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Research Article

Antioxidant, Antimicrobial Activities, and Characterization of Phenolic Compounds of Thyme (*Thymus vulgaris* L.), Sage (*Salvia officinalis* L.), and Thyme–Sage Mixture Extracts

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The antioxidant properties of sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.), and their mixtures were examined using the 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) scavenging activity and ferric-reducing activity of plasma (FRAP) methods. The antimicrobial activity of the plant extracts against four bacterial strains (*Escherichia coli, Staphylococcus aureus, Bacillus cereus,* and *Salmonella typhomorium*) was determined using the agar well diffusion method. Results showed the highest overall rate of increase in total phenolic (13.67 mg·GAE/g·DW), and flavonoid (6.75 mg·QUE/g·DW) contents in the thyme–sage mixture extract compared with the thyme and sage extracts. As found by GC-MS analysis of methanolic extracts, thymol, apigenin, rosmarinic acid, and carvacrol were the most abundant phenolic compounds in the thyme–sage extract. The lowest EC50 ($DPPH^{\bullet}$, 55.51 $\mu g \cdot ml^{-1}$) and the highest FRAP value (95.51 mM Fe (II) mg^{-1} extract) were recorded in the extract of the thyme–sage mixture compared with sage and thyme extracts, and butylated hydroxytoluene solution (BHT). The highest antimicrobial activity against *E. coli, S. aureus, B. cereus,* and *S. typhomorium* was observed in the thyme–sage mixture with the inhibition zone diameters of 22.13, 28.67, 31.25, and 23.65 mm, respectively. It is concluded that the extract obtained from the thyme–sage mixture has more potential to be used in the pharmaceutical and food industry as a natural antibacterial and antioxidant agent.

1. Introduction

The term antioxidant refers to a wide variety of compounds that could neutralize the harmful effects of free radicals through different mechanisms. Besides, these compounds can postpone or block the oxidation process by inhibiting the initial or diffusion stage of chain reactions [1]. Active radicals are active atoms or molecules that potentially have a high affinity for other surrounding compounds due to the status of their last atomic layer. If their activity is not terminated, it can lead to cell and tissue destruction, including disorders such as cancer and heart disease [2, 3]. One of the methods to prevent the detrimental effects of free radicals is the application of antioxidants. There are various antioxidants, such as polyphenols (including isoflavones, flavones, flavonol, flavanones, flavane, biflavane, anthocyanin, rosmarinic acid, carnosic acid, ascorbic acid) that have a potential conservative effect against different kinds of active radicals [4]. There are many shreds of evidence confirming the antinutritive effects of synthetic antioxidants, such as butylated hydroxytoluene (BHT). Moreover, the risk of cancer and liver damage in laboratory animals is one of the drawbacks of using artificial antioxidants. Therefore, it seems inevitable to apply natural antioxidants with less toxic and more effective properties to prevent cell damage induced by reactive

oxygen species (ROS), including free radicals, peroxides, and lipid peroxides [4]. Furthermore, secondary plant-derived metabolites such as polyphenols can potentially eliminate free radicals. They can be found in various parts of the plant, such as the leaves, seeds, fruit, skin, and root. Thus, medicinal plants can be regarded as a valuable source of potential chemicals with many practical effects, such as antimicrobial, antiseptic, carminative, and antioxidant properties [5].

Thyme (*Thymus vulgaris* L.) is one of the medicinal plants used widely as it provides high antioxidant and antibacterial properties. The most important compounds synthesized by this plant are carvacrol and thymol, which not only have antioxidant properties but also inhibit microorganisms as a result of a reduction in vital intracellular substances and disruption of bacterial enzyme systems, as well as an increase in cell membrane permeability [6]. Another plant that has many medicinal properties is sage (*Salvia officinalis* L.), which is the largest genus of plants from the Lamiaceae family with nearly 900 species [7]. Sage is distributed throughout three distinct regions: the Mediterranean, Iran, and some parts of Europe. This plant has long been used in traditional medicine to treat colds, gastrointestinal disorders, bronchitis, cancers, and *tuberculosis* due to its antibacterial, antitumor, antifungal, and anti-inflammatory properties [8]. In general, the antibacterial and antioxidant potential of sage has been attributed to the presence of thujone, camphor, and 1,8-cineole [9].

