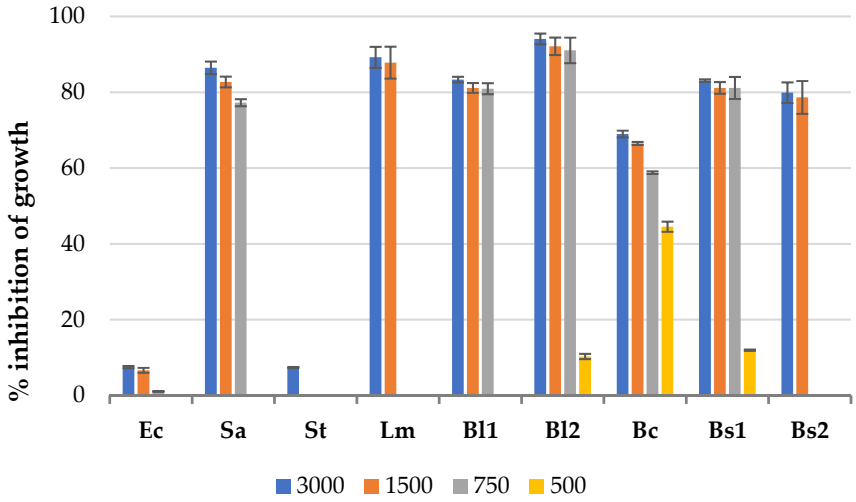

Figure 3. Antimicrobial activity (% inhibition of growth at 48 h) of Greek sage distillation SR extracts against pathogenic and spoilage bacteria using different concentration levels (mg/L) of the extracts.

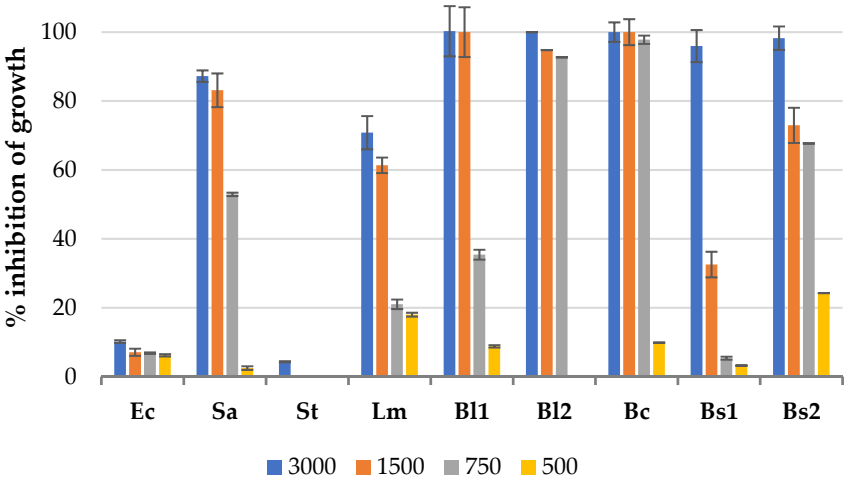

Figure 4. Antimicrobial activity (% inhibition of growth at 48 h) of spearmint SR extracts against pathogenic and spoilage bacteria using different concentration levels (mg/L) of the extracts.

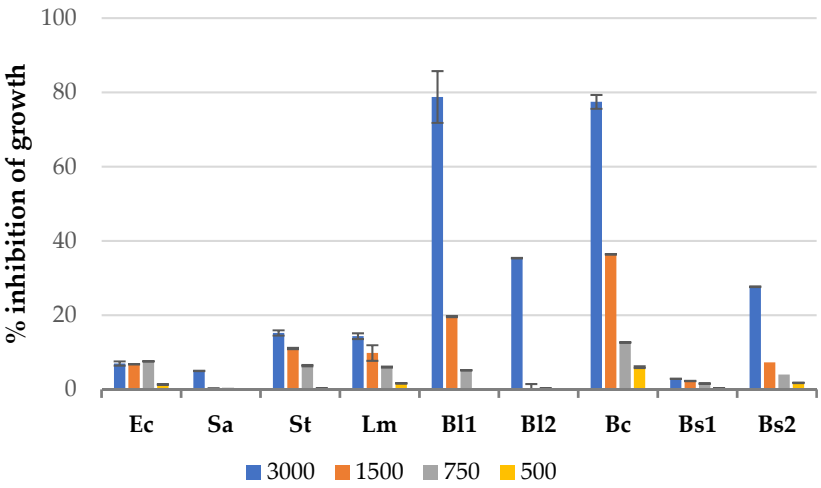

Figure 5. Antimicrobial properties (% inhibition of growth at 48 h) of Greek oregano SR extracts against pathogenic and spoilage bacteria using different concentration levels (mg/L) of the extracts.

