Rosemary and Sage Outperformed Six other Culinary Herbs in Antioxidant and Antibacterial Properties

Eric W.C. Chan*, Lei Quan Kong, Kar Yen Yee, Wen Yee Chua and Tze Ying Loo

Faculty of Applied Sciences, UCSI University, 56000 Cheras, Kuala Lumpur, Malaysia

Abstract: The antioxidant and antibacterial properties of Labiatae culinary herbs are well documented but the effects of different drying methods are poorly studied. In this study, the antioxidant and antibacterial properties of fresh and oven-dried herbs of oregano, marjoram, rosemary, sage, basil, thyme, peppermint and spearmint were compared with available commercial herbs. Antioxidant properties of total phenolic content, total flavonoid content, caffeoylquinic acid content, free radical scavenging activity, ferric reducing power and ferrous ion chelating ability were assessed using the Folin-Ciocalteu, aluminium chloride, molybdate, DPPH radical scavenging, potassium ferricyanide and ferrozine assays, respectively. Antibacterial properties were assessed using the disc diffusion assay based on mean diameter of inhibitory zone and minimum inhibitory dose. The two drying treatments were oven drying at 50°C (OD) and microwave pre-treatment followed by oven drying at 50°C (MOD). Fresh rosemary and oven-dried oregano had the strongest antioxidant properties. For most herbs, oven drying resulted in loss of antioxidant values compared to fresh herbs with the exception of oregano. Values of oven-dried oregano, spearmint, thyme, peppermint and basil were higher than commercial samples, while those of oven-dried rosemary were lower. Of the six commercial herbs, rosemary had the highest values, followed by oregano, spearmint, thyme, peppermint and basil. All herbs showed no antibacterial activity against Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Rosemary, sage, peppermint and spearmint inhibited the growth of Gram-positive *Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus*. Rosemary and sage had stronger antibacterial properties than green and black teas of *Camellia sinensis*. When used in combination, rosemary and sage can have enhanced antioxidant and antibacterial effects, which are desirable in developing nutraceutical products, and in controlling rancidity and bacterial growth in food.

Keywords: Labiatae, phenolic content, antioxidant activity, polyphenol oxidase, antibacterial properties.

1. INTRODUCTION

Historically, herbs and spices have been used for flavouring food, as traditional medicine and as preservatives [1]. Besides being used in food to impart flavour, pungency and colour, they also have antioxidant, antimicrobial, nutritional and pharmaceutical properties [2].

There is no clear distinction between herbs and spices [3]. Herbs are herbaceous plants grown in sub-tropical or temperate climate. They are green leafy material with a pleasant taste. Spices are grown in the tropics and are dried material produced from seed, bark, root, fruit, or flower of shrubs and trees. They are usually brown, black or red in colour with a pungent smell. Some plants can be both herb and spice, e.g. cilantro (leaves) is herb and coriander (seeds) is spice.

Plants of the family Labiatae are annual or perennial herbs that are densely glandular and aromatic [4]. Leaves are simple and opposite, and stems are four-angled. Flowers are hermaphrodite and form whorls that are arranged in spikes, heads, racemes, or cymes. They are widely used for flavouring and as teas or traditional medicines. Some species are also used as sources of essential oils.

Of the common species used for flavouring, oregano is a favourite for seasoning pizza and other Italian dishes [2]. Rosemary is used for flavouring meat and poultry dishes. Thyme adds a pungent taste to meat and vegetables, and is the main ingredient for garnishing soups and stews. Basil is a classic complement to tomatoes, and is used to flavour salads, sauces and vegetables. Sage is widely used for flavouring meat dishes, soups, sausages and canned food [4]. Marjoram, with a sharp and spicy taste, is used for flavouring eggs, vegetables, soups, stews, etc. Peppermint has a characteristic, sweetish, strong aroma with a cooling after-taste and is widely used in flavouring chewing gums, sugar confectionery, ice creams, desserts, baked goods, tobacco and alcoholic beverages. It is also used for flavouring pharmaceutical and oral preparations e.g. mouth rinse and toothpaste. Spearmint is often used to flavour vegetables, soups, sauces and salads. It is also used in the flavouring of chewing gums, toothpastes and other oral products.

Research has shown that Labiatae herbs contain potent antioxidant compounds that can provide significant protection against chronic diseases such as heart disease and cancer [1,5]. These compounds may protect cholesterol from oxidation, inhibit cyclooxygenase and lipoxygenase enzymes, inhibit lipid peroxidation, or have antiviral or anti-tumour activity. Essential oils of culinary herbs can inhibit mevalonate

*Address corresponding to this author at the Faculty of Applied Sciences, UCSI University, 56000 Cheras, Kuala Lumpur, Malaysia; Tel: +60391018880; Fax: +60391023606; E-mail: chanwc@ucsi.edu.my

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synthesis and thereby suppress cholesterol synthesis and tumour growth.

Although the antioxidant and antibacterial properties of Labiatae herbs are well known, the effects of drying treatments are poorly studied. In this study, the antioxidant and antibacterial properties of fresh herbs of eight Labiatae herbs were analysed and evaluated. The effects of oven drying with and without microwave pre-treatment were also assessed with comparison to commercial herbs.

