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Antioxidant Activity of Basil

H.R. Juliani and J.E. Simon*

INTRODUCTION

The commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest (Rice-Evans et al. 1997). Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or beverages and the prevention of diseases (Scalbert and Williamson 2000). These effects have been attributed to antioxidant components such as plant phenolics, including flavonoids and phenylpropanoids among others (Rice-Evans et al. 1996).

Basils (*Ocimum* spp., Lamiaceae) contain a wide range of essential oils rich in phenolic compounds (Simon et al. 1990; Phippen and Simon 2000) and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins (Phippen and Simon 1998). The objective of this study was to evaluate the antioxidant activity of basil extracts and essential oils.

METHODOLOGY

Five green basil cultivars and breeding lines including 'Italian Large Leaf' (Johnny's Selected Seeds), 'Sweet' (Rutgers ON92CBT93-19), 'Cinnamon' (*Ocimum basilicum*, Rutgers SPSMEC-98), 'Sweet Dani Lemon' (*O. citriodorum*, Johnny's Selected Seeds), and 'Holy' (*O. sanctum*, Johnny's Selected Seeds), plus four purple basil cultivars, 'Dark Opal' (Richters), 'Osmin Purple', 'Purple Ruffles', and 'Red Rubin' basil (*O. basilicum*, Johnny's Selected Seeds). For comparison purposes, 'Greek' oregano (*Origanum vulgare*) (Rutgers SPS01-01) and green tea (*Camellia sinensis*) (The Vert de Chine Green Tea, Shangai, China) were also assessed as products recognized for their high antioxidant activity.

Sample Preparation

The ethanolic extracts were prepared by grinding two grams of leaf to a fine powder under liquid nitrogen and extracting with 80% ethanol (with 0.1% HCl for purples basils). Essential oils (EO) were extracted by hydrodistillation in a Clevenger-type apparatus (Charles and Simon 1990) of basil leaves that had been dried for 96 hr at 38°C. Yield (in ml) was related to percentages of dry weight samples.

The ethanolic extracts were tested for in vitro antioxidant activity using two screens. In the ABTS screen the antioxidant activity was related to Trolox (a water soluble analogue of vitamin E) and expressed as μmol of Trolox per gram of leaf dry weight (DW) (TEAC, Trolox equivalent antioxidant activity). In the FRAP screen the activity was related to ascorbic acid (vitamin C) and expressed as μmol ascorbic acid per gram of leaf DW (AEAC, ascorbic acid equivalent antioxidant activity). Total phenolics were also measured and expressed as gallic acid equivalents (GAE, mg of gallic acid per gram of leaf DW) (Gao et al. 2000).

The essential oils were also tested using this two screens but the activity was expressed as μmol (Trolox and ascorbic acid) per ml of oil. The antioxidant activity of the ethanolic extracts was considered as 100% antioxidant activity and the contribution of the essential oil to this percentage was then measured using both assays (ABTS and FRAP).

The oils were analyzed by gas chromatography coupled to a mass and FID detectors. (Agilent GC System 6890 Series, Mass Selective Detector, Agilent 5973 Network, FID detector). Samples were injected with an autosampler (Agilent 7683 Series). The inlet temperature was 180°C, HP5-MS (30 m, 0.25 ID, 0.25 μm) column, programmed temperature, 60°C 1 min, 4°C/min, 200°C 15 min. The helium flow rate was 1 ml/min. Individual compound identifications were made by matching spectra with those from mass spectral library (Wiley 275.L), the identity of each compound was confirmed by its Kovats index (Jennings and Shibamoto 1980). Data were analyzed statistically by analysis of variance (ANOVA) followed by the LSD test, with the level of significance set at 5%.

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RESULTS AND DISCUSSION

Ethanolic Extracts

Total phenolics were higher in the purple basils than in the green cultivars (Table 1). 'Dark Opal' basil contained the highest concentration (126.2 mg phenolics/g dry weight), in contrast to the other purple cultivars 'Red Rubin' (95.1 mg) and 'Osmin Purple' (81.7 mg). The green cultivars evaluated yielded significantly lower total phenols, varying from 35.6 mg in 'Cinnamon' to 62.9 mg in 'Italian Large Leaf'.

The antioxidant activity of the purple basils, as measured by TEAC, was higher for 'Dark Opal', 'Red Rubin', and 'Purples Ruffles', than for 'Osmin Purple' (Table 1). Antioxidant activity was much lower in the green basils. Antioxidant activity as measured by the second screen, AEAC, showed the same trends, with 'Dark Opal', 'Purples Ruffles', and 'Red Rubin' exhibiting the highest activity, significantly lower activity in 'Osmin Purple', and the lowest activity observed in the green basils (Table 1).