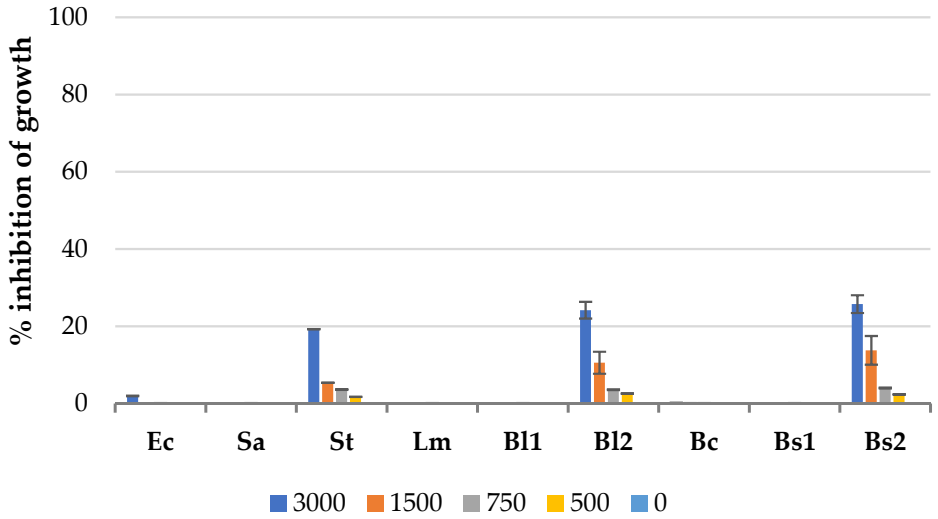

Figure 6. Antimicrobial properties (% inhibition of growth at 48 h) of lemon balm SR extracts against pathogenic and spoilage bacteria using different concentration levels (mg/L) of the extracts.

Overall, the Gram-positive bacteria (Sa, Lm, Bc, Bs1, Bs2, Bl1, and Bl2) were more susceptible than the Gram-negative ones (Ec and St); Gram-positive bacteria have been previously reported to be more sensitive to plant extracts than Gram-negative bacteria [17], although some researchers found that specific plant extracts (rosemary, roselle, clove, and thyme) significantly alter the cell membrane structure of both Gram-positive and Gram-negative bacteria, causing a substantial decrease in cytoplasmic pH [40]. This is consistent with earlier studies [29] regarding the antibacterial activity of SRs of rosemary incorporated into an agar, and it can be attributed to variations in bacteria cell membrane structures. The porins found in Gram-negative bacteria possibly restrict the entry of some solutes and make them less vulnerable to antibacterial chemicals, making the outer peptidoglycan layer of those bacteria an effectual permeability barrier [41].

The rosemary, Greek sage, and spearmint SR extracts exhibited certain antibacterial activity against Gram-positive bacteria (*S. aureus*, *L. monocytogenes*, and bacilli strains) (Figures 2–4). Specifically, the rosemary SR extract (Figure 2) caused a reduction in the cell densities of all the Gram-positive bacteria. The bacilli strains were inhibited between 98 and 100% at a concentration of 3000 mg/L; Bc and Bl2 were reduced by >90% and Bl1 > 80% even at a concentration of 750 mg/L. The Bs1 and Bs2 cell densities were reduced by 96% and 98% at 3000 mg/L, 33% and 73% at 1500 mg/L, and 5% and 68% at 750 mg/L, respectively. A considerable reduction of 87 and 71% at 3000 mg/L and 83% and 61% at 1500 mg/L was observed for Sa and Lm, respectively. Additionally, Sa was reduced by 53% at a concentration of 750 mg/L.

The Greek sage SR extract (Figure 3) caused a reduction in the cell densities of the Gram-positive bacteria between 69 and 90% at a concentration of 3000 mg/L. The pathogenic Sa and Lm strains were reduced by >80%, even at a concentration of 1500 mg/L, while the bacilli were reduced between 69 and 94% at a concentration of 3000 mg/L.

The spearmint SR extract caused a reduction in the cell densities of the Gram-positive bacteria by >70% at the highest level of the tested concentrations (3000 mg/L) (Figure 4). Generally, the best extract in terms of antibacterial efficiency against pathogenic Gram-positive bacteria was Greek sage, followed by spearmint and rosemary. With regard to its antibacterial activity against bacilli, rosemary was more effective, exhibiting an inhibition of growth (cell densities) of over 80% even at low concentrations (750 mg/L), followed by Greek sage and spearmint.

This study validates earlier findings in the literature, according to which an increase in extract concentration (%) is directly related to an increase in antibacterial activity [42]. Luca et al. [29] also reported that a post-distillation SR extract of rosemary obtained with

UAE using, as a solvent, a methanol/water 75/25 (*v*/*v*) mixture exhibited significant antimicrobial action against *S. aureus* and *B. cereus* but had no effect against pathogenic Gram-negative bacteria, including *S.* Typhimurium and *E. coli* strains. Ziani et al. [27] noted the antibacterial activity of *Rosmarinus tournefortii* SR extracts against *Listeria innocua* and *E. coli* and revealed that the ethanol/water concentration had an effect on antimicrobial activity. They reported that 20% ethanol was the most effective against *L. innocua* and *E. coli*, whereas decreasing the ethanol concentration from 50 to 20% resulted in increased antibacterial activity, probably due to the higher polarity of the extracted compounds [40]. Other findings in the literature also indicate that ethanolic/alcoholic extracts [43,44] display higher antimicrobial activity than the corresponding aqueous extracts, presumably due to variations in the compositions of the extracted materials from the SRs.

The oregano (Figure 5) and lemon balm (Figure 6) SR extracts presented similar antibacterial activity patterns against the tested strains, i.e., a lack of antibacterial effect against all the pathogenic bacteria. However, the Greek oregano extracts exhibited moderate inhibition against some *Bacillus* strains, Bl1 and Bc, at 3000 mg/L. These results are in accordance with previous studies [29], where a lack of antibacterial activity of oregano SRs against *E. coli*, *S.* Typhimurium, and the two *B. subtilis* strains was reported, along with limited activity against *B. cereus* and the two *B. licheniformis* strains, for which only at the maximum concentration was any effect observed (20 mg/mL).