2. MATERIALS AND METHODS

2.1. Plant Samples

Fresh herbs were obtained from local supermarkets (Kuala Lumpur, Malaysia). These herbs were grown in Genting Highlands (Pahang, Malaysia). They were oregano (*Origanum vulgare* L.), marjoram (*Origanum majorana* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), basil (*Ocimum basilicum* L.), thyme (*Thymus vulgaris* L.), peppermint (*Mentha piperita* L.) and spearmint (*Mentha spicata* L.). Commercial herbs of oregano, rosemary, basil, thyme and spearmint (McCORMICK), and peppermint (BOH) purchased from the supermarkets (Kuala Lumpur, Malaysia) were used as standards for comparison.

2.2. Drying Protocols

Two drying protocols were used. For each species, one batch of herbs (15 g) was dried in an universal oven (UFB500, Memmert, Germany) for 5.5 h at 50^{o}C (OD). Another batch (15 g) was pre-treated in a microwave oven (R-397J(S), Sharp, Malaysia) for 30 sec before oven drying for 5.5 h at 50^{o}C (MOD).

2.3. Extraction

For antioxidant properties, fresh herbs (1 g) and oven-dried herbs (0.3 g) were powdered with liquid nitrogen in a mortar and extracted with 50 ml of methanol with continuous shaking (150 rpm) for 1 h at room temperature. Extracts were filtered under suction and stored at 4°C for further analysis.

For antibacterial activity, fresh herbs (10 g) or oven-dried herbs (3 g) were powdered with liquid nitrogen in a mortar and extracted with 100 ml of methanol, three times for 1 h each time. The mixture was swirled continuously at 120 rpm in an orbital shaker. Extracts were filtered under suction and stored at 4°C for further analysis.

2.4. Phenolic Content

Total phenolic content (TPC) was assessed using the Folin-Ciocalteu (FC) assay [6]. Extracts (300 µl) were introduced into test tubes wrapped with aluminium foil followed by 1.5 ml of FC reagent (10 times dilution) and 1.2 ml of sodium carbonate (7.5%, w/v). After standing for 30 min in the dark, absorbance was measured at 765 nm. TPC was expressed as gallic acid equivalent (GAE) in mg per 100 g of sample.

Total flavonoid content (TFC) was evaluated using the aluminium chloride assay [7]. Extract (1 ml) is added into test tubes containing 4 ml of water. Subsequently, 0.3 ml of 5% sodium nitrite was added followed by 0.3 ml of 10% aluminium chloride. Sodium hydroxide solution (2 ml, 1 M) was then added, followed by 2.4 ml of water to make up to 10 ml. The mixtures were mixed well and incubated at room temperature for 10 min. Absorbance was read at 415 nm against a sample blank consisting of 1 ml of the respective extracts with 9 ml of water. TFC was expressed as quercetin equivalent (QE) in mg/100 g of sample.

Caffeoylquinic acid content (CQAC) was quantified using the molybdate assay [8]. Molybdate reagent was prepared by dissolving 16.5 g sodium molybdate, 8.0 g dipotassium hydrogen phosphate and 7.9 g potassium dihydrogen phosphate in 1 litre of water. The reagent (2.7 ml) was added to the plant extract (0.3 ml), mixed and incubated at room temperature for 10 min before absorbance was measured at 370 nm against a sample blank consisting of 0.3 ml of the respective extracts with 2.7 ml of water. CQAC was expressed as mg chlorogenic acid equivalent (CGAE)/100 g of sample.

2.5. Antioxidant Activity

Radical scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [6]. Different dilutions of extracts (1 ml) were added to 2 ml of DPPH (5.9 mg per 100 ml methanol). Absorbance was measured at 517 nm after 30 min. IC_{50} was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg ascorbic acid (AA)/100 g of sample. AEAC was calculated as $IC_{50}(a)/IC_{50}(s) \times 10^5$ (a = ascorbic acid, s = sample) where IC_{50} of ascorbic acid was 0.00387 mg/ml.

Ferric reducing power (FRP) was measured using the potassium ferricyanide assay [9]. Different dilutions of extracts (1 ml) were added to 2.5 ml phosphate

buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%, w/v). The mixture was incubated at 50°C for 20 min. After adding trichloroacetic acid solution (2.5 ml, 10%, w/v), the mixture was separated into aliquots of 2.5 ml, and diluted with 2.5 ml of water. To each diluted aliquot, 500 ml of ferric chloride solution (0.1%, w/v) was added. After 30 min, absorbance was measured at 700 nm. FRP of extracts was expressed as mg GAE/100 g. The calibration equation for gallic acid was y = 16.767x (R^2 = 0.9974), where y is the absorbance and x is the GA concentration in mg/ml.

Ferrous ion chelating (FIC) ability was determined using the ferrozine assay [9]. $FeSO_4$ (0.1 mM, 1 ml) was mixed with different dilutions of extracts (1 ml), followed by ferrozine (0.25 mM, 1 ml). Absorbance (A) was measured at 562 nm after 10 min. The ability of extracts to chelate ferrous ions was calculated as chelating effect % = (1 - $A_{extract}/A_{control}$) x 100. FIC ability was expressed as half-maximal chelating efficiency concentration (CEC_{50}) in mg/ml, or the effective concentration of the extract to chelate ferrous ions by 50%.

