Antioxidant and antibacterial properties of some fresh and dried Labiatae herbs

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ABSTRACT

Introduction: Although the antioxidant and antibacterial properties of Labiatae herbs are well known, the effects of different drying methods are yet to be determined. 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 investigated, in comparison with commercial brands of dried herbs. **Methods:** Antioxidant properties of total phenolic content, total flavonoid content, caffeoylquinic acid content, free radical scavenging activity, and ferric reducing power were assessed using the Folin-Ciocalteu, aluminium chloride, molybdate, DPPH radical scavenging, and potassium ferricyanide assays, respectively. Antibacterial properties were assessed using the disc-diffusion assay based on minimum inhibitory dose (MID). Bacteria tested were Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, and Gram-positive *Bacillus cereus*, *Micrococcus luteus*, and *Staphylococcus aureus*. The three drying treatments were oven drying at 50°C (OD_{50}), oven drying at 80°C (OD_{80}), and oven drying at 50°C with microwave pre-treatment (MOD_{50}). **Results:** Fresh and commercial rosemary, and oven-dried oregano had the strongest antioxidant properties. Generally, MOD_{50} herbs had the strongest antioxidant properties followed by OD_{50} and OD_{80} herbs. Oven-dried rosemary had lower phenolic content and antioxidant activity than commercial rosemary, while oven-dried oregano, spearmint, thyme, peppermint, and basil had higher values. All herbs showed no antibacterial activity against Gram-negative *E. coli*, *P. aeruginosa*, and *S. typhi*. Rosemary, sage, peppermint, and spearmint inhibited the growth of Gram-positive *B. cereus*, *M. luteus*, and *S. aureus*. Compared to green and black teas of *Camellia sinensis*, rosemary and sage have stronger antibacterial properties. **Conclusion:** Labiatae herbs can have enhanced antioxidant and antibacterial effects when used in combination. Further research is needed to study the synergistic behaviour of these herbs.

Keywords: oregano, marjoram, rosemary, sage, basil, thyme, peppermint, spearmint.

INTRODUCTION

Herbs and spices are commonly used for flavouring food and as traditional medicines as traditional medicines for generations. There is no clear distinction between them.[1] 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.

Plants of the family Labiatae are annual or perennial herbs that are densely glandular and aromatic.[2] 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.

Among the common species used for flavouring, oregano is a favourite seasoning for pizza and other Italian dishes.[3] Rosemary is used for flavouring meat and poultry dishes. Thyme adds a pungent taste to meat and vegetables, and is

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the most 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.[2] 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 in flavouring pharmaceutical and oral preparations e.g. mouth rinse and toothpaste. Spearmint is often used to flavour vegetables, soups, meat and fish sauces, and salads. It is also used in the flavouring of chewing gums, toothpastes, and other oral products.

Although the antioxidant and antibacterial properties of Labiatae herbs are well known, the effects of different drying methods 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 different drying methods were assessed with comparison to commercial brands of dried herbs.

MATERIALS AND METHODS

Herb samples

Fresh herbs produced by Genting Garden in Genting Highlands were purchased from the Jusco and Cold Storage Supermarkets in the Mid-Valley Megamall in Kuala Lumpur, 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 (COM) of oregano, rosemary, basil, thyme, peppermint, and spearmint were used as standards for comparison.

Drying protocols

The drying protocol used was oven drying. In oven drying, 15 g of herb was dried in an universal oven (Memmert, Germany, Model UFB500) for 5.5 h at 50°C (OD_{50}) and for 3.5 h at 80°C (OD_{80}). The effects of microwave pre-treatment prior to oven drying were also assessed. To assess the effects of microwave pre-treatment, 15 g of herb was heated in a microwave oven (Sharp, Malaysia, Model R-397J(S), 230–240 V, 50 Hz) for 30 sec followed by oven drying for 5.5 h at 50°C (MOD_{50}).

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) and 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.