Although lemon balm (Figure 6) exhibited the lowest antibacterial activity, it had some effect against the Gram-negative bacteria, causing a reduction of 19% in the cell densities of St compared to the control at a concentration of 3000 mg/L. With regard to the Gram-positive bacteria, Bs2 had a reduction of 26 and 14% at the two higher concentrations used (3000 and 1500 mg/L, respectively), and Bl2 exhibited a reduction of 24% at 3000 mg/L.

According to previous studies, the antibacterial properties of MAPs' EOs and extracts [45–47] are attributed mainly to their phenolic compounds. Therefore, the variation in their chemical compositions of EOs and solvent extracts may reflect the differences in their antimicrobial activities [43]. Since the level of phenolic compounds in EOs is positively correlated to their antibacterial activity [41], the limited antibacterial activity of the oregano and lemon balm SR extracts is likely explained by the fact that the majority of the essential oil from oregano has been removed during the distillation process, along with the recognized potent antibacterial properties of carvacrol and thymol [48–50] and the fact that the main constituents present in the post-distillation SR extract are not effective against the studied bacteria. Therefore, the chemical composition of the SR extracts may be important information in relation to the antibacterial activity of each extract.

3.2. Total Phenolic Content, Total Flavonoid Content, and Antioxidant Activity of SR Extracts

Phenolic compounds are an important class of secondary plant metabolites, with bioactive potential linked to their antioxidant and antibacterial characteristics. As previously reported, post-distillation SRs from MAPs are important sources of bioactive compounds. Since distillation mainly aims to recover volatiles, the majority of non-volatile phenolic compounds present in the tissues of MAPs are not extractable, so they remain in the SRs. The total phenolic content (TPC), expressed as mg gallic acid equivalent per g of extract (mg GAE/g), and the total flavonoid content (TFC), expressed as mg catechin equivalent per g of extract (mg CATE/g), of the five SR extracts are shown in Figure 7. The ethanol/water extracts of the five SRs had significant TPC and TFC values. The findings show that the extracts of lemon balm and spearmint SRs presented the highest TPC (255 mg GAE/g) and TFC (395 mg CATE/g) values, respectively. The differences observed between the two extracts were not statistically significant ($p < 0.05$), neither for the TPC nor for the TFC. On the other hand, the lowest TPC (138 mg GAE/g) and TFC (220 mg CATE/g) values were found for the rosemary SR extracts ($p < 0.05$). The Greek sage and oregano SR extracts had similar TPC values (175 and 185 mg GAE/g, respectively) ($p < 0.05$), while in terms of the TFC, the Greek sage extract presented higher values than oregano ($p < 0.05$), 270 and 225 mg CATE/g, respectively. To the best of the authors' knowledge, there are no data in the literature directly comparing the composition

of the post-distillation SR extracts of these MAPs in terms of the TPC and TFC. An extract of Greek sage SRs obtained by UAE with 67.9% ethanol after optimization studies was reported in our earlier study [22] to have a TPC of 192 mg GAE/g and a TFC of 272 mg CATE/g. We have also reported in a previous study [24] that 70% methanolic extracts of spearmint and lemon balm, extracted using an ultrasound bath, had lower values of TPC and TFC than those found for the 50% ethanolic extracts in the present study. With regard to rosemary, Luca et al. [29] reported that an extract obtained from rosemary post-hydrodistillation SRs had a TPC of 57.68 mg GAE/g and a TFC of 19.86 mg CATE/g. These values are lower than those in our study, possibly due to the different method applied for the distillation of EOs, the extraction method applied, the type of solvent (methanol versus ethanol), and the chemical composition of the initial material [22,23].

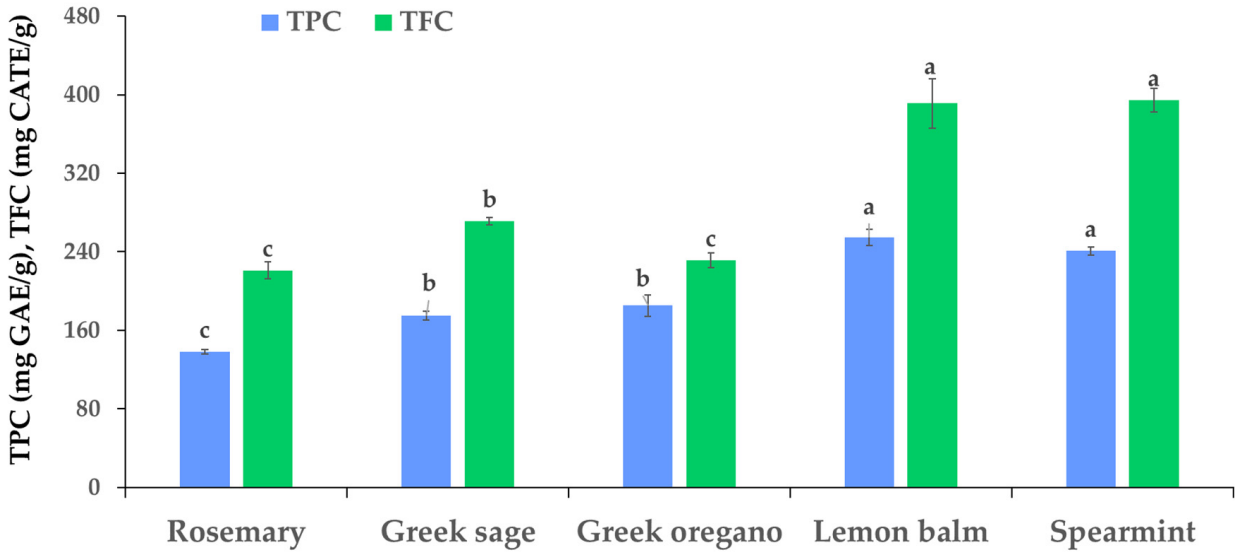

Figure 7. Total phenolic content (TPC) and total flavonoid content (TFC) in 50% ethanolic extracts from post-distillation solid residues of rosemary, Greek sage, Greek oregano, lemon balm, and spearmint retained after the recovery of essential oil by steam distillation. Different letters among columns with the same color indicate statistically significant differences according to Duncan's post hoc test ($p < 0.05$).