2.6. Polyphenol Oxidase Activity

Polyphenol oxidase (PPO) activity of extracts was assayed with (+)-catechin as substrate [10]. Plant samples (1 g) were powdered with liquid nitrogen in a mortar and washed three times with 50 ml of cold acetone (-20°C) to remove chlorophyll and other phytochemicals. Washed herbs were recovered by centrifugation to yield acetone powder. PPO was extracted from 0.1 g of acetone powder with 7.5 ml of 0.2 M sodium sulphate. Mixture was vortexed and filtered using cotton wool. The solution was then separated into three test tubes of 1.2 ml of aliquots. Two test tubes of aliquots were added with 300 µl of L-DOPA and another one with 300 µl of distilled water as blank. The presence of PPO activity was indicated by an increase in absorbance at 380 nm after 30 min.

2.7. Antibacterial Properties

Antibacterial properties of fresh and oven-dried herbs were measured using the disc-diffusion method [6,11]. Bacteria tested were Gram-positive *Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus*, and Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Bacterial cultures were obtained from stock cultures of the Microbiology Laboratory of UCSI University. The stock plate for each bacteria was prepared by streaking a single pure colony onto Mueller-Hinton agar (MHA) using a sterile inoculating loop. The plates were then incubated for 24 hr at 37°C and kept at 4°C until use.

Inoculums (100 µl) were spread evenly onto 20 ml MHA set in 90 mm Petri dishes using a sterile cotton swab. Sterilised paper discs (6 mm diameter) were impregnated with plant samples (2 mg per disc) using a micropipette and firmly placed onto the inoculated agar, ensuring even distribution to avoid overlapping of zones. Streptomycin susceptibility discs (10 µg) were used as positive controls. After incubation overnight at 37ºC, the mean diameter of inhibitory zone (DIZ) in millimetres was measured using a vernier calliper. Minimum inhibitory dose (MID) in mg/disc, or the minimum amount of extract per disc required to show a zone of inhibition, was recorded.

2.8. Statistical Analysis

All experiments were done in triplicate (n = 3) and results were expressed as means ± standard deviation (SD). Analysis of variance (ANOVA) was analysed using the Tukey Honestly Significant Difference (HSD) test based on significant difference of $p < 0.05$. Correlation coefficient (R^2) was calculated and plotted using the Microsoft Excel Analysis ToolPak. R^2 is the percentage of data that is closest to the line of best fit, represented by a regression equation.

3. RESULTS AND DISCUSSION

3.1. Antioxidant Properties

Antioxidant properties of fresh herbs (fresh weight) are shown in Table **1**. Rosemary had the highest phenolic content of TPC (1440 ± 94 mg GAE/100 g), TFC (340 ± 75 mg QE/100 g) and CQAC (703 ± 57 mg CGAE/100 g), and the strongest primary antioxidant activity of AEAC (1630 ± 93 mg AA/100 g) and FRP (1350 ± 97 mg GAE/100 g). Spearmint and thyme had the strongest secondary antioxidant activity with CEC_{50} values of 3.5 ± 0.4 and 3.9 ± 0.2 mg/ml, respectively. Ranking based on phenolic content was rosemary > thyme > sage > marjoram > oregano > spearmint > peppermint ~ basil. Ranking based on primary antioxidant activity was rosemary > thyme > marjoram > oregano > sage > spearmint > peppermint ~ basil. Ranking based on secondary antioxidant activity of CEC_{50} was spearmint ~ thyme > marjoram ~ oregano ~ peppermint > rosemary ~ basil ~ sage.

The potent primary antioxidant activity of rosemary may be attributed to its phenolic constituents. Major

Table 1: Phenolic Content and Antioxidant Activity of Fresh Herbs (Fresh Weight)

Fresh herb	Phenolic content			Antioxidant activity		
	TPC	TFC	CQAC	AEAC	FRP	CEC_{50}
Rosemary	1440 ± 94[a]	340 ± 75[a]	703 ± 57[a]	1630 ± 93[a]	1350 ± 97[a]	8.5 ± 1.9[c]
Thyme	1160 ± 59[b]	580 ± 11[a]	548 ± 49[b]	1210 ± 67[c]	1350 ± 80[a]	3.9 ± 0.2[a]
Sage	858 ± 121[a]	313 ± 32[a]	524 ± 135[a]	832 ± 121[a]	534 ± 90[a]	9.5 ± 1.6[c]
Marjoram	1010 ± 77[c]	196 ± 18[c]	261 ± 28[d]	1350 ± 62[b]	893 ± 48[b]	4.6 ± 0.5[b]
Oregano	857 ± 43[d]	189 ± 18[c]	297 ± 19[c]	799 ± 67[d]	781 ± 55[b]	5.1 ± 0.4[b]
Spearmint	655 ± 90[e]	165 ± 47[d]	259 ± 19[d]	580 ± 96[e]	448 ± 45[c]	3.5 ± 0.4[a]
Peppermint	338 ± 52[f]	124 ± 28[e]	139 ± 31[e]	288 ± 58[f]	233 ± 49[d]	5.5 ± 0.9[b]
Basil	299 ± 64[f]	109 ± 2[e]	182 ± 51[e]	264 ± 64[f]	247 ± 52[d]	9.2 ± 0.9[c]