There was a strong relationship between the total phenolic content and the antioxidant activity expressed as TEAC (R^2=0.93) and FRAP (R^2=0.82). These results suggest that the antioxidant activity in basils is largely due to the presence of phenolic components. The same relationship was also observed between phenolics and antioxidant activity in rosehip extracts (Gao et al. 2000).

Essential Oils

Among the essential oils extracted from the basil cultivars, the highest antioxidant activity was found in the Sweet basil essential oils, with significantly lower activity observed in the essential oils from 'Dark Opal' and 'Osmin purple', and much lower activity in the 'Lemon', 'Purple Ruffles', Italian Large Leaf', 'Cinnamon', and 'Holy Basil' oils (Table 2).

Table 1. Phenolic content and antioxidant activity of basil, oregano, and tea.

Cultivar	Phenolics (GA^z/g DW)	Antioxidant activity: µmol Trolox/g DW	Antioxidant activity: µmol AA/g DW
Basil			
Cinnamon	35.6 g^y	199 g	282 gh
Dark Opal	126.2 a	547 bc	726 a
Holy	51.1 f	297 fg	420 efg
Italian Large Leaf	62.9 ef	354 ef	459 def
Sweet Dany Lemon	55.8 ef	206 g	254 h
Osmin Purple	81.7 cd	440 de	582 bcd
Purple Ruffles	92.6 bc	497 bcd	694 ab
Red Rubin	95.1 bc	562 b	803 a
Sweet	55.7 ef	296 fg	401 fg
Oregano			
Greek	92.6 bc	670 a	544 cde
Tea			
Green	256.4 h	3028 h	2205 i

^{z}Gallic acid.

^{y}Values sharing the same letter within a column do not differ statistically according to LSD test (p=0.05).

Table 2. Content and antioxidant activity of basil and oregano essential oils and contribution of essential oils (%) to total antioxidant activity in ABTS and FRAP assays.

Cultivar	Essential oil content (ml EO/100 g DW)	Antioxidant activity		Contribution of EO	
		μmol Trolox/ml EO	μmol AA/ml EO	% ABTS	% FRAP
Basil					
Cinnamon	1.4 abcd	171 e	394 de	1.2	2.0
Dark Opal	1.1 bdef	751 c	434 de	1.5	0.7
Holy	1.0 def	127 ef	269 de	0.4	0.7
Italian Large Leaf	1.7 a	59 ef	75 fg	0.3	0.3
Sweet Dany Lemon	1.1 bcde	41 f	52 fg	0.2	1.1
Osmin Purple	0.9 ef	997 b	876 c	1.9	1.0
Purple Ruffles	1.1 cdef	50 ef	22 g	0.1	0.0
Red Rubin	0.6 f	79 ef	78 fg	0.1	0.0
Sweet	1.1 bcde	1105 b	2125 a	4.1	5.9
Oregano					
Greek	1.6ab	1577 a	1447 b	5.0	4.3

^{z}Values sharing the same letter within a column do not differ statistically according to LSD test (p=0.05).

The chemical composition showed a close relationship between the relative percentage of eugenol and the antioxidant activity in both assays (Table 3). All basil oils contained less than 18% eugenol. The highest antioxidant activity was observed in oregano essential oil, due to its high levels of carvacrol (70%). The ‘Italian Large Leaf’, ‘Purple Ruffles’, ‘Cinnamon’, and ‘Lemon’ basil oils showed a very low antioxidant activity, and all contained low concentrations of eugenol.

In all basils, the essential oil contribution to the total antioxidant activity was low, varying from 0.05% in ‘Purples Ruffles’ to 5.9% in ‘Sweet’ basil (FRAP) and from 0.1% in ‘Purples Ruffles’ to 4% (ABTS) in ‘Sweet’ basil. In ‘Greek’ oregano, the essential oil contribution to the overall antioxidant activity was also found to be only ca. 5%. These results strongly suggest that the main antioxidant activity from these plants does not arise from their essential oils, but rather from other phenolics such as flavonoids in green basils and anthocyanins in purple basils.

In sweet basil, although the antioxidant activity of the ethanolic extract was low, the activity of the oil itself was the highest, as this oil contained the highest amount of eugenol relative to all other samples. However, the contribution of this oil to the antioxidant activity of the ethanolic extract was around 5%, due to the modest concentration of eugenol (18% relative to total essential oil).