The antimicrobial and antioxidant properties of thyme and sage have been demonstrated by many studies [10]. However, there are limited studies on the antioxidant potential and antimicrobial activity of the combined extracts of medicinal plants [11]. The majority of combined medicinal plant research to date has mostly focused on the combination of plant essential oils. To our knowledge, no study has investigated the possible synergistic effect produced by the combination of *Thymus vulgaris* and *Salvia officinalis* extracts. Hence, this study aimed to investigate the chemical composition of extracts of sage, thyme, and thyme–sage mixtures by hyphenated gas chromatography–mass spectrometry (GC-MS) and their antibacterial and antioxidant activities using well-known testing paradigms.

2. Materials and Methods

2.1. Chemicals and Reagents. 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), butylated hydroxytoluene (BHT), 2,4,6-tris(2-pyridyl)- s-triazine (TPTZ), sodium acetate, sodium carbonate, sodium hydroxide, gallic acid, ethanol, methanol, ferric chloride, quercetin, aluminum chloride, Mueller–Hinton agar (MHA), tryptic soy broth (TSB), and Folin–Ciocalteu reagents were provided by Sigma–Aldrich Chemical Co.

2.2. Soil and Plant Sampling. At the flowering stage, random samples of thyme and sage were collected from the Botanical Garden of the Medicinal Botanic Center, Tehran, Iran (35°41′N, 51°19′E), where the mean annual rainfall, temperature, and relative humidity of the area are about 210 mm, 17.4°C, and 41%. The plants were identified using a valid botanical reference [12]. The voucher specimens of *Salvia officinalis* L. and *Thymus vulgaris* L. were deposited at the Herbarium of the Medicinal Plants and Drugs Research Institute, Tehran, Shahid Beheshti University, Iran (MPH-864 and MPH 789).

Above-ground portions of the plants were cut at 3–4 cm above the soil surface and air-dried to a constant weight at 23°C ± 2. The soil sample was also taken from the agricultural land (0–30 cm surface layer), air-dried, and passed through a 2 mm stainless steel sieve. Available *K* was analyzed in the solution extracted by 1 M, ammonium acetate ($NH_4CH_3CO_2$) [13]. Soil pH and electrical conductivity (EC) were measured according to the methodology described by Thomas and Rhoades, respectively [14, 15]. Soil organic carbon was determined by the Walkley–Black method [16]. Total *N* and available *P* were also measured by the Kjeldahl and Olsen methods, respectively [17, 18] (Table 1).

2.3. Plant Extract. All air-dried thyme and sage were ground, and their extracts were then obtained using an ultrasound-assisted extraction procedure [19], where 100 g of each ground sample was mixed with 500 ml of methanol (70%), and the mixture was sonicated in an ultrasonic bath (Elmas model 690/H) with a frequency of 35 kHz at 35°C ± 2 for thirty minutes. The temperature was maintained by circulating cold water. Excess solvent was evaporated using an evaporator rotary (40°C). The concentrated extracts were stored and freeze-dried at −18°C. The extract of the thyme–sage mixture was also prepared by mixing 50 g of each ground sample of thyme and sage.

2.3.1. Determination of Total Phenolic Content. Total phenolic contents in the extracts of thyme, sage, and their mixture were determined using the Folin–Ciocalteu reagent [20], where 0.5 mL of extract was mixed with 2.5 mL of reagent (1 : 10 reagents to distilled water ratio) and 2 mL of sodium bicarbonate solution (7.5%, w/v). The mixture was kept at room temperature for two hours. The absorbance of each sample was measured at a wavelength of 760 nm using a spectrophotometer (UV-VIS 2100, Unico, U.S.A). A standard curve was prepared using different concentrations of gallic acid. The content of total phenolics was expressed as mg of gallic acid equivalents (GAE) per Gram of dry weight (DW).

2.3.2. Determination of Total Flavonoid Content. Total flavonoid contents in the extracts of thyme, sage, and their mixture were determined using the aluminum chloride colorimetric method [21], where 0.25 mL of the extracts was mixed with 1.25 mL of distilled water. Then was added 0.075 mL of $NaNO_2$ (5%). After six minutes, the mixture was mixed with 0.15 mL of aluminum chloride (10%) and kept at room temperature for five minutes. Finally, distilled water was added to the mixture, followed by adding 0.5 mL of sodium hydroxide (4%). The absorbance of each sample was measured at a wavelength of 510 nm using

Table 1: Soil chemical characteristics of the agricultural land.