Data on phenolic content and antioxidant activity are means ± standard deviations. Within the same column, different superscripts (a−f) are significantly different at $p < 0.05$, as measured by the Tukey HSD test. Units: total phenolic content (TPC) = mg GAE/100 g, total flavonoid content (TFC) = mg QE/100 g, caffeoylquinic acid content (CQAC) = mg CGAE/100 g, ascorbic acid equivalent antioxidant capacity (AEAC) = mg AA/100 g, ferric reducing power (FRP) = mg GAE/100 g and chelating efficiency concentration (CEC_{50}) = mg/ml. Abbreviations: GAE = gallic acid equivalent, QE = quercetin equivalent, CGAE = chlorogenic acid equivalent, AA = ascorbic acid and HSD = honestly significant difference.

phenolic compounds with antioxidant properties in rosemary are carnosic acid, carnosol, rosmanol and rosmarinic acid, with carnosol and carnosic acid being the two most important constituents which are frequently studied [2,12]. Rosmarinic acid may also be an important antioxidant as its radical scavenging activity is stronger than caffeic acid, chlorogenic acid, α-tocopherol, ferulic acid and butylated hydroxytoluene (BHT) [13]. The content of carnosic acid, rosmanol and rosmarinic acid in fresh rosemary was 127, 124 and 33 mg/100 g, respectively [14]. The content of rosmarinic acid, rosmanol, carnosol and carnosic acid in freeze-dried rosemary was 1286, 1113, 802 and 655 mg/100 g, respectively [15]. Rosmanol and carnosol exhibited antioxidant activity more than four and two times higher activity than BHT, respectively [16]. Carnosol and carnosic acid accounted for over 90% of the antioxidant activity of rosemary [17,18]. In addition, rosemary also contains luteolin and caffeic acid, which are known antioxidants [19].

Contrary to findings of this study, ranking of TPC and oxygen radical absorbance capacity (ORAC) of phosphate buffer extracts in fresh weight was marjoram ~ oregano > thyme ~ rosemary ~ peppermint ~ basil ~ sage > spearmint [14]. TPC and ORAC values of marjoram and oregano were 5.5 and 3.5 times those of rosemary and thyme, respectively. Ranking of ORAC of methanol extracts in dry weight was marjoram > basil > oregano > sage ~ thyme > rosemary [20]. TPC and DPPH radical scavenging values of hot water extracts in dry weight of peppermint, oregano and sage have been reported to be much higher than rosemary, marjoram and basil [21].

In general, there was strong correlation between TPC, AEAC and FRP of fresh herbs. The correlation coefficient (R^2) between TPC and AEAC was 0.944, and between TPC and FRP was 0.899. However, the correlation between TPC and CEC_{50} was very weak with R^2 of 0.003. Both AEAC and FRP are measures of the hydrogen- and electron-donating ability of primary antioxidants, respectively [22]. Primary antioxidants prevent oxidative damage by directly scavenging free radicals. This would mean that herbs with higher phenolic content would also have stronger primary antioxidant activities. FIC ability measures the ability of secondary antioxidants to chelate metal ions [22]. They act indirectly by preventing the formation of free radicals through Fenton's reaction. Species with strong CEC_{50} were not those with high phenolic content and primary antioxidant activity. This indicates that herbs with potent primary antioxidant activity may not necessary have strong secondary antioxidant activities.

Antioxidant properties of oven-dried and fresh herbs (fresh weight) are shown in Table **2**. For most herbs, oven drying resulted in declines in phenolic content and antioxidant activity compared to fresh herbs. Oregano was the only exception where phenolic content and primary antioxidant activity showed significant increase. Increase of OD oregano ranged from 4% (TPC) to 80% (CQAC) while increase of MOD oregano ranged from 6% (FRP) to 72% (CQAC). Secondary antioxidant activity (CEC_{50}) of oven-dried oregano, however, declined significantly from 5.1 ± 0.4 mg/ml (fresh) to 12 ± 1.0 mg/ml (OD) and 13 ± 1.0 mg/ml (MOD), respectively.

Table 2: Phenolic Content and Antioxidant Activity of Fresh and Oven-Dried Herbs (Fresh Weight)