Antioxidant properties

Fresh and oven-dried herbs were analysed for phenolic content (total phenolic content, total flavonoid content, and caffeoylquinic acid content), and antioxidant activity (radical scavenging activity and ferric reducing power).

Total phenolic content (TPC) was assessed using the Folin-Ciocalteu (FC) assay.[4] 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 incubating 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.[5] 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 determined at 415 nm against a sample blank 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.[6] 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. Absorbance was measured at 370 nm against a sample blank 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.

Radical scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.[4] 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.[7] 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 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.

Antibacterial properties

Antibacterial properties of fresh and oven-dried herbs were measured using the disc-diffusion method.[4,8] Bacterial species tested were Gram-positive *Bacillus cereus*, *Micrococcus luteus*, and *Staphylococcus aureus*, and Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*. Inoculums (100 µl) were spread evenly onto 20 ml Mueller-Hinton agar 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. The minimum inhibitory dose (MID) or minimum amount of extract in mg/disc required to show a zone of inhibition was recorded after incubation overnight at 37ºC.

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's Honestly Significant Difference (HSD) test, based on significant difference of $p < 0.05$.

RESULTS AND DISCUSSION

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 antioxidant activity of AEAC (1630 ± 93 mg AA/100 g) and FRP (1350 ± 97 mg GAE/100 g). Ranking based on phenolic content was rosemary > thyme > sage > marjoram > oregano > spearmint > peppermint ~ basil. Ranking based on antioxidant activity was rosemary > thyme > marjoram > oregano > sage > spearmint > peppermint ~ basil.

The potent antioxidant properties of rosemary may be attributed to its phenolic constituents. Major phenolic compounds with antioxidant properties in rosemary are carnosic acid, carnosol, rosmanol, and rosmarinic acid.[3] In rosemary, carnosol and carnosic acid are the two most important constituents, frequently studied for antioxidant activity.[9] Rosmarinic acid may also be an important antioxidant, but it occurs in greater quantity in other plants. The content of carnosic acid, rosmanol, and rosmarinic acid in fresh samples was 127, 124, and 33 mg/100 g, respectively.[10] The content of rosmarinic acid, rosmanol, carnosol, and carnosic acid in freeze-dried rosemary was 1286, 1113, 802, and 655 mg/100 g, respectively.[11] Rosmanol and carnosol exhibited antioxidant activity more than four and two times higher activity than butylated hydroxytoluene (BHT), respectively.[12] Carnosol and carnosic acid contributed most of the antioxidant activity of rosemary.[13] Other phenolic compounds found in rosemary are luteolin and caffeic acid.[14]

Percentage gain or loss in antioxidant properties of oven-dried in comparison with fresh herbs (fresh weight) is shown in Table 1. For most herbs, oven drying resulted in declines in phenolic content and antioxidant activity. Declines ranged from 26% to 72% (rosemary), from 13% to 87% (thyme), from 10% to 72% (spearmint), from 2% to 82% (peppermint), and from 40% to 95% (basil). In some cases, the declines were not significant (NS) at $p < 0.05$, reflecting little or change in antioxidant properties. Generally, declines were most drastic in OD_{80} herbs followed by OD_{50} herbs and MOD_{50} herbs.

Table 1 Phenolic content and antioxidant activity of fresh herbs, and percentage loss (–) or gain (+) in values of oven-dried herbs in comparison with fresh herbs (fresh weight)