Soil	Total N ($g{\cdot}kg^{-1}$)	Available P ($g{\cdot}kg^{-1}$)	Available K ($g{\cdot}kg^{-1}$)	Organic carbon ($g{\cdot}kg^{-1}$)	pH	EC ($dS{\cdot}m^{-1}$)
Medicinal botanic center	2.54	0.21	0.36	25.69	7.4	0.87

a spectrophotometer (UV-VIS 2100, Unico, U.S.A). Different concentration of quercetin was used to prepare the standard curve. The total flavonoid content was expressed as mg quercetin (QUE) equivalents per g dry weight (DW).

2.3.3. GC-MS Analysis. The plant extracts were analyzed by gas chromatography. The plant extracts were injected into a GC-MS system consisting of an Agilent 6890 UK gas chromatograph equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.2 μm film thickness). Temperature arrangements were set according to the following conditions: initiate at 50°C and hold for three minutes; from 50 to 200°C at 5°C/min (holding for 3 min), from 200°C to 300°C and then hold for three minutes. The injector temperature was also set at 290°C [22]. The phytochemical compounds of the plant extracts were identified by comparing the retention times and mass spectra with those of standards. The abundance of the compounds was calculated from the calibration curves.

2.3.4. DPPH•-Scavenging Assay. The free radical scavenging activity of the extracts and butylated hydroxytoluene (BHT, as a synthetic antioxidant) was investigated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical scavenging method [23]. 2 mL of each prepared solution was added to 2 mL methanolic solution of DPPH• (0.15 mM) and incubated for 30 min at a constant 25°C ± 2 (in darkness). The absorbance of incubated solutions was determined at 517 nm using a spectrophotometer (UV-VIS 2100, Unico, U.S.A). The blank solution was made from a solution consisting of 2 mL DPPH• solution mixed with a 2 mL methanol solution (95%). The percentage of DPPH• inhibition was then calculated by the following equation:

$$\%\text{Inhibition of DPPH}^{\bullet} = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100, \quad (1)$$

where A blank and A sample are the absorbances of the blank solution at $t = 0$ and the absorbance of the extract or BHT solution, respectively. Extract concentration reducing the absorbance of DPPH• by 50% (EC50) was calculated using the graph plotted the inhibition percentage against the concentration of the extract.

2.3.5. Ferric-Reducing Antioxidant Power Assay. The procedure for the determination of ferric-reducing antioxidant power was measured according to the methodology described by Sudha et al. where the ferric-reducing activity of plasma (FRAP) reagent was prepared by adding 2.5 mL of a 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution to 2.5 mL of 10 mM $FeCl_3{\bullet}6H_2O$, 40 mM HCl, plus 25 mL of 300 mM acetate buffer (pH 3.6). Then, a 900 μL FRAP reagent was added to 30 μL of each extract and BHT solution and mixed with 90 μL of water. The mixtures were centrifuged at 650 rpm for ten minutes, and their absorbance was measured at 593 nm using a UV-VIS spectrophotometer [24].

2.3.6. Antibacterial Bioassay. The antibacterial activity of the extracts was determined by the agar-well diffusion technique according to the methodology described by Wald et al. [25], where *Escherichia coli*, *Salmonella typhimurium, Bacillus cereus, and Staphylococcus aureus* bacteria were grown in tryptic soy broth (TSB) medium at 37°C for 24 h. Then, 0.1 mL of standardized inoculum (10^6 CFU/mL; 0.5 MacFarland) of each bacterium was spread on the surface of Mueller–Hinton agar (MHA). Then, 6 mm diameter wells were punched into the solidified agar media, and subsequently, 50 μL of each extract was poured into the wells and then incubated at 37°C for 48 h. After incubation, the diameters of the growth inhibition zones around each well were measured in mm by mechanical calipers.

2.4. Statistical Analysis. Comparisons of DPPH• scavenging, ferric-reducing, and antimicrobial activity between extracts of thyme, sage, and thyme–sage mixture were analyzed and compared using LSD tests in the statistical analysis system (SAS 9.2) at $P < 0.05$.