Herb	Phenolic content			Antioxidant activity		
	TPC	TFC	CQAC	AEAC	FRP	CEC_{50}
Rosemary						
Fresh	1440 ± 94[a]	340 ± 75[a]	703 ± 57[a]	1630 ± 93[a]	1350 ± 97[a]	8.5 ± 1.9[a]
OD	754 ± 24[b]	161 ± 21[b]	297 ± 27[c]	1200 ± 49[b]	661 ± 45[c]	10 ± 2.0[b]
MOD	673 ± 27[c]	153 ± 13[b]	356 ± 20[b]	1150 ± 67[b]	857 ± 53[b]	8.8 ± 1.0[a]
Thyme						
Fresh	1160 ± 59[a]	580 ± 11[a]	548 ± 49[a]	1210 ± 67[a]	1350 ± 80[a]	3.9 ± 0.2[a]
OD	456 ± 28[c]	198 ± 14[c]	181 ± 37[b]	452 ± 56[c]	318 ± 36[c]	7.0 ± 1.0[b]
MOD	819 ± 36[b]	234 ± 19[b]	477 ± 42[a]	909 ± 51[b]	757 ± 46[b]	7.0 ± 1.2[b]
Sage						
Fresh	858 ± 121[a]	313 ± 32[a]	524 ± 135[a]	832 ± 121[a]	534 ± 90[a]	9.5 ± 1.6
OD	316 ± 40[c]	184 ± 15[b]	130 ± 2[c]	356 ± 26[b]	351 ± 12[b]	ND
MOD	544 ± 44[b]	202 ± 14[b]	327 ± 37[b]	715 ± 53[a]	655 ± 56[a]	ND
Marjoram						
Fresh	1010 ± 77[a]	196 ± 18[b]	261 ± 28[b]	1350 ± 62[a]	893 ± 48[a]	4.6 ± 0.5[a]
OD	830 ± 133[b]	237 ± 22[a]	125 ± 11[c]	326 ± 35[c]	333 ± 58[b]	15 ± 3.0[c]
MOD	1020 ± 152[a]	210 ± 42[ab]	419 ± 66[a]	1040 ± 94[b]	780 ± 106[a]	9.0 ± 2.0[b]
Oregano						
Fresh	857 ± 43[b]	189 ± 18[b]	297 ± 19[b]	799 ± 67[b]	781 ± 55[b]	5.1 ± 0.4[a]
OD	894 ± 65[b]	261 ± 28[a]	534 ± 96[a]	1130 ± 7[a]	1200 ± 94[a]	12 ± 1.0[b]
MOD	1020 ± 18[a]	213 ± 8[b]	510 ± 30[a]	1100 ± 41[a]	830 ± 31[b]	13 ± 1.0[b]
Spearmint						
Fresh	655 ± 90[a]	165 ± 47[a]	259 ± 19[a]	580 ± 96[a]	448 ± 45[a]	3.5 ± 0.4[a]
OD	271 ± 37[c]	132 ± 21[a]	122 ± 16[b]	274 ± 35[b]	209 ± 33[b]	15 ± 3.0[c]
MOD	348 ± 21[b]	143 ± 30[a]	229 ± 45[a]	312 ± 7[b]	335 ± 79[a]	9.0 ± 2.0[b]
Peppermint						
Fresh	338 ± 52[a]	124 ± 28[a]	139 ± 31[a]	288 ± 58[a]	233 ± 49[a]	5.5 ± 0.9[a]
OD	133 ± 12[c]	119 ± 10[a]	47 ± 4[b]	105 ± 15[c]	99 ± 9[b]	13 ± 3.0[b]
MOD	270 ± 5[b]	106 ± 18[a]	142 ± 11[a]	207 ± 5[b]	211 ± 9[a]	12 ± 2.0[b]
Basil						
Fresh	299 ± 64[a]	109 ± 2[a]	182 ± 51[a]	264 ± 64[a]	247 ± 52[a]	9.2 ± 0.9
OD	46 ± 1[c]	40 ± 3[c]	17 ± 2[c]	32 ± 1[c]	36 ± 1[c]	ND
MOD	109 ± 17[b]	65 ± 3[b]	70 ± 10[b]	71 ± 9[b]	104 ± 16[b]	ND

Data on phenolic content and antioxidant activity are means ± standard deviations. Within the same column, different superscripts (a−c) are significantly different at $p < 0.05$, as measured by the Tukey HSD test. ANOVA does not apply between species. Units: total phenolic content (TPC) = mg GAE/100 g, total flavonoid content (TFC) = mg QE/100 g, caffeoylquinic acid content (CQAC) = mg CGAE/100 g, ascorbic acid equivalent antioxidant capacity (AEAC) = mg AA/100 g, ferric reducing power (FRP) = mg GAE/100 g and chelating efficiency concentration (CEC_{50}) = mg/ml. Abbreviations: GAE = gallic acid equivalent, QE = quercetin equivalent, CGAE = chlorogenic acid equivalent, AA = ascorbic acid, ND = not detected and HSD = honestly significant difference.

The present findings are consistent with other studies on the effects of thermal treatments on plant samples which led to loss of antioxidant properties. Decrease in phenolic content and antioxidant activity has been reported in herbs [23,24], and in fruits and vegetables [25-27]. The decrease in antioxidant values following thermal treatments has been attributed to thermal degradation of phytochemicals, enzymatic degradation of phenolic compounds and loss of antioxidant enzyme activities [23,25]. Declines in phenolic content and antioxidant activity are usually accompanied by the loss of other bioactive properties [26].

Contrary to and in support of findings of this study, vacuum oven drying of Labiatae herbs of rosemary, oregano, marjoram, sage, basil and thyme resulted in higher ORAC values than fresh herbs [19]. Air-dried oregano, peppermint and lemon balm had significantly higher TPC and radical scavenging activity than fresh

herbs [28]. In this study, only oregano showed increase in phenolic content and primary antioxidant activity, but decrease in secondary antioxidant activity.