Herb	Phenolic content			Antioxidant activity	
	TPC	TFC	CQAC	AEAC	FRP
Rosemary					
Fresh	1440 ± 94[a]	340 ± 75[b]	703 ± 57[a]	1630 ± 93[a]	1350 ± 97[a]
OD_{50}	−48%	−53%	−58%	−26%	−51%
OD_{80}	−60%	−66%	−72%	−36%	−51%
MOD_{50}	−53%	−55%	−49%	−29%	−37%
Thyme					
Fresh	1160 ± 59[b]	580 ± 11[a]	548 ± 49[b]	1210 ± 67[c]	1350 ± 80[a]
OD_{50}	−61%	−66%	−67%	−63%	−76%
OD_{80}	−78%	−85%	−82%	−84%	−87%
MOD_{50}	−29%	−60%	−13% (NS)	−25%	−44%
Sage					
Fresh	858 ± 121[d]	313 ± 32[b]	524 ± 135[b]	832 ± 121[d]	534 ± 90[c]
OD_{50}	−63%	−41%	−75%	−57%	−34%
OD_{80}	−69%	−52%	−75%	−78%	−55%
MOD_{50}	−37%	−35%	−38%	−14% (NS)	+23%
Marjoram					
Fresh	1010 ± 77[c]	196 ± 18[c]	261 ± 28[d]	1350 ± 62[b]	893 ± 48[b]
OD_{50}	−18%	+21%	−52%	−76%	−63%
OD_{80}	−35%	−19%	−2% (NS)	−65%	−31%
MOD_{50}	+1% (NS)	+7% (NS)	+61%	−23%	−13% (NS)
Oregano					
Fresh	857 ± 43[d]	189 ± 18[c]	297 ± 19[c]	799 ± 67[d]	781 ± 55[b]
OD_{50}	+4% (NS)	+38%	+80%	+41%	+54%
OD_{80}	−28%	−24%	−33%	−30%	−16%
MOD_{50}	+19%	+13% (NS)	+72%	+38%	+6% (NS)
Spearmint					
Fresh	655 ± 90[e]	165 ± 47[d]	259 ± 19[d]	580 ± 96[e]	448 ± 45[c]
OD_{50}	−59%	−20% (NS)	−53%	−53%	−53%
OD_{80}	−65%	−10% (NS)	−59%	−72%	−61%
MOD_{50}	−47%	−13% (NS)	−12% (NS)	−46%	−25%
Peppermint					
Fresh	338 ± 52[f]	124 ± 28[e]	139 ± 31[e]	288 ± 58[f]	233 ± 49[d]
OD_{50}	−61%	−4% (NS)	−66%	−64%	−58%
OD_{80}	−76%	−28%	−82%	−85%	−73%
MOD_{50}	−20%	−15% (NS)	−2% (NS)	−28%	−9% (NS)
Basil					
Fresh	299 ± 64[f]	109 ± 2[e]	182 ± 51[e]	264 ± 64[f]	247 ± 52[d]
OD_{50}	−85%	−63%	−91%	−88%	−85%
OD_{80}	−89%	−70%	−93%	–95%	−86%
MOD_{50}	−64%	−40%	−62%	−73%	−58%

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's 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, and ferric reducing power (FRP) = mg GAE/100 g. Abbreviations: GAE = gallic acid equivalent, QE = quercetin equivalent, CGAE = chlorogenic acid equivalent, AA = ascorbic acid, NS = not significant, and HSD = honestly significant difference.

Loss of antioxidant properties was the least in MOD_{50} herbs (Table 1). This can be attributed to the brief microwave pre-treatment of 30 sec before oven drying at 50°C, which was adequate to inactivate the activity of polyphenol oxidase (PPO). It has been reported that microwave drying has the ability to inactivate PPO because microwaves inhibit the binding of polyphenols to the leaf matrix.[15] Microwave inactivation of PPO has been used in the manufacture of green tea. In microwave treatment, 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.[16]

Other herbs showed both declines or gains. OD_{50} and MOD_{50} oregano were the only exceptions where antioxidant properties showed gains in phenolic content and antioxidant activity. Increase of OD_{50} oregano ranged from 4% (TPC) to 80% (CQAC), while increase of MOD_{50} oregano ranged from 6% (FRP) to 72% (CQAC).