Phenols are essential components of plants, and the hydroxyl groups on phenols give them their ability to scavenge free radicals [51]. The antioxidant capacity of the five extracts, as determined by different assays (ABTS, DPPH, and FRAP), is presented in Figure 7. Similar trends were noted in terms of antioxidant activity, consistent with those of the TPC, since phenolics are the main antioxidant compounds in the SR extracts. The differences among the samples were even more profound. More specifically, as observed in Figure 8, the lemon balm SR extracts presented the highest antioxidant activity in all three assays, followed by spearmint and Greek oregano, respectively ($p < 0.05$). The Greek sage SR extracts exceeded the antioxidant capacity of the rosemary ones when measured by the FRAP and ABTS assays ($p < 0.05$), but the differences in the values of the DPPH assay were not statistically significant. The concentration of the major bioactive compounds is one of the key factors that determines the biological activity (e.g., antioxidant) of the SR extracts [20]. However, the present results could also indicate that although some of the extracts may contain similar amounts of total phenolics, their respective bioactivity or their ability to scavenge free radicals can slightly differ. This is attributed to the groups of phenolic compounds present in the respective plant materials and their concentration in the extracts. Although extracts from post-distillation SRs of MAPs usually exhibit comparatively lower antioxidant activity compared to the original plant extracts, considerable amounts of such bioactive antioxidant compounds can also be recovered from the distilled by-products [18], as is shown by the present findings.

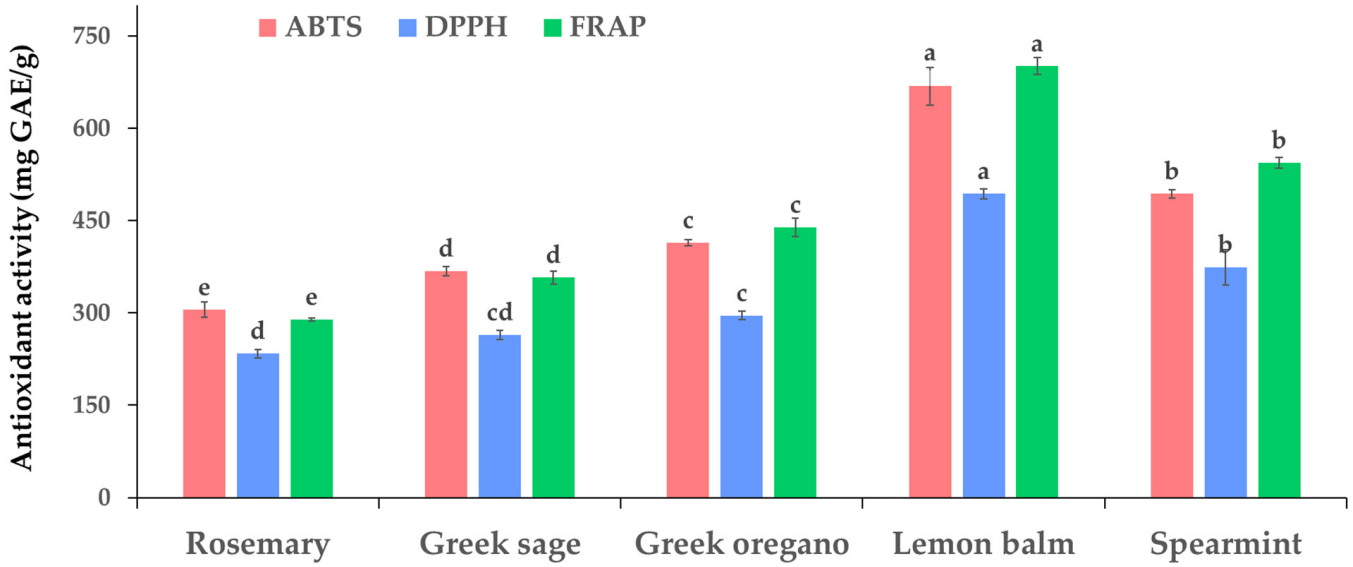

Figure 8. Antioxidant activity evaluated by ABTS, DPPH, and FRAP assays in ethanolic extracts from distillation SRs of rosemary, Greek sage, Greek oregano, lemon balm, and spearmint retained after the recovery of essential oil by steam distillation. Different letters among columns with the same color indicate statistically significant differences according to Duncan's test ($p < 0.05$).

3.3. Phenolic Composition of SR Extracts

As mentioned above, the observed differences in the TPC and TFC values of the SR extracts could be attributed to different parameters, with the most important being the different chemical profiles of the bioactive compounds of the individual MAPs under investigation. Therefore, it would be interesting to further explore the chemical components of the five extracts in order to explain the variation in their antioxidant and antibacterial activity, which can possibly be attributed to specific compounds.