Increase in phenolic content and antioxidant activity following thermal treatments has also been reported in tomatoes [29], sweet corn [30], ginseng [31], persimmon peels [32] and Shiitake mushroom [33]. Explanations for the increase in antioxidant values included the release of bound phenolic compounds by the breakdown of cellular constituents and the formation of new compounds with enhanced antioxidant properties [34,35].

Moisture loss, phenolic content and antioxidant activity of oven-dried and commercial herbs (dry weight) are shown in Table **3**. Moisture loss ranged from 69.7 to 90.1% for OD herbs and from 73.4% to 89.3% for MOD herbs. Based on phenolic content and antioxidant activity, ranking of oven-dried herbs was oregano > marjoram > rosemary > thyme ~ spearmint > sage > peppermint > basil.

In most cases, MOD herbs yielded significantly higher phenolic content and antioxidant activity than OD herbs. This is can be attributed to the brief microwave pre-treatment (30 sec) before oven drying

Table 3: Moisture Loss, Phenolic Content and Antioxidant Activity of Oven-Dried and Commercial Herbs (Dry Weight)

Dried herb	Moisture loss (%)	Phenolic content			Antioxidant activity		
		TPC	TFC	CQAC	AEAC	FRP	CEC_{50}
Rosemary							
OD	69.7	2490 ± 80^{b}	530 ± 68^{b}	979 ± 89^{c}	3960 ± 163^{b}	2180 ± 148^{b}	3.1 ± 0.7^{b}
MOD	73.4	2530 ± 102^{b}	574 ± 50^{b}	1340 ± 77^{b}	4340 ± 252^{a}	3220 ± 200^{a}	2.2 ± 0.1^{a}
COM	NA	3700 ± 245^{a}	1350 ± 44^{a}	1700 ± 38^{a}	4530 ± 364^{a}	2920 ± 147^{a}	3.7 ± 0.3^{b}
Sage							
OD	75.3	1280 ± 161^{b}	745 ± 61^{b}	525 ± 9^{b}	1440 ± 105^{b}	1420 ± 50^{b}	ND
MOD	82.4	3090 ± 248^{a}	1150 ± 79^{a}	1860 ± 211^{a}	4060 ± 302^{a}	3720 ± 320^{a}	9.1 ± 3.1^{a}
Oregano							
OD	85.4	6120 ± 447^{b}	1790 ± 192^{a}	3660 ± 660^{a}	7760 ± 48^{a}	8200 ± 643^{a}	1.8 ± 0.1^{b}
MOD	85.0	6800 ± 120^{a}	1420 ± 53^{b}	3400 ± 203^{a}	7360 ± 273^{b}	5530 ± 206^{b}	1.9 ± 0.2^{b}
COM	NA	2670 ± 194^{c}	820 ± 43^{c}	834 ± 71^{b}	2090 ± 140^{c}	2040 ± 184^{c}	1.3 ± 0.0^{a}
Marjoram							
OD	85.0	5530 ± 884^{a}	1580 ± 148^{a}	833 ± 72^{b}	2170 ± 233^{b}	2220 ± 384^{b}	1.3 ± 0.1^{b}
MOD	82.9	5990 ± 891^{a}	1230 ± 245^{b}	2450 ± 387^{a}	6100 ± 549^{a}	4560 ± 619^{a}	1.0 ± 0.1^{a}
Spearmint							
OD	88.4	2340 ± 317^{b}	1140 ± 183^{a}	1050 ± 139^{b}	2360 ± 298^{a}	1800 ± 282^{b}	1.7 ± 0.4^{b}
MOD	87.7	2830 ± 174^{a}	1160 ± 244^{a}	1860 ± 365^{a}	2540 ± 59^{a}	2720 ± 643^{a}	1.1 ± 0.2^{a}
COM	NA	2050 ± 204^{b}	674 ± 38^{b}	924 ± 107^{b}	1870 ± 395^{b}	1490 ± 168^{b}	3.4 ± 0.3^{c}
Thyme							
OD	77.3	2010 ± 123^{b}	871 ± 63^{b}	798 ± 161^{b}	1990 ± 247^{b}	1400 ± 157^{b}	1.7 ± 0.2^{b}
MOD	79.1	3920 ± 170^{a}	1120 ± 89^{a}	2280 ± 200^{a}	4350 ± 243^{a}	3620 ± 222^{a}	1.4 ± 0.1^{a}
COM	NA	1760 ± 57^{c}	661 ± 93^{c}	513 ± 11^{c}	1310 ± 53^{c}	942 ± 13^{c}	9.5 ± 1.4^{c}
Peppermint							
OD	86.3	974 ± 85^{b}	868 ± 73^{a}	341 ± 30^{c}	768 ± 113^{b}	723 ± 63^{b}	1.8 ± 0.4^{b}
MOD	89.0	2450 ± 41^{a}	964 ± 167^{a}	1290 ± 98^{a}	1880 ± 42^{a}	1920 ± 80^{a}	1.3 ± 0.0^{a}
COM	NA	804 ± 100^{b}	620 ± 42^{b}	835 ± 195^{b}	807 ± 89^{b}	631 ± 32^{b}	5.8 ± 0.2^{c}
Basil							
OD	90.1	466 ± 9^{b}	404 ± 26^{b}	170 ± 18^{b}	325 ± 11^{b}	367 ± 9^{b}	ND
MOD	89.3	1020 ± 156^{a}	611 ± 26^{a}	656 ± 96^{a}	660 ± 88^{a}	973 ± 149^{a}	ND
COM	NA	464 ± 50^{b}	339 ± 98^{b}	207 ± 30^{b}	300 ± 25^{b}	293 ± 31^{c}	ND