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,[16,17] and in fruits and vegetables.[18–20]

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.[16,18] Declines in phenolic content and antioxidant activity are usually accompanied by the loss of other bioactive properties.[19]

Contrary to 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.[14] Air-dried oregano, peppermint and lemon balm had significantly higher TPC and radical scavenging activity than fresh herbs.[21] In this study, only oregano showed increase in phenolic content and antioxidant activity.

Antioxidant properties of oven-dried herbs (dry weight) in comparison with commercial herbs are shown in Table 2. Moisture loss ranged from 69.7% to 90.1% for OD_{50}, from 72.3% to 92.8% for OD_{80}, and from 73.4% to 89.3% for MOD_{50}. Ranking of oven-dried herbs, based on phenolic content and antioxidant activity, was oregano > marjoram > rosemary > thyme ~ spearmint > sage > peppermint > basil.

Table 2 Moisture loss, phenolic content, and antioxidant activity of oven-dried herbs in comparison with commercial herbs (dry weight)

Dried herb	Moisture loss (%)	Phenolic content			Antioxidant activity	
		TPC	TFC	CQAC	AEAC	FRP
Oregano						
OD_{50}	85.4	6120 ± 447^{b}	1790 ± 192^{a}	3660 ± 660^{a}	7760 ± 48^{a}	8200 ± 643^{a}
OD_{80}	85.2	4180 ± 243^{c}	1070 ± 65^{c}	1720 ± 63^{b}	3220 ± 275^{c}	4140 ± 246^{c}
MOD_{50}	85.0	6800 ± 120^{a}	1420 ± 53^{b}	3400 ± 203^{a}	7360 ± 273^{b}	5530 ± 206^{b}
COM	NA	2670 ± 194^{d}	820 ± 43^{d}	834 ± 71^{c}	2090 ± 140^{d}	2040 ± 184^{d}
Marjoram						
OD_{50}	85.0	5530 ± 884^{a}	1580 ± 148^{a}	833 ± 72^{c}	2170 ± 233^{c}	2220 ± 384^{b}
OD_{80}	85.1	4400 ± 235^{b}	961 ± 213^{c}	1330 ± 100^{b}	3760 ± 306^{b}	4380 ± 383^{a}
MOD_{50}	82.9	5990 ± 891^{a}	1230 ± 245^{b}	2450 ± 387^{a}	6100 ± 549^{a}	4560 ± 619^{a}
Rosemary						
OD_{50}	69.7	2490 ± 80^{b}	530 ± 68^{b}	979 ± 89^{d}	3960 ± 163^{b}	2180 ± 148^{b}
OD_{80}	72.3	2080 ± 426^{b}	410 ± 89^{c}	709 ± 117^{c}	3780 ± 306^{b}	2380 ± 340^{b}
MOD_{50}	73.4	2530 ± 102^{b}	574 ± 50^{b}	1340 ± 77^{b}	4340 ± 252^{a}	3220 ± 200^{a}
COM	NA	3700 ± 245^{a}	1350 ± 44^{a}	1700 ± 38^{a}	4530 ± 364^{a}	2920 ± 147^{a}
Spearmint						
OD_{50}	88.4	2340 ± 317^{b}	1140 ± 183^{a}	1050 ± 139^{b}	2360 ± 298^{a}	1800 ± 282^{b}
OD_{80}	87.4	1790 ± 251^{c}	1180 ± 83^{a}	837 ± 159^{b}	1280 ± 253^{b}	1380 ± 208^{b}
MOD_{50}	87.7	2830 ± 174^{a}	1160 ± 244^{a}	1860 ± 365^{a}	2540 ± 59^{a}	2720 ± 643^{a}
COM	NA	2050 ± 204^{bc}	674 ± 38^{b}	924 ± 107^{b}	1870 ± 395^{b}	1490 ± 168^{b}
Thyme						
OD_{50}	77.3	2010 ± 123^{b}	871 ± 63^{b}	798 ± 161^{b}	1990 ± 247^{b}	1400 ± 157^{b}
OD_{80}	81.9	1400 ± 92^{d}	492 ± 30^{d}	538 ± 58^{c}	1040 ± 61^{d}	990 ± 60^{c}
MOD_{50}	79.1	3920 ± 170^{a}	1120 ± 89^{a}	2280 ± 200^{a}	4350 ± 243^{a}	3620 ± 222^{a}
COM	NA	1760 ± 57^{c}	661 ± 93^{c}	513 ± 11^{c}	1310 ± 53^{c}	942 ± 13^{c}
Sage						
OD_{50}	75.3	1280 ± 161^{b}	745 ± 61^{b}	525 ± 9^{c}	1440 ± 105^{b}	1420 ± 50^{b}
OD_{80}	81.0	1410 ± 86^{b}	794 ± 29^{b}	718 ± 60^{b}	943 ± 106^{c}	1260 ± 80^{c}
MOD_{50}	82.4	3090 ± 248^{a}	1150 ± 79^{a}	1860 ± 211^{a}	4060 ± 302^{a}	3720 ± 320^{a}
Peppermint						
OD_{50}	86.3	974 ± 85^{b}	868 ± 73^{a}	341 ± 30^{c}	768 ± 113^{b}	723 ± 63^{b}
OD_{80}	89.9	809 ± 95^{b}	882 ± 38^{a}	249 ± 68^{c}	438 ± 65^{c}	612 ± 110^{b}
MOD_{50}	89.0	2450 ± 41^{a}	964 ± 167^{a}	1290 ± 98^{a}	1880 ± 42^{a}	1920 ± 80^{a}
COM	NA	804 ± 100^{b}	620 ± 42^{b}	835 ± 195^{b}	807 ± 89^{b}	631 ± 32^{b}
Basil						
OD_{50}	90.1	466 ± 9^{b}	404 ± 26^{b}	170 ± 18^{b}	325 ± 11^{b}	367 ± 9^{c}
OD_{80}	92.8	473 ± 29^{b}	452 ± 36^{b}	177 ± 6^{b}	163 ± 2^{c}	472 ± 22^{b}
MOD_{50}	89.3	1020 ± 156^{a}	611 ± 26^{a}	656 ± 96^{a}	660 ± 88^{a}	973 ± 149^{a}
COM	NA	464 ± 50^{b}	339 ± 98^{b}	207 ± 30^{b}	300 ± 25^{b}	293 ± 31^{d}

