International Food Research Journal 20(3): 1113-1117 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Relationship between leaf position and antioxidant properties in three basil species

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Article history

Received: 24 November 2012
Received in revised form: 24 December 2012
Accepted:28 December 2012

Keywords

Antioxidant
herbs
holy basil
lemon basil
sweet basil

Abstract

Variation in antioxidant contents and activities in leaves from the top to the base of holy basil, lemon basil and sweet basil shoots was investigated. The contents of chlorophyll a and chlorophyll b were not different in all leaf positions while those of β-carotene, ascorbic acid, phenolics and flavonoids depended on both the location of leaves and plant species. Holy basil contained a high amount of phenolics in all leaf positions but sweet basil leaves were rich in flavonoids from the top and middle regions of the plants which might be related to the high value of DPPH free radical scavenging activity but not the ferrous iron chelating ability. In contrast, the bottom leaves of lemon basil had the highest DPPH scavenging activity while the middle leaf of this herb had the highest activity of ferrous ion chelation. This ability in lemon basil leaves was also obviously higher than that in the other two basils.

Introduction

In plants, leaf is one of the promising sources of antioxidants. An excellent classical example is tea leaves which have long been customarily consumed in China for more than 2,000 years (Cabrera *et al*., 2006). Holy, lemon and sweet basils are well-known in traditional Thai cuisine. Recently, use of the basil leaves in food may also have potential health benefits which could be attributed to their antifungal, anti-inflammatory, antimicrobial and antioxidant activities (Nahak *et al*., 2011).

Many factors are known to influence the amount and activity of leaf antioxidants. For example, there were studies showing the influence of genotype, season, sun- and shade acclimation and leaf age on leaf antioxidative potential in safflower, *Moringa oleifera*, sour orange trees and Aloe barbadensis (Schwanz *et al*., 1996; Iqbal and Bhanger, 2006; Golkar *et al*., 2009; Amareswari *et al*., 2012).

Leaves of several different *Ocimum* species were found to have antioxidants and antioxidative capabilities (Juliani and Simon, 2002; Hakkim *et al*., 2008). However, little is known about the possible variation in leaf antioxidant properties in relation to leaf position. This is of interest to plant physiologists and the consumers who want to be better informed regarding antioxidant contents and activities in leaves. Therefore, the present research aimed to investigate this problem. Some of the common antioxidants and antioxidative activities including chlorophylls a and b, ß-carotene, ascorbic acid, phenolic, flavonoids, reducing power, ferrous ion chelating and 1 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging capabilities were extracted from leaves in three different positions of the three basils and quantified in the present study.

Materials and Methods

Plant materials

Holy basil (*Ocimum sanctum* L.), lemon basil (*O.* × *citriodorum* Vis.) and sweet basil (*O. basilicum* L.) were purchased from a local market in the Nonthaburi province, Thailand. Leaves were taken for various biochemical analyses from three regions (top, middle and bottom) of the plants (Figure 1). Afterwards, plant height, range of each leaf position and leaf size were measured.

Determination of antioxidative components

Basil leaves (1 g) were homogenized in 5 mL of acetone: hexane (2:3) mixture. The absorbances of the leaf extracts were determined spectrophotometrically at 663, 645, 505 and 453 nm (Nagata and Yamashita, 1992). The concentrations (mg/100 mL) of the

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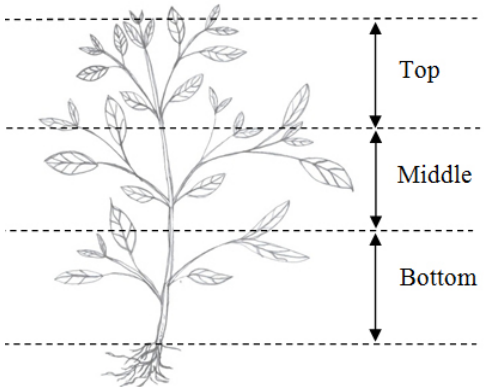

Figure 1. Sampling of leaves in three different regions from the top to the base of the basil plant

pigments were calculated using the following formulae:

$$\text{Chlorophyll a} = 0.999 \times \text{Abs.663} - 0.0989 \times \text{Abs.645}$$

$$\text{Chlorophyll b} = -0.328 \times \text{Abs.663} + 1.77 \times \text{Abs.645}$$

$$\beta\text{-Carotene} = 0.216 \times \text{Abs.663} - 1.22 \times \text{Abs.645} - 0.304 \times \text{Abs.505} + 0.452 \times \text{Abs.453}$$

Determination of ascorbic acid, phenolics, flavonoids, and reducing power capacity was based on the method of Chang *et al*. (2006) with slight modifications. Ascorbic acid analysis: Basil leaves (2.5 g) were homogenized in 10 mL of 1% (w/v) metaphosphoric acid solution. The homogenate was centrifuged at 6000 g for 15 min. One mL of the supernatant was mixed with 4 mL of 0.5 mM 2,6-dichlorophenolindophenol (DIP) and absorbance of the mixture was measured spectrophotometrically at 515 nm.