3. Results

3.1. Extract Yields, Total Phenol, and Total Flavonoid Contents. Figure 1 presents the total phenolic and flavonoid contents, and extract yield of the thyme, sage, and sage-thyme mixture. The results revealed that the type of plant and plant mixture significantly caused a change in the total phenolic and flavonoid contents, and extract yield of the sage, thyme, and thyme–sage mixture. Total phenolic and flavonoid contents were significantly higher in the extract of the thyme–sage mixture (total phenolic content: 13.67 mg·GAE/g·DW; flavonoid content: 6.75 mg·QUE/g·DW) than in thyme extract (total phenolic content: 8.89 mg·GAE/g·DW; flavonoid content: 3.87 mg·QUE/g·DW) and the sage extract (total phenolic content: 6.43 mg·GAE/g·DW; flavonoid content: 4.11 mg·QUE/g·DW). Similarly, extract yield was highest in the extract of the thyme-sage mixture (25.80%) compared to the thyme extract (21.81%) and the sage extract (19.80%). Except for the flavonoid contents in sage extract, which showed significantly higher flavonoid contents compared to thyme extract, the total phenolic contents and extract yield were higher in thyme extract than in sage extract.

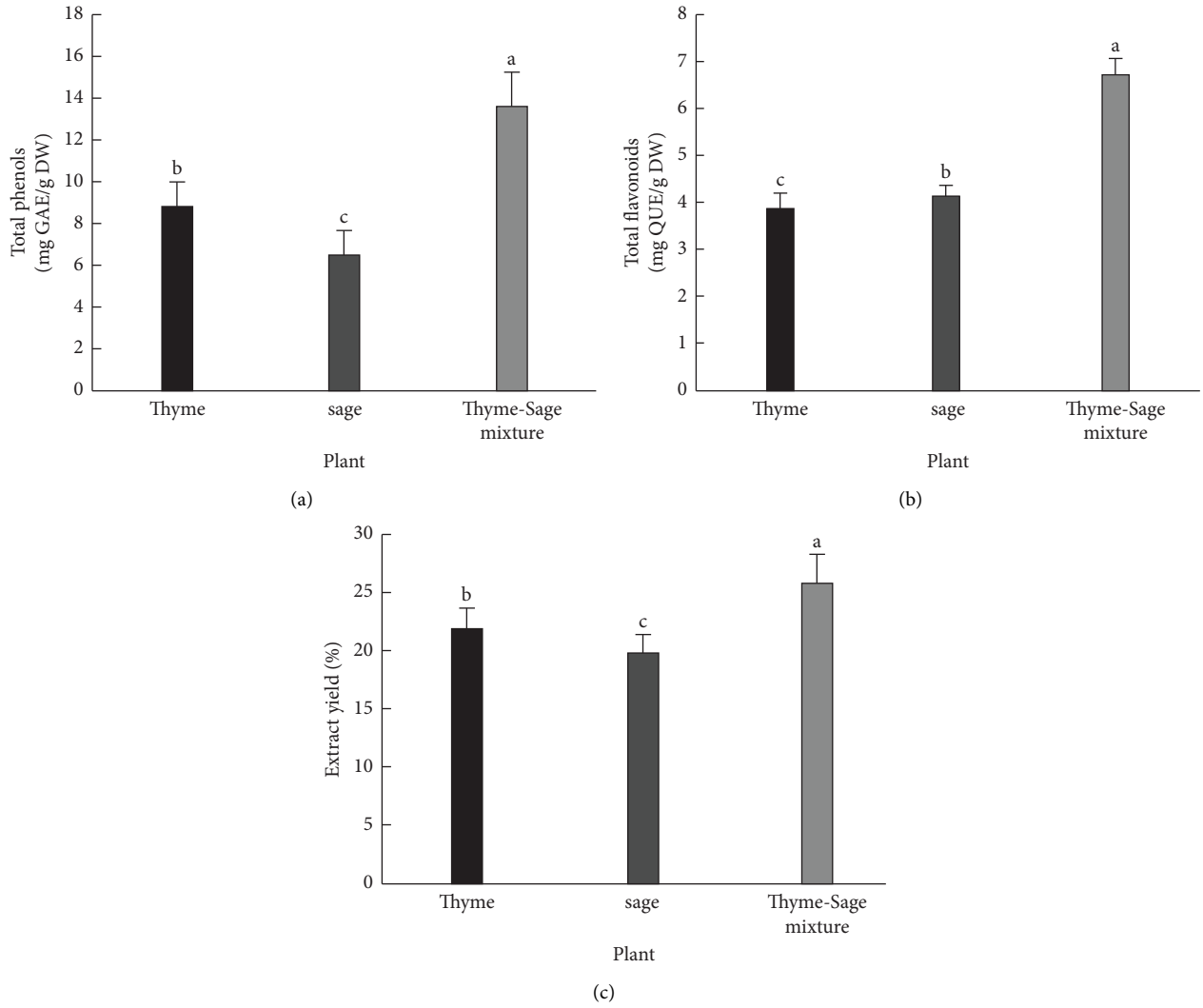

FIGURE 1: Total phenolic (a) and flavonoid contents (b), and extract yields (c) of thyme, sage, and thyme–sage mixture. Results are means ± standard deviations ($n = 3$).