Data on phenolic content and antioxidant activity are means ± standard deviations. Within the same column of each species, different superscripts (a−c) are significantly different at $p < 0.05$, as measured by the Tukey HSD test. ANOVA does not apply between species. Units: total phenolic content (TPC) = mg GAE/100 g, total flavonoid content (TFC) = mg QE/100 g, caffeoylquinic acid content (CQAC) = mg CGAE/100 g, ascorbic acid equivalent antioxidant capacity (AEAC) = mg AA/100 g, ferric reducing power (FRP) = mg GAE/100 g and chelating efficiency concentration (CEC_{50}) = mg/ml. Abbreviations: OD = oven drying at 50°C, MOD = microwave pre-treatment before oven drying at 50°C, COM = commercial, GAE = gallic acid equivalent, QE = quercetin equivalent, CGAE = chlorogenic acid equivalent, AA = ascorbic acid, NA = not available, ND = not detected and HSD = honestly significant difference.

which was adequate to inactivate PPO activity. Qualitative determination of PPO activity indicated that only MOD herbs were inactivated. The resulting solutions of MOD extracts were colourless, whereas those of OD extracts were orange to red, suggesting the presence of PPO activity.

It has been reported that microwave drying of leaf samples has the ability to inactivate PPO because microwaves inhibit the binding of polyphenols to the leaf matrix [10]. It is commonly used for inactivation of PPO in manufacturing of green tea. In microwave drying, the absorption of microwave energy by water molecules in the plant samples is rapid, and inactivation of degradative enzymes is faster compared to the conventional oven [23].

Values of OD and MOD oregano, spearmint, thyme, peppermint and basil were higher than commercial samples, while those of rosemary were lower. Of the six commercial herbs, rosemary had the highest values, followed by oregano, spearmint, thyme, peppermint and basil. In commercial herbs, there was also strong correlation between TPC, AEAC and FRP. The correlation coefficient (R^2) between TPC and AEAC was 0.910, and between TPC and FRP was 0.981. The correlation between TPC and CEC_{50} was also very weak with R^2 of 0.240.

3.2. Antibacterial Properties

All fresh, oven-dried and commercial herbs showed no antibacterial activity against Gram-negative *E. coli*, *P. aeruginosa* and *S. typhi*, which are generally less susceptible to antibiotics than Gram-positive bacteria.

Gram-negative bacteria are known to have an outer membrane of lipoprotein and lipopolysaccharide, which is selectively permeable and can regulate access of antimicrobials into the underlying cell structures [36,37]. This renders them generally less susceptible to plant extracts than Gram-positive bacteria. The Gram-positive bacteria cell wall is composed of a thick, multi-layered peptidoglycan sheath outside the cytoplasmic membrane [37]. Antibiotics can exert toxicity by inhibiting the peptidoglycan synthesis in the bacteria cell wall, inhibiting protein and nucleic acid synthesis, and by disrupting membrane leading to leakage of cytoplasmic contents.

Antibacterial properties of fresh herbs are shown in Table **4**. Sage and rosemary inhibited the growth of all three Gram-positive bacteria of *B. cereus*, *M. luteus* and *S. aureus* with DIZ of 10 to 16 mm and MID of 0.063 to 0.500 mg/ml. Thyme inhibited the growth of *M. luteus* and *S. aureus*. Oregano inhibited the growth of only *S. aureus*. Marjoram, spearmint, peppermint and basil showed no antibacterial activity.

Antibacterial properties of oven-dried and commercial herbs are shown in Table **5**. Rosemary and sage inhibited the growth of all three Gram-positive bacteria. DIZ and MID of rosemary were 8 to 15 mm and 0.063 to 0.125 mg/ml while those of sage were 11 to 17 mm and 0.063 to 0.125 mg/ml, respectively. OD oregano inhibited *S. aureus*. Oven-dried peppermint, spearmint, thyme, marjoram and basil exhibited no antibacterial activity. Of the six commercial herbs, rosemary, peppermint and spearmint inhibited all three Gram-positive bacteria with rosemary being the most potent with DIZ of 14 to 15 mm and MID of 0.063. Oregano and thyme inhibited *B. cereus* and *S. aureus*, while basil exhibited no antibacterial activity.