Forty-four major compounds were identified by LC–DAD-MS in the SRs of Greek oregano (O), lemon balm (L), spearmint (SP), rosemary (R), and Greek sage (S), as shown in Table 1. Although the major compounds of the five plants' extracts from distillation SRs were similar, a substantial variation in their chemical profiles across the species was found (Table 1). Figure 9 displays representative LC-MS chromatograms of the phenolic components detected in the rosemary, Greek sage, Greek oregano, lemon balm, and spearmint extracts. As shown in Table 1, in the phenolic extracts of the aforementioned SRs, 37 out of the 44 main phenolic compounds, representing more than 84% of the total detected peaks, were successfully identified. The mass and UV spectra of the peaks that were identified from data in the literature are given in Figures S2 and S3, respectively (Supplementary data). Rosmarinic acid (peak 22), carnosol (peaks 40, 41), and carnosic acid (peak 42) comprised a significant percentage of the total compounds that were identified.

More specifically, the predominant identified compounds in all the SR extracts were rosmarinic acid (peak 22), followed by salvianolic acid isomers (peaks 9, 15, 17, 20, 23, 25, 26, 28, 31, 32). The Greek sage and rosemary extracts were distinguished by the presence of rosmanol isomers (peaks 37, 38, 39, 43). All SR extracts contained quinic acid (peak 3), danshensu (peak 5), gallocatechin isomer (peak 7), caffeic acid (peak 8), and salvianolic acid A (peak 26). The flavonoids luteolin-7-O-glucuronide (peak 13), luteolin-7-O-rutinoside (peak 10), and luteolin (peak 30) were found in the extracts of spearmint, Greek sage, and rosemary. Hesperidin was only found in the extracts of rosemary and spearmint, while luteolin-7-O-glucoside (peak 12) was present in the lemon balm extract and isorhamnetin-3-O-D-glucoside (peak 14) in both rosemary and Greek sage extracts.

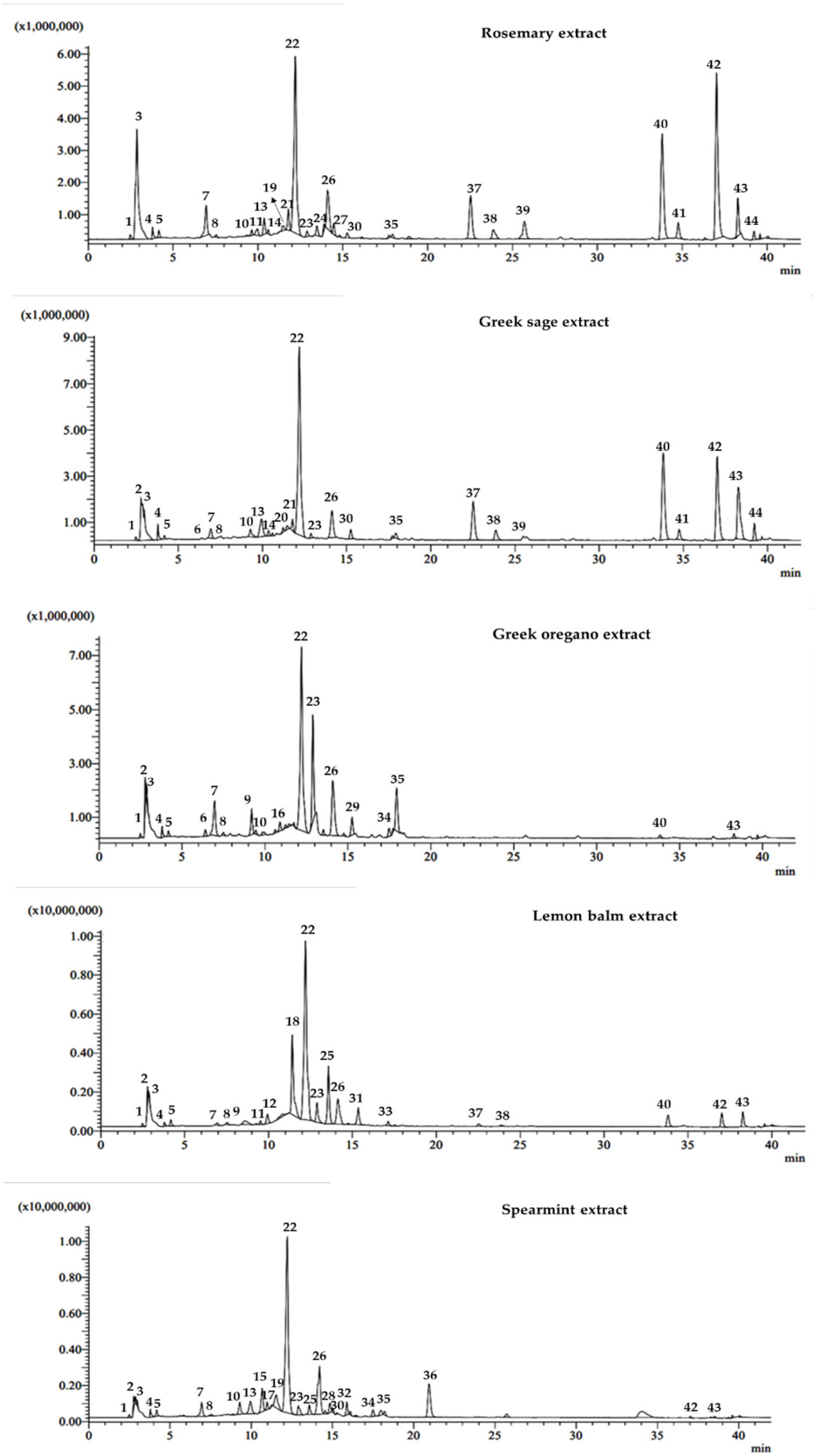

Figure 9. Mass chromatograms with negative ion mode recordings for 50% ethanol extracts from post-distillation SRs of rosemary, Greek sage, Greek oregano, lemon balm, and spearmint retained after the extraction of essential oil from the respective plant tissues by steam distillation.