3.2. Phytochemical Compounds. The GC-MS analysis of methanolic extracts of thyme, sage, and thyme–sage mixture is presented in Table 2. Representative chromatograms are shown in Figure 2. Thymol was the predominant phenolic compound in the thyme extract. Thymol constituted 21.65% of the total extracted compounds, followed by carvacrol (17.34%) and cinnamic acid (9.1%). The GC-MS analysis of methanolic extracts of sage showed that the dominant phenolic compound was apigenin (25.23%), followed by apigenin-7-glucoside (15.43%) and rosmarinic acid (15.15%). Moreover, the qualitative-quantitative analysis of the thyme–sage mixture revealed that the total extracted compounds increased by 91.27% with respect to the thyme and sage extracts. Thymol was the dominant compound in the thyme–sage extract (more than 16% of the total extracted compounds), followed by apigenin (15.45%), rosmarinic acid (12.06%), and carvacrol (10.50%).

3.3. Antioxidant Activity of the Extracts. Comparison of DPPH$^{\bullet}$ radical scavenging activity of plant extracts and BHT solution revealed that the thyme–sage mixture had the lowest EC50 (55.51 $\mu g \cdot ml^{-1}$), followed by thyme, sage, and BHT in ascending order (69.39, 77.21, and 86.58 $\mu g \cdot ml^{-1}$, respectively) (Table 3).

In addition, the value of the ferric-reducing activity of plasma (FRAP) in the methanolic extracts of thyme, sage, and their mixture was evaluated and compared with that of BHT. Results showed that the sage extract and BHT solution had the lowest amounts of FRAP. However, there was no significant difference between the methanolic sage extract and the BHT solution. In contrast, the thyme–sage extract showed a significantly higher FRAP value than other methanolic extracts and BHT solutions.

3.4. Antibacterial Activity. The plant extracts were investigated for antibacterial activity against four bacterial strains (Table 4). The results revealed that among the studied plant extracts, the highest antibacterial activity against *E. coli*, *B. cereus*, *S. typhimurium*, and *S. aureus* was found in the thyme–sage mixture with the inhibition zone diameters

Table 2: Phenolic compounds of thyme, sage, and thyme–sage mixture methanolic extract.

Compounds	Rt* (min)	% Of the total		
		Thyme	Sage	Thyme–sage mixture
Caffeic acid	6.43	0.87	0.16	0.39
β-pinene	7.98	0.32	0.75	0.43
Sabinene	9.34	0.84	0.84	0.38
Carnosol	11.45	—	1.58	0.17
Ocimene	12.98	0.48	—	0.33
p-coumaric acid	13.54	0.56	—	0.56
Ferulic acid	15.65	1.66	1.12	1.05
Caffeoylquinic acid derivative	18.95	2.32	—	1.12
Carnosic acid	22.76	4.57	1.46	1.03
Rosmarinic acid	26.45	6.71	15.15	12.06
Thymol	29.42	21.65	—	16.65
Quercetin-7-o glucoside	30.17	2.65	—	0.85
Cinnamic acid	31.56	9.11	—	6.32
Carvacrol	34.56	17.34	3.56	10.50
Ferulic acid derivative	39.22	1.67	0.26	1.03
Methyl rosmarenate	42.32	2.13	1.58	1.21
Methyl carnosate	46.65	—	1.98	1.18
Apigenin	49.12	3.22	25.23	15.45
Luteolin-7-o-rutinose	52.78	1.75	9.38	4.13
Cirsiliol	53.82	1.22	0.23	1.35
Apigenin-7-glucoside	54.92	3.33	15.43	8.65
Salvigenin	55.24	—	1.43	1.03
Naringnin	55.65	3.29	—	2.09
Luteolin	56.32	2.43	6.55	3.07
Hesperidin	56.88	1.86	—	0.24
Total extracted compounds (%)		89.98	86.69	91.27

*RT: retention time.

of 22.13, 31.25, 23.65, and 28.67 mm, respectively, while the lowest antibacterial activity was observed in the sage extract against *B. cereus* (21.73 mm), *S. aureus* (19.12 mm), and *E. coli* (16.76 mm) (except for *S. typhimurium*).