Green tea is extremely rich in polyphenolic compounds which can constitute up to 300 mg/g of material (Robertson 1992). ‘Dark Opal’ basil contained 126 mg, half of the phenolics of our tea sample (256 mg). The antioxidant activity of purple basils was highest, similar to that of ‘Greek’ oregano. The phenolic content and antioxidant activity of basils were also similar to red and black raspberry (Wang and Lin 2000) and higher than rosehips (Gao et al. 2000).

Given the high relative antioxidant activity of selected basils, these plants could constitute new sources of antioxidant phenolics in the diet, providing 125 mg of gallic acid equivalents, 85–125 mg of Trolox, or 106–140 mg of ascorbic acid equivalents per gram of dry weight. Using biofractionation, current studies are now elucidating the specific basil compounds that contribute to the antioxidant activity.

Table 3. Chemical composition of basil and Greek oregano essential oils.

		Relative amounts by cultivar (%)									
Compounds[z]	Retention index	Cinnamon basil	Dark Opal basil	Holy basil	Italian Large Leaf basil	Osmin Purple basil	Purple Ruffle basil	Red Rubin basil	Sweet basil	Sweet Dani Lemon basil	Greek oregano
α-Pinene	936	0.10	0.21		0.15[4]	0.18	0.18	0.08	0.10		
Camphene	951		0.11	0.10	tr	tr			0.10		
Sabinene	975		0.27		tr	0.23	0.26	0.14	0.10		0.96
β-Pinene	978	0.50	0.62	tr	0.80	0.53	0.61	0.34	0.10		
Myrcene	991	0.26	0.91		0.50	0.77	0.93	0.46	0.10		
1,8-Cineole	1034	3.60	9.08	0.12	7.70	9.81	7.00	9.60	0.80		0.10
cis Ocimene	1039	0.10							0.10		0.72
β Ocimene	1050	0.60	0.53			0.16			2.60		0.53
γ-Terpinene	1060	0.30	tr			tr	tr				7.66
Terpinolene	1088	0.15	1.17			1.45	0.79	tr	0.20	0.10	0.10
Linalool	1098	13.35	53.42	1.34	21.5	55.3	22.1	63.9	36.00		
Camphor	1146	0.44	1.33	0.04	0.60	0.79			1.10		
Borneol	1166	0.10		3.23					0.70		0.90
Terpineol 4	1179	1.40	0.13	0.09		0.22	0.13	0.14	0.30	1.40	0.26
α-Terpineol	1190	0.60	0.99	0.06		1.20	0.69	0.98	0.10	0.10	0.10
Methylchavicol	1199	13.10		0.65	44.9		52.3	0.13		6.19	
Nerol	1233					tr				4.30	
Neral	1248									25.90	0.10
Trans-Geraniol	1257				0.10	1.40				1.20	
Geranial	1274				0.10					33.16	0.10
Bornylacetate	1286	0.20	0.27		tr	0.33	tr		0.20	0.10	
Thymol	1292										0.50
Carvacrol	1309										70.0
α-Cubebene	1351	0.15	0.10	0.14					0.10	0.18	
Eugenol	1358	0.15	7.29	3.40	0.60	8.24	0.29	0.65	18.20	0.20	
α-Copaene	1375	0.11	0.24	3.40		0.15	0.09	0.19	0.20	0.50	
β-Cubebene	1390			2.10	0.13	0.72	0.57		0.10	0.40	
β-Elemene	1392			2.20				1.36	1.10	0.30	
Methylcinnamate	1394	45.43									
Methyleugenol	1404	0.10	3.93	67.45	0.90	3.39	0.17	0.18	0.70		
β-Caryophyllene	1420	0.10	1.43	0.10		0.95	0.09	0.12	0.17	4.90	1.20
α-Bergamontene	1436	0.10		0.10		0.51				0.90	
α-Guaiene	1440	0.10						7.23			
α-Humulene	1455	0.10	0.09	2.40	0.40	1.02	0.06	0.06	0.40	0.80	
β-Farnesene	1458	0.70	1.11			0.18	0.31	0.40		0.40	
Germacrene D	1482	2.99	2.60	9.90	2.10	2.55	1.49	3.00	5.33	6.80	0.31
β-Selinene	1488									2.40	
α-Selinene	1496									2.26	
Biclogermacrene	1496	1.66			0.80	1.11	0.69	1.07	2.20		
δ-Guaiene	1506	2.30		3.24	0.70	1.38		2.02	2.40	0.18	
β-Bisabolene	1509		1.87							0.10	1.18
γ-Cadinene	1515	1.44			1.15						
α-Amorphene	1516							1.16	3.26		
7 epi-a-Salinene	1518		1.32							0.10	
δ-Cadinene	1525		0.26	0.60	0.26		0.37	0.76	0.50	0.27	0.10

[z]Compounds are listed in order of elution on HP-5MS.

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