Table 1. List of tentative major compounds identified by LC–DAD-MS in the essential oil post-distillation solid residues of Greek oregano (O), lemon balm (L), spearmint (SP), rosemary (R) and Greek sage (S) extracts.

Peak	RT (min)	UV λmax (nm)	[M-H]- (*m*/*z*)	Tentative Identification	Ref.	Extract
1	2.48	-	289	unknown	-	O, L, SP, R, S
2	2.79	256	387	unknown	-	O, L, SP, S
3	2.89	277	191	quinic acid	st	O, L, SP, R, S
4	3.79	279	191	citric acid	st	O, L, SP, R, S
5	4.17	281	197	danshensu	[52,53]	O, L, SP, R, S
6	6.39	270, 335	593	vicenin-2	st	O, S
7	6.93	239, 284, 314	305	gallocatechin isomer	[54,55]	O, L, SP, R, S
8	7.49	320	179	caffeic acid	st	O, L, SP, R, S
9	9.21	252, 285, 344	537(493)	salvianolic acid isomer	[56,57]	O, L
10	9.28	260, 345	593	luteolin-7-O-rutinoside	st	O, SP, R, S
11	9.63	239, 275	597	yunnaneic acid F	[52,58]	L, R
12	9.93	253, 366	447	luteolin-7-O-glucoside	st	L
13	9.95	260, 345	461	luteolin-7-O-glucuronide	st	SP, R, S
14	10.36	272, 345	477	isorhamnetin-3-O-D-glucoside	st	R, S
15	10.62	254, 283, 341	717(579)	salvianolic acid L	[53,59]	SP
16	10.91	239, 284, 333	578(303)	unkown	-	O
17	10.98	232, 285, 334	717(537)	salvianolic acid isomer	[53,59]	SP
18	11.42	329	439	sulphated rosmarinic acid	[58]	L
19	11.45	283	609	hesperidin	st	SP, R
20	11.62	241, 286, 321	555	salvianolic acid K	[60]	S
21	11.72	331	461	hispidulin-7-O-glucoside	[61]	R, S
22	12.22	329, 285sh	359	rosmarinic acid	st	O, L, SP, R, S
23	12.88	287, 325	717(537)	salvianolic acid B	st	O, L, SP, R, S
24	13.47	243, 269, 337	503	caffeoyl-hexosyl-hexose	[52]	R
25	13.53	239, 293, 338	537(493)	lithospermic acid A	[53,58]	L, SP
26	14.08	239, 299 (240)	493(137)	salvianolic acid A (IS)	[53,58]	O, L, SP, R, S
27	14.45	243, 269, 337	503	caffeoyl-hexosyl-hexose	[52]	R
28	14.56	245, 286, 334	715(537)	salvianolic acid isomer	[53]	SP
29	15.26	287	287	eriodictyol	st	O
30	15.27	282	285	luteolin	st	SP, R, S
31	15.35	243, 286, 318	715(493)	salvianolic acid C	[57]	L
32	15.88	241, 286, 321	717(519)	salvianolic acid E	[53,59]	SP
33	17.13	-	583	unknown	-	L
34	17.49	259, 294, 334	329	unknown	-	O, SP
35	17.94	288	271	naringenin	st	O, SP, R, S
36	18.20	289, 345	359	rosmarinic acid derivative	[53]	SP
37	22.53	280	345	rosmanol isomer	[52,60]	L, R, S
38	23.87	280	345	rosmanol isomer	[52,60]	L, R, S
39	25.48	280	345	rosmanol isomer	[52,60]	S
40	33.79	280	329(285)	carnosol	st	O, L, R, S
41	34.76	280	329(285)	carnosol	st	R, S
42	37.02	280	331(287)	carnosic acid	st	L, SP, R, S
43	38.28	279	345	rosmanol isomer	[52,53,60]	O, L, SP, R, S
44	39.22	263, 286	317	unkown	-	R, S

IS, internal standard (salicylic acid); in parentheses (), the fragments observed.

After identification by LC/ESI-MS, the majority of the phenolic compounds were quantified, and the most significant ones are shown in Table 2. The major phenolic compounds in the rosemary and Greek sage extracts were the phenolic diterpenoids (carnosol and carnosic acid), followed by rosmarinic acid. Spearmint, Greek oregano, and lemon balm did not contain the diterpenoids. Instead, the main phenolic compounds in those extracts were rosmarinic acid and salvianolic acid isomers. More specifically, spearmint, followed by lemon balm, Greek sage, Greek oregano, and rosemary, had the highest content of rosmarinic acid. In an extensive review by Skendi et al. [25], it was reported that

rosmarinic acid is abundant in post-distillation SRs from MAPs. Similarly, it was reported that the level of rosmarinic acid was higher in lemon balm and spearmint [24], as well as in thyme [62] SR extracts; these results are in line with the present study. Rosmarinic acid was also found in high concentrations in the residual wastewaters obtained from the EO distillation of sage and rosemary (135.3 ± 12.3 mg/100 mL and 46.8 ± 9.4 mg/100 mL, respectively), according to the study of Celano et al. [52]. Following the extraction of EOs, the amount of rosmarinic acid in the SR extracts is affected by the degree of partial degradation caused by the high temperatures used, as well as by its solubilization in the distillation water and subsequent elimination via the waste water stream. The phenolic compounds might be subjected to the same general process, highlighting the significance of using a multistep biorefining approach to extract these precious chemicals [24]. Greek oregano had the highest content of salvianolic acid isomers, followed by lemon balm and spearmint.