Moreover, *E. coli* and *S. typhimurium* were less sensitive to the inhibitory activity of the thyme, sage, and thyme–sage mixture.

4. Discussion

The ferric-reducing activity of plasma (FRAP) and 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) free-radical scavenging activity were used to determine the antioxidant activities of the plant extracts. The $DPPH^{\bullet}$ scavenging activity is based on the antioxidant's ability to reduce the number of $DPPH^{\bullet}$ molecules equal to the number of their available hydroxyl groups, while the FRAP assay measures the capacity of antioxidants to reduce Fe^{3+} to Fe^{2+} [26]. In general, a higher rate of increase in antioxidant capacity of the thyme–sage mixture than sage and thyme extracts and BHT solution could be inferred from the significant increase in phenolic and flavonoid contents of the thyme–sage mixture. The higher total phenolic (13.67 mg·GAE/g·DW) and flavonoid contents (6.75 mg·QUE/g·DW) in the thyme–sage mixture may be attributable to the presence of a wide variety of phenolic compounds in the sage and thyme extracts, which in turn, resulted in more antioxidant capacity. The results obtained for total phenolic content in sage (6.43 mg·GAE/g·DW) and thymus (8.89 mg·GAE/g·DW) extracts are almost similar to those reported by Robey et al. [27]. Also, in other examined sage and thyme extracts the results showed that the total phenolic and flavonoid contents were dependent on the differences between plant species, environmental factors, and extraction methods. So, Srećković et al. found that the total phenolic and flavonoid contents in the methanolic extracts of *Salvia pratensis* L. were 128.94 mg·GAE/g extract and 68.46 mg·QUE/g extract, respectively [28]. In another study reported by Kocak et al., the total phenolic and flavonoid contents in methanolic extracts of *Salvia cadmica* Boiss were obtained at 64.98 μmol GAEs/g dry plant and 12.96 μmol REs/g dry plant, respectively [29].

The antioxidant potential of the combined extracts is not only controlled by the concentration of phenolic compounds, but it is also dependent on the structure and the synergistic interactions between phenolic compounds against the oxidation process [11]. The differences between the antioxidant potential of thyme–sage extract and individual plant extracts could be explained by the number and position of substituted hydroxyl or methoxyl groups and glycosylation around the flavonoid skeleton [30]. Since total phenolic and flavonoid contents do not give a complete picture of the quality and quantity of the phenolic and flavonoid constituents, GC-MS analyses can provide the most helpful information on individual phenolic