The antibacterial properties of Labiatae herbs against have been studied. Against Gram-positive *Listeria monocytogenes*, *B. cereus* and *S. aureus*, and Gram-negative *Salmonella anatum* and *E. coli*, ranking based on DIZ was oregano (13.3 mm), thyme (7.3 mm), sage (6.9 mm), rosemary (6.7 mm) and basil (5.5

Table 4: Antibacterial Activity of Fresh Herbs based on Diameter of Inhibitory Zone (DIZ) and Minimum Inhibitory Dose (MID)

Fresh herb	DIZ (mm)			MID (mg/disc)		
	B. cereus	*M. luteus*	*S. aureus*	*B. cereus*	*M. luteus*	*S. aureus*
Sage	16 ± 0.2	14 ± 0.1	11 ± 0.1	0.063	0.125	0.125
Rosemary	10 ± 0.9	13 ± 0.2	10 ± 0.1	0.125	0.125	0.500
Thyme	−	9.7 ± 0.6	8.0 ± 0.0	−	2.000	2.000
Oregano	−	−	8.0 ± 1.0	−	−	2.000

Data on phenolic content and antioxidant activity are means ± standard deviations. Abbreviations: *B.* = *Bacillus*, *M.* = *Micrococcus* and *S.* = *Staphylococcus*. Amount of extract = 2 mg/disc. Fresh peppermint, spearmint, marjoram and basil showed no antibacterial activity.

Table 5: Antibacterial Activity of Oven-Dried and Commercial Herbs based on Diameter of Inhibitory Zone (DIZ) and Minimum Inhibitory Dose (MID)

Dried herb	DIZ (mm)			MID (mg/disc)		
	B. cereus	*M. luteus*	*S. aureus*	*B. cereus*	*M. luteus*	*S. aureus*
Rosemary						
OD	8 ± 0.5	14 ± 1.6	10 ± 0.3	0.125	0.125	0.125
MOD	10 ± 0.6	14 ± 0.9	11 ± 2.1	0.063	0.063	0.063
COM	15 ± 2.2	14 ± 0.3	15 ± 0.9	0.063	0.063	0.063
Sage						
OD	12 ± 0.6	16 ± 1.5	11 ± 0.5	0.125	0.125	0.125
MOD	14 ± 0.3	17 ± 1.2	14 ± 0.5	0.063	0.125	0.125
Peppermint						
OD	–	–	–	–	–	–
MOD	–	–	–	–	–	–
COM	8.0 ± 0.0	7.7 ± 0.1	9.3 ± 0.1	0.250	1.000	0.125
Oregano						
OD	–	–	7.6 ± 0.3	–	–	2.000
MOD	–	–	–	–	–	–
COM	8.0 ± 0.5	–	15 ± 0.3	1.000	–	0.250
Spearmint						
OD	–	–	–	–	–	–
MOD	–	–	–	–	–	–
COM	10 ± 0.2	7.8 ± 0.1	9.7 ± 0.1	0.500	1.000	1.000
Thyme						
OD	–	–	–	–	–	–
MOD	–	–	–	–	–	–
COM	10 ± 1.0	–	12 ± 0.6	0.500	–	0.500

Data on phenolic content and antioxidant activity are means ± standard deviations. Abbreviations: *B.* = *Bacillus*, *M.* = *Micrococcus*, *S.* = *Staphylococcus*, OD = oven drying at 50°C, MOD = microwave pre-treatment before oven drying at 50°C and COM = commercial. Amount of extract = 2 mg/disc. Oven-dried and commercial basil, and oven-dried marjoram showed no antibacterial activity.

mm) [38]. Oregano inhibited *S. anatum* and *E. coli* but not basil. Thyme, sage and rosemary inhibited *S. anatum* but not *E. coli*. Contrary to findings of the present study, a review of antimicrobial activity of spices [39] reported that *M. luteus* was inhibited by oregano but not by rosemary and sage. The disparity in results could be due differences in the sample preparation, extraction, bacterial strains and extract concentration.

Most antimicrobial phytochemicals in herbs and spices consist of substituted phenolic rings [3]. The OH groups in phenolic compounds are thought to be related to their microbial inhibitory action. There is also evidence that compounds with increasing number of OH groups in the compound exhibits greater toxicity to microbes.

In this study, rosemary and sage (fresh, oven-dried and commercial) inhibited the growth of *B. cereus*, *M. luteus* and *S. aureus* with MID of 0.06 to 0.25. MID of hot water extracts of green teas of *Camellia sinensis* ranged from 0.06 to 2.00 against the three Gram-positive bacteria [6]. MID of black teas was 0.50 against *B. cereus*, 0.13 against *M. luteus*, with no activity against *S. aureus*. It can therefore be inferred that rosemary and sage had stronger antibacterial properties than teas of *C. sinensis*.

4. CONCLUSION

Of the eight species of Labiatae herbs, fresh and commercial rosemary had the highest phenolic content and antioxidant activity. For most herbs, oven drying resulted in loss of antioxidant values compared to fresh herbs with the exception of oregano. MOD herbs yielded significantly higher phenolic content and antioxidant activity than OD herbs. Values of OD and MOD oregano, spearmint, thyme, peppermint and basil were higher than commercial samples, while those of rosemary were lower. Of the six commercial herbs, rosemary had the highest values, followed by oregano, spearmint, thyme, peppermint and basil. Rosemary,

sage, peppermint and spearmint inhibited all three Gram-positive bacteria of *B. cereus*, *M. luteus* and *S. aureus*. Rosemary and sage had stronger antibacterial properties than green and black teas of *C. sinensis*. When used in combination, rosemary and sage can have enhanced antioxidant and antibacterial effects, which are desirable which are desirable in developing nutraceutical products, and in controlling rancidity and bacterial growth in food.

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