Table 2. Quantification of major phenolic components (mg/g of extract) in distillation SRs of rosemary, Greek sage, Greek oregano, lemon balm, and spearmint following steam distillation for the extraction of essential oils using LC-DAD-MS.

Extracts	Rosmarinic Acid	Phenolic Diterpenoids	Salvianolic AcidIsomers
Rosemary	53.31 ± 3.81 ^{E}	393.09 ± 29.51 ^{A}	8.03 ± 0.26 ^{D}
Greek sage	79.57 ± 7.91 ^{C}	155.42 ± 11.27 ^{B}	3.23 ± 0.15 ^{D}
Greek oregano	66.38 ± 1.78 ^{D}	-	41.78 ± 1.88 ^{A}
Lemon balm	95.42 ± 4.02 ^{B}	-	32.78 ± 1.28 ^{B}
Spearmint	109.90 ± 6.32 ^{A}	-	17.36 ± 4.27 ^{C}

Statistically significant differences according to Duncan's test are shown by different superscript letters in each column ($p < 0.05$).

Some flavonoids, such as luteolin-7-O-glucuronide (0.11–2.25 mg/g) and luteolin-7-O-glucuronide (trace–3.66 mg/g), were also quantified in minor quantities in the rosemary, Greek sage, and spearmint extracts, while hesperidin was quantified in the rosemary and spearmint extracts. These compounds have been related previously with the antibacterial activity of the extracts [63].

In general, an extract's antioxidant activity is correlated with its content of phenolic compounds. This is because the majority of phenolic compounds can act as antioxidants through various mechanisms, increasing the overall antioxidant activity of the respective extract [64]. However, in the case of antimicrobial/antibacterial activity, a slightly different pattern is observed. Although plant extracts have the ability to inhibit the growth of both pathogenic and spoilage microorganisms, the sensitivity of microorganisms to the extracts depends mostly on the type and molecular structure of the phenolic components as well as on the type and strain of the microorganism. So, it is obvious that the concentration of total phenolics present in an extract is not as crucial as their type. This is also confirmed in the present study, since the lemon balm SR extract presented the weakest antibacterial activity, although its total phenolic and flavonoid contents were higher compared to the other extracts (Figure 7). On the other hand, the spearmint SR extract, which contained equivalent amounts of phenolics to lemon balm, was more effective against bacteria (Figure 4). These two extracts presented quite different phenolic profiles, especially in terms of flavonoid content. Moreover, it appears that among the phenolic components, there is synergistic action against microorganisms, since studies have shown that plant extracts enriched with many compounds show a stronger antimicrobial effect compared to individual pure compounds [65].

In the case of phenolic acids (e.g., rosmarinic acid) and their derivatives, their antimicrobial activity is performed through bactericidal actions. Since these compounds are weak organic acids and quite lipophilic, their physicochemical characteristics affect their diffusion and solubility through microbial membranes, resulting in different antimicrobial activities. As reported in the work of Ecevit et al. [66], some phenolic acids passively diffuse

through the cell membrane and tend to acidify the cytoplasm. In this way, cell membrane disruption occurs, which causes the leakage of essential intracellular constituents into the extracellular space, resulting in microbial cell death. As reported by Nieto et al. [67], rosmarinic acid and the phenolic diterpenes carnosol and carnosic acid may interact with the cell membrane. This interaction is responsible for a series of changes in the microbial cell, such as alterations in its genetic material and electron transport, cellular component leakage, and changes in the production of fatty acids. This could explain the effectiveness of the SR extracts of rosemary, Greek sage, and spearmint, which presented the highest antibacterial activity against the Gram-positive tested microorganisms, since these extracts were found to contain higher concentrations of the above-mentioned compounds (Table 2). The lipophilic ends of lipoteichoic acid in the Gram-positive cell wall facilitate the penetration of lipophilic compounds into these bacteria. Instead, Gram-negative bacteria show greater resistance to the action of such compounds, which is attributed to the existence of the outer membrane. The outer membrane proteins and/or lipopolysaccharides present in this group of bacteria can limit the rate of diffusion of hydrophobic compounds inside a bacterial cell [68].

Apart from phenolic acids, flavonoids are also an important group of antimicrobial agents. According to a number of studies, these compounds may exhibit bacteriostatic and bactericidal properties. Their capacity to assemble into complexes with the bacterial cell wall and impede bacterial development is linked to their bacteriostatic actions. Specifically, they can impede nucleic acid synthesis, microbial cell energy metabolism, or cytoplasmic membrane function to restrict cell development [66]. According to Cowan [69], the antimicrobial efficacy of plant extracts is linked to their flavonoid contents. In addition, many flavonoids have the ability to form complexes with various proteins both inside the bacterial cell wall and in the extracellular environment, thus exhibiting anti-infective activity [67]. Although detected in minor quantities, the flavonoids present in the SR extracts of rosemary, Greek sage, and spearmint may have contributed to their antibacterial activity by acting synergistically with the other phenolic compounds (Table 1).