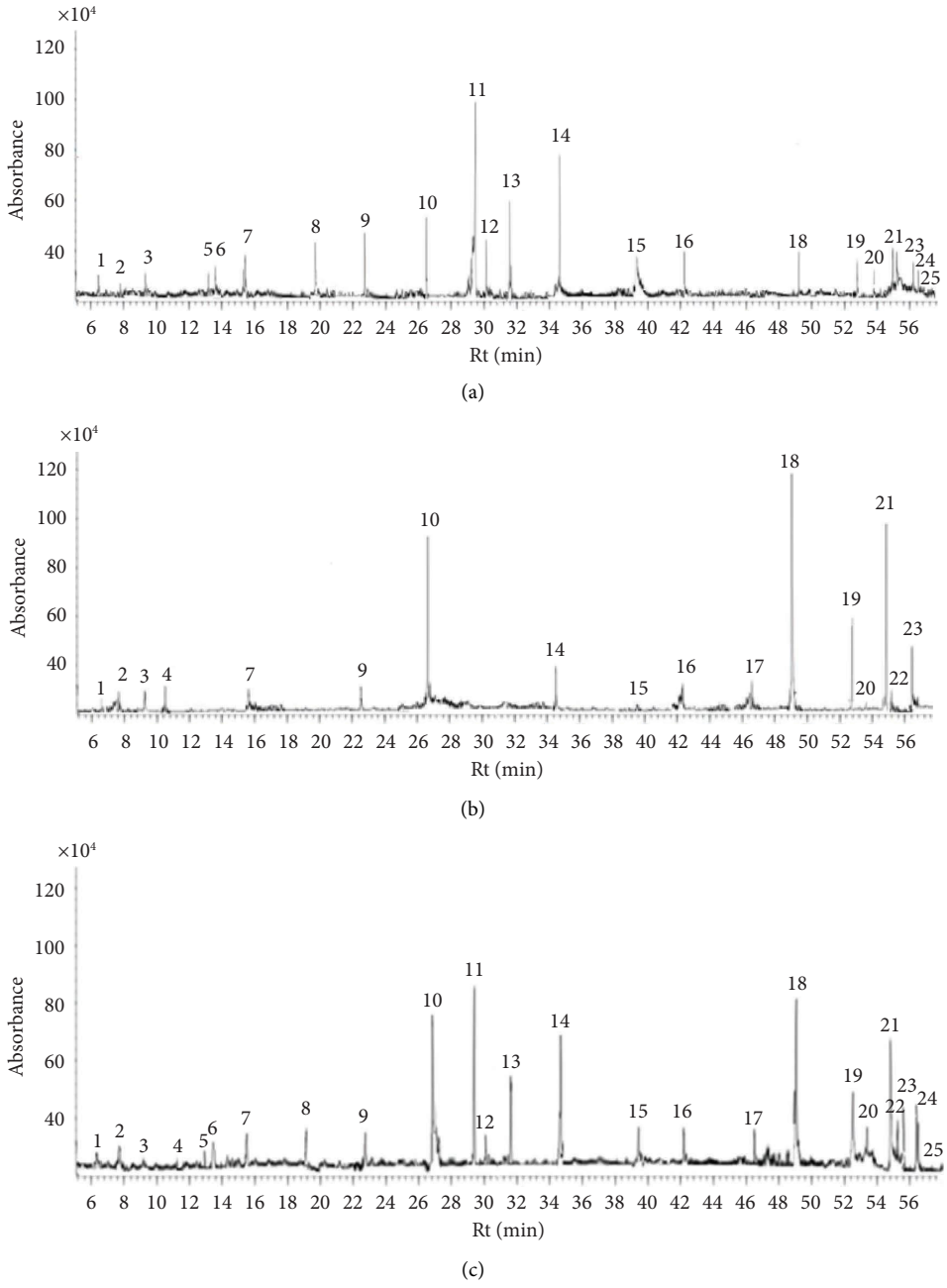

Figure 2: GC-MS chromatograms of thyme, (a) sage, (b) and thyme–sage mixture (c) methanolic extract. 1: Caffeic acid, 2: β-pinene, 3: sabinene, 4: carnosol, 5: ocimene, 6: p-coumaric acid, 7: ferulic acid, 8: caffeoylquinic acid derivative, 9: carnosic acid, 10: rosmarenic acid, 11: thymol, 12: quercetin-7-o glucoside, 13: cinnamic acid, 14: carvacrol, 15: ferulic acid derivative, 16: methyl rosmarenate, 17: methylcarnosate, 18: apigenin, 19: luteolin-7-o-rutinose, 20: cirsiliol, 21: apigenin-7-glucoside, 22: salvigenin, 23: naringnin, 24: luteolin, and 25: hesperidin.

Table 3: The mean ± SD ($n = 3$) of comparison between the antioxidant activities of thyme, sage, thyme–sage extract, and BHT solution.

Plan extract/BHT solution	DPPH$^{\bullet}$ (EC50) ($\mu g \cdot ml^{-1}$)	FRAP (mM Fe(II) mg^{-1} extract)
Thyme	69.39 ± 3.01^{c}	75.39 ± 3.01^{b}
Sage	77.21 ± 2.61^{b}	69.21 ± 2.61^{c}
Thyme–sage mixture	55.51 ± 3.23^{d}	95.51 ± 7.23^{a}
BHT	86.58 ± 2.89^{a}	66.58 ± 2.89^{c}

*Different letters in each group indicate significant differences ($P < 0.05$).

Table 4: The mean ± SD of antimicrobial activity of sage, thyme, and thyme–sage mixture extracts against *E. coli, S. aureus, B. cereus*, and *S. typhomorium* bacteria.