Data on phenolic content and antioxidant activity are means ± standard deviations Within the same column of each species, different superscripts (a–d) are significantly different at $p < 0.05$, as measured by the Tukey's 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, and ferric reducing power (FRP) = mg GAE/100 g. Abbreviations: OD_{50} = oven drying at 50°C, OD_{80} = oven drying at 80°C, MOD_{50} = oven drying at 50°C with microwave pre-treatment, COM = commercial, GAE = gallic acid equivalent, QE = quercetin equivalent, CGAE = chlorogenic acid equivalent, AA = ascorbic acid, NA = not available, and HSD = honestly significant difference.

In an earlier study, of eight Labiatae species analysed, fresh oregano has been reported to have the highest phenolic content and oxygen radical absorbance capacity.[10] Freeze-dried oregano has also been reported to have significantly higher phenolic content and total equivalent antioxidant capacity than rosemary, sage, thyme, and basil.[11] Major phenolic compounds in oregano were rosmarinic acid (2563 mg/100 g) and caffeoyl derivatives (1324 mg/100 g).

Among the commercial herbs, rosemary had the highest phenolic content and antioxidant activity, followed by oregano, spearmint, thyme, peppermint, and basil (Table 2). MOD_{50} herbs generally had the strongest antioxidant properties, followed by OD_{50} and OD_{80} herbs. In rosemary, oven drying resulted in lower phenolic content and antioxidant activity compared to commercial rosemary. Oven-dried oregano, spearmint, thyme, peppermint, and basil generally had higher values than commercial samples.