Phenolics analysis: Basil leaves (10 g) were homogenized in 10 mL of ethanol (80%, v/v). The homogenate was centrifuged at 12,000 g for 20 min. One mL of the supernatant was mixed with 5 mL of Folin-Ciocalteu reagent (1:9, v/v in water), and 4 mL of 7.5% (w/v) sodium carbonate. This reaction mixture was incubated in a hot water bath (30°C) for 1 h, transferred to an ice bath for 1 h and then absorbance was read at 760 nm. Standard curve was constructed using different concentrations of gallic acid.

Flavonoids analysis: Leaves (1 g) were soaked in 5 mL of methanol. After that, it was filtered through Whatman No.1 filter paper under suction. One ml of the filtrate was mixed with 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL distilled water, and was left at room temperature for 30 min before absorbance was measured at 415 nm and compared with standard curve of rutin.

Antioxidant activity assays

Reducing power assay: Basil leaves (1 g) were soaked in 9 mL of methanol, and left at room temperature for 3 h before it was filtered through Whatman No.1 filter paper under suction. Five ml of the methanolic extract were mixed with 1.25 mL of 0.2 M phosphate buffer (pH 6.6) and 1.25 mL of 1% (w/v) potassium ferricyanide, incubated in a hot water bath (50°C) for 20 min and then transferred to an ice bath for cooling down before addition of 1.25 mL of 10% (w/v) of trichloroacetic acid. The resultant cleared zone (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride and was left at room temperature for 10 min before absorbance was read at 700 nm.

Ferrous iron chelating ability assay: Leaves (1 g) were soaked in 9 mL of methanol, kept at room temperature for 3 h and then filtered through Whatman No.1 filter paper under suction. The methanolic extract (1 mL) was mixed with 3.7 mL methanol, 0.1 mL of 2 mM ferrous chloride and 0.2 mL of 5 mM ferrozine and left at room temperature for 10 min before absorbance at 562 nm (Chang *et al.*, 2006) was taken. For control, ferrous chloride and ferrozine were mixed with methanol instead of methanolic leaf extract. Ferrous iron chelating power was calculated using the following formula:

$$\text{Ferrous iron chelating ability (\%)} = [(1 - \text{Abs. of sample})/\text{Abs. of control}] \times 100$$

DPPH scavenging activity assay: Basil leaves (1 g) were soaked in 9 mL of methanol, left at room temperature for 3 h and then filtered through Whatman (No.1) filter paper under suction. The methanolic extract (4 mL) was mixed with 1 mL of 1 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) and kept at room temperature for 30 min. before absorbance was read at 517 nm (Chang *et al*., 2006). For control, 10 mM DPPH was mixed with methanol instead of methanolic leaf extract. The percentage of scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = [(1 - \text{Abs. of sample})/\text{Abs. of control}] \times 100$$

Statistical analysis

The analysis of variance (ANOVA) for antioxidant component and activity values and comparison of means by Duncan's multiple range test (DMRT) were both performed at $P < 0.05$ using SPSS for Windows (SPSS Inc.).

Results

Characteristics of basil plants

Among the three basils that were investigated, sweet basil plants were found to be the shortest (Table 1). However, compared to the other two basils, lemon basil leaves in all three positions (Figure 1) were the smallest (Table 1) even though lemon basil had highest range in every location (Table 1).

Table 1. Plant height, leaf size (width x length) and range of top, middle and bottom parts of three basils

Characters	Plant species		
	Holy basil	Lemon basil	Sweet basil
Plant height (cm)	35.90 ± 1.34	39.34 ± 1.70	30.74 ± 2.10
Position range (cm)[1]			
Top	30.90 ± 1.34 - 35.90 ± 1.34	33.30 ± 1.92 - 39.34±1.70	26.98 ± 0.98 - 30.74 ± 2.10
Middle	16.40 ± 1.34 - 30.90 ± 1.34	16.70 ± 1.20 - 33.30 ± 1.92	14.42 ± 0.63 - 26.98±0.98
Bottom	0.00 ± 0.00 - 16.40 ± 1.34	0.00 ± 0.00 - 16.70 ± 1.20	0.00 ± 0.00 - 14.42 ± 0.63
Leaf area (cm^2)			
Top	21.64 ± 8.08	9.66 ± 3.50	14.92 ± 2.30
Middle	42.51 ± 7.13	14.44 ± 5.46	19.11 ± 3.62
Bottom	32.70 ± 6.43	7.24 ± 2.08	15.30 ± 1.86

Data are means ± SD from 15 replications.
[1]The basil plants were separated into 3 parts.
The Bottom range of plant contained leaves from the 1st and 2nd nodes (up from the root). The Middle range of plant contained leaves from the 3rd and 4th nodes.
The Top range of plant contained the rest (generally 5th and 6 th nodes with tip of plant). For example, the data form 15 holy basil plants had
Bottom range from 0.00 cm to 16.40 cm (15 values in each nodal position of 15 plants) and SD was from mean of each value [(15 data of the lower node) to (15 data of the higher node)].