Bacterial strain	Plant extract	Inhibition zone (mm)
Bacillus cereus	Thyme	24.87 ± 2.47^{b}
	Sage	21.73 ± 1.65^{c}
	Thyme–sage mixture	31.25 ± 2.93^{a}
Escherichia coli	Thyme	18.43 ± 0.96^{b}
	Sage	16.76 ± 1.23^{c}
	Thyme–sage mixture	22.13 ± 1.34^{a}
Salmonella typhomorium	Thyme	17.11 ± 2.43^{b}
	Sage	17.32 ± 2.79^{b}
	Thyme–sage mixture	23.65 ± 1.38^{a}
Staphylococcus aureus	Thyme	23.56 ± 2.11^{b}
	Sage	19.12 ± 1.48^{c}
	Thyme–sage mixture	28.67 ± 3.31^{a}

*Different letters in each group indicate significant differences ($P < 0.05$).

compounds. As found by GC-MS analyses, the combination of thyme and sage resulted in a 5.28 and 1.43% increase in total phenolic compounds compared with sage and thyme extracts, respectively. Among the phenolic compounds determined in thyme and sage extracts, apigenin, and thymol were the principal flavonoid and phenolic compounds, respectively. Apigenin and thymol represented more than 25% and 21% of the phenolic compounds extracted from the sage and thyme, respectively. Such results are in agreement with those reported by Wojdylo et al. and Shan et al. [31, 32]. These compounds can be considered the main contributing factors to the increased antioxidant potential of the thyme–sage mixture. Tural and Turhan found that the increased antioxidant capacity of the mixed essential oils obtained from thyme and laurel was attributed to the thymol and carvacrol content of thyme [11]. Robey et al. revealed that the increased $DPPH^{\bullet}$ radical scavenging activity of sage methanolic solution extract was most likely controlled by flavonoid compounds such as apigenin [27]. An increase in the $DPPH^{\bullet}$ radical scavenging activity of *Thymus pulegioides* L. as a consequence of the presence of thymol and carvacrol was also observed by Ložienė et al. [33]. Benyoucef et al. found that *Thymus fontanesii* and *Rosmarinus officinalis* essential oils blend had a higher antioxidant capacity (IC50: 7.2 mg/L) compared with *Thymus fontanesii* (IC50: 13.7 mg/L) and *Rosmarinus officinalis* (IC50: 24.5 mg/L) essential oils. These differences resulted from the association of thymol and p-cymene with 1,8-cineole, borneol, and verbenone [34].

In the case of antibacterial activity, the higher antibacterial activity of the thyme–sage mixture than sage and thyme may also be affected by the increased total phenol and flavonoid contents as a result of the combination of thyme and sage. In many studies, the antibacterial activity of the thyme extract was positively related to thymol, carvacrol, and cinnamic acid compounds, in contrast, the antibacterial activity of the sage extract was explained by rosmarinic acid, apigenin, and apigenin-7-glucoside compounds [11]. It could be concluded that combining thyme with sage resulted in increased total extracted compounds in the thyme–sage mixture, which in turn increased antibacterial activity. These compounds may inhibit bacterial enzymes, increase cell membrane permeability, or disturb the synthesis of the structural component and the genetic substance functionally [35]. Gadisa et al. found that a combined essential oil obtained from *Blepharis cuspidata* and *Thymus schimper* had the strongest antibacterial activity against *E. coli,* with an inhibition zone in diameter of 29 mm as a result of the increased synergistic interactions [36]. A study by Walsh et al. found that extracts containing phenolic compounds such as thymol, carvacrol, and eugenol at high levels had a robust antimicrobial effect on pathogenic microorganisms [37]. The diameters of the inhibition zones of the extracts are different according to their components and bacteria strains. Afridi et al. found that *Mentha piperita* L. essential oil had inhibitory effects on *B. cereus, S. typhi,* and *S. aureus* with inhibition zone diameters of 15, 14, and 17 mm, respectively [38]. Generally, *S. typhimurium* and *E. coli* are less sensitive to the inhibitory activity of thyme, sage, and thyme–sage mixtures than *B. cereus* and *S. aureus*. Gram-negative bacteria are known to be more resistant to antibacterial compounds than Gram-positive bacteria, which is consistent with the findings of Breijyeh et al. [39]. The presence of lipopolysaccharides and lipoproteins in the cellular walls of Gram-negative bacteria that form a barrier to restrict hydrophobic compounds entry could directly be contributed to the resistance [40].

5. Conclusions

As observed, all studied extracts had antimicrobial activity and antioxidant capacity. Among the extracts, the highest reducing power, total antioxidant capacity, and antimicrobial activity were obtained from the thyme–sage mixture, which can be attributed to the increased phenolic and flavonoid contents. These results represent a basis for further research on the potential use of the combination of thyme and sage as natural antioxidants and antimicrobial agents, both in the food and pharmaceutical fields.

Data Availability

Data are available upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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