Fresh and commercial rosemary had the highest phenolic content and antioxidant activity but oven-dried rosemary ranked third. This suggested that thermal drying has adverse effects on its antioxidant properties. The commercial brand of rosemary may be freeze-dried and this can explain its strong antioxidant properties as heat is not involved. In terms of phenolic content and antioxidant activity, fresh oregano ranked fifth while dried and commercial oregano ranked first and second. Similarly, fresh marjoram ranked fourth while dried marjoram ranked second. It is likely that thermal drying may have enhanced the antioxidant properties of oregano and marjoram.

Increase in phenolic content and antioxidant activity following thermal treatments has been reported in tomatoes, sweet corn, ginseng, persimmon peels, and Shiitake mushroom.[22–26] Explanations for the increase in antioxidant values have been attributed to the release of bound phenolic compounds by the breakdown of cellular constituents and to the formation of new compounds with enhanced antioxidant properties.[27,28]

Antibacterial properties

All fresh, commercial and oven-dried 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 have a selectively permeable outer membrane of lipoprotein and lipopolysaccharide, which can regulate access of antimicrobials into the underlying cell structures.[29,30] This renders them generally less susceptible to plant extracts than Gram-positive bacteria.

Antibacterial properties of fresh herbs are shown in Table 3. Sage and rosemary inhibited the growth of all three Gram-positive bacteria of *B. cereus*, *M. luteus*, and *S. aureus*. 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 commercial herbs are shown in Table 4. Rosemary, peppermint, and spearmint inhibited all three Gram-positive bacteria. Rosemary was the strongest with MID of 0.06 mg/disc for all three bacterial species. Oregano and thyme inhibited *B. cereus* and *S. aureus* while basil exhibited no antibacterial activity.

In a comparative study of rosmarinic acid content in some Labiatae herbs, the content of rosmarinic acid in spearmint, thyme and peppermint were 58.5 ± 1.4, 23.5 ± 0.5, and 28.2 ± 0.3 mg/g.[31] The content of rosmarinic acid was the lowest in rosemary (7.2 ± 0.1 mg/g). This implies that the potent antibacterial properties of rosemary may be due to phenolic constituents other than rosmarinic acid.

Antibacterial properties of oven-dried herbs are shown in Table 4. OD_{50}, OD_{80}, and MOD_{50} rosemary inhibited the growth of all three Gram-positive bacteria. MID of OD_{80} and MOD_{50} rosemary was 0.06 mg/disc, and OD_{50} rosemary was 0.13 mg/disc. OD_{50}, OD_{80}, and MOD_{50} sage inhibited all three Gram-positive bacteria. MID ranged from 0.06 to 0.25 mg/disc. OD_{50} oregano showed weak inhibitory activity against *S. aureus*. Peppermint, spearmint, thyme, marjoram, and basil exhibited no antibacterial activity against the three Gram-positive bacteria.

Table 3 Antibacterial activity of fresh culinary herbs based on minimum inhibitory dose

Fresh herb	Minimum inhibitory dose (mg/disc)		
	B. cereus	*M. luteus*	*S. aureus*
Sage	0.06	0.13	0.13
Rosemary	0.13	0.13	0.50
Thyme	–	2.00	2.00
Oregano	–	–	2.00
Marjoram	–	–	–
Spearmint	–	–	–
Peppermint	–	–	–
Basil	–	–	–

Abbreviations: B. = Bacillus, M. = Micrococcus, S. = Staphylococcus, and – = no activity. Amount of extract = 2 mg/disc.