Overall, phenolic compounds can cause disruption to the cytoplasmic membrane through their association with proteins, resulting in a loss of control of the chemosmotic mechanism, thus leading to cell death [70]. Furthermore, disruption of the bacterial membrane can cause the leakage of intracellular components, such as proteins, nucleotides, and small cellular molecules, e.g., potassium and phosphate ions [71]. Finally, phenolic compounds can affect protein biosynthesis and alter some metabolic processes in bacterial cells, while it has been reported that they inhibit DNA synthesis by suppressing gyrase enzyme activity as well as ATP synthesis [65,72]. Nevertheless, the exact mechanism of the antimicrobial action of phenolic components is not clear. This is due to the wide diversity in chemical structures, which means that there could be many possible mechanisms of antimicrobial activity. In addition, in plant extracts, the interactions between their components can greatly influence their activity. In general, phenolic compounds act similarly to essential oil components, primarily affecting the cell wall and membrane integrity of bacterial cells; the latter equates to reduced cell resistance to adverse conditions such as high or low osmotic pressure and temperature [65]. Even in this case, the phospholipid-rich cell wall of Gram-negative bacteria makes them resistant to the action of phenolic components due to the reduced permeability of this lipophilic membrane by macromolecules. The high resistance of Gram-negative bacteria to phenolic components may also be related to enzymes in the cytoplasm, which have the ability to inactivate incoming molecules [73].

On the other hand, it should be noted that some phenolic compounds may promote the growth of microorganisms instead of exerting antimicrobial activity. For example, O-glycosylated polyphenols (such as diosmin) and quinic acid esters (such as chlorogenic acid), through enzymatic hydrolysis with bacterial enzymes, may release glucose or quinic acid, which can act as growth-promoting factors [74]. Plant polyphenols are a family of substances that may also fit the definition of prebiotics, although much more research for the target microbial host is necessary [75].

It should be noted that the present research has focused primarily on the antibacterial activity of extracts obtained from SRs of MAPs native to the Mediterranean and examining pathogenic and spoilage bacteria that can be found in bakery products. The lack of studies regarding the antifungal activity of SRs and their extracts, as well as their antibacterial activity against other spoilage microorganisms or useful bacterial cultures, further points to the need for future work in this area.

4. Conclusions

In the present study, the antibacterial and antioxidant properties of phenolic extracts obtained from SRs retained after the EO distillation of five species, namely Greek oregano, rosemary, spearmint, lemon balm, and Greek sage, were evaluated. Based on the results, the phenolic extracts were quite effective against Gram-positive species. The rosemary and Greek sage extracts exhibited the strongest antibacterial activities against all the Gram-positive bacterial strains tested (*L. monocytogenes*, *S. aureus*, *B. subtilis*, *B. licheniformis*, and *B. cereus*), even at a concentration of 750 mg/L, while the extracts of spearmint and Greek oregano were less effective for the Gram-positive bacteria and only had an effect at the highest concentration used. The lemon balm extract did not exhibit any inhibitory effect; however, it had the highest phenolic concentration, and it showed moderate antioxidant activity, along with spearmint. Although the Greek oregano and lemon balm extracts exhibited the lowest antibacterial activity, they may be used as antioxidant components in food products. Major phenolic components were identified by LC/MS in all the SR extracts. In the rosemary and Greek sage extracts, the primary recognized compounds were rosmarinic acid, carnosol, and carnosic acid, whereas in the Greek oregano, spearmint, and lemon balm extracts, there were salvianolic acid isomers and rosmarinic acid.

The solid distillation residues of aromatic plants contain a variety of bioactive substances, primarily polyphenols, which can be further utilized in food products, cosmetics, and pharmaceutical preparations. The examined SR extracts have the potential to be utilized as antimicrobial substances in food formulations for enhancing the shelf life of products and improving their nutritional value (e.g., by increasing their antioxidant potency). To prevent the growth of strains of the genus *Bacillus* that cause the roping of bakery items, these extracts could be added to the dough during bread production. Taking into account industrial requirements, UAE, as an eco-friendly "green" technique, can be economically feasible for the production of extracts at a lower cost than conventional methods. However, further studies on the application of extracts in formulations of bread products would be required to fully explore the in situ functionalities of these materials and thereby contribute to the potential valorization of post-distillation residues from MAPs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr12010140/s1, Figure S1. Image of 96-well microtiter plates at 0 (left) and 48 h (right) for SR oregano extract. Each bacterium is incubated in three consecutive positions. In the places where there is growth of the bacterium after 48 h, the black line is not visible. Figure S2. Mass spectra recorded in the negative ion mode of peaks identified by literature data. Figure S3. UV spectra of peaks identified by literature data.

Author Contributions: Conceptualization, M.I., A.L. and P.C.; methodology, E.B., M.H. and M.I.; software, M.I.; validation, E.B., M.H., M.I. and P.C.; formal analysis, E.B. and M.H.; investigation, E.B. and S.C.; data curation, M.I., E.B. and M.H.; writing—original draft preparation, E.B., S.C. and M.H.; writing—review and editing, A.L., P.C., M.I. and C.G.B.; visualization, E.B. and M.I.; supervision, M.I.; project administration, M.I.; funding acquisition, C.G.B. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are contained within the article.

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

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