Table 4 Antibacterial activity of oven-dried culinary herbs in comparison with commercial herbs

Dried herb	Minimum inhibitory dose (mg/disc)		
	B. cereus	*M. luteus*	*S. aureus*
Rosemary			
OD_{50}	0.13	0.13	0.13
OD_{80}	0.06	0.06	0.06
MOD_{50}	0.06	0.06	0.06
COM	0.06	0.06	0.06
Sage			
OD_{50}	0.13	0.13	0.13
OD_{80}	0.13	0.25	0.25
MOD_{50}	0.06	0.13	0.13
Peppermint			
OD_{50}	–	–	–
OD_{80}	–	–	–
MOD_{50}	–	–	–
COM	0.25	1.00	0.13
Spearmint			
OD_{50}	–	–	–
OD_{80}	–	–	–
MOD_{50}	–	–	–
COM	0.50	1.00	1.00
Thyme			
OD_{50}	–	–	–
OD_{80}	–	–	–
MOD_{50}	–	–	–
COM	0.50	–	0.50
Oregano			
OD_{50}	–	–	2.00
OD_{80}	–	–	–
MOD_{50}	–	–	–
COM	1.00	–	0.25
Marjoram			
OD_{50}	–	–	–
OD_{80}	–	–	–
MOD_{50}	–	–	–
Basil			
OD_{50}	–	–	–
OD_{80}	–	–	–
MOD_{50}	–	–	–
COM	–	–	–

Abbreviations: B. = Bacillus, M. = Micrococcus, S. = Staphylococcus, – = no activity, OD_{50} = oven drying at 50°C, OD_{80} = oven drying at 80°C, MOD_{50} = oven drying at 50°C with microwave pre-treatment, and COM = commercial. Amount of extract = 2 mg/disc.

The antibacterial properties of five freeze-dried Labiatae herbs against Gram-positive *Listeria monocytogenes*, *B. cereus*, and *S. aureus*, and Gram-negative *Salmonella anatum* and *E. coli* have been studied.[32] Ranking of antibacterial activity based on mean diameter of inhibitory zone was oregano (13.3 mm), thyme (7.3 mm), sage (6.9 mm), rosemary (6.7 mm), and basil (5.5 mm). Oregano inhibited *S. anatum* and *E. coli* but not basil. Thyme, sage, and rosemary inhibited *S. anatum* but not *E. coli*. The disparity between these results and those of the present study could be due to differences in the methods of plant preparation and extraction, and in the strains of bacteria tested.

From this study, rosemary and sage (fresh, commercial, and oven-dried) inhibited the growth of *B. cereus*, *M. luteus*, and *S. aureus* with MID ranging from 0.06 to 0.25 mg/disc. MID of hot water extracts of green teas of *Camellia sinensis* ranged from 0.06 to 2.00 mg/disc against the three Gram-positive bacteria.[4] MID of black teas was 0.50 mg/disc against *B. cereus*, 0.13 mg/disc against *M. luteus*, with no activity against *S. aureus*. It can therefore be inferred that rosemary and sage have stronger antibacterial properties compared to tea infusions of *C. sinensis*.

CONCLUSION

Of the eight species of Labiatae herbs, fresh and commercial rosemary, and oven-dried oregano had the highest phenolic content and antioxidant activity. Fresh, commercial and dried basil had the weakest antioxidant properties. MOD_{50} herbs generally had the strongest antioxidant properties, followed by OD_{50} and OD_{80} herbs. Oven-dried rosemary had lower values than commercial rosemary while oven-dried oregano, spearmint, thyme, peppermint, and basil had higher values than commercial samples. All herbs showed no antibacterial activity against Gram-negative *E. coli*, *P. aeruginosa*, and *S. typhi*. Rosemary, sage, peppermint, and spearmint inhibited Gram-positive *B. cereus*, *M. luteus*, and *S. aureus*. Rosemary and sage have stronger antibacterial properties compared to green and black teas of *C. sinensis*. When used in combination, Labiatae herbs can have enhanced antioxidant and antibacterial effects, which are desirable in controlling rancidity and bacterial growth in food. The synergistic behaviour of these herbs would require further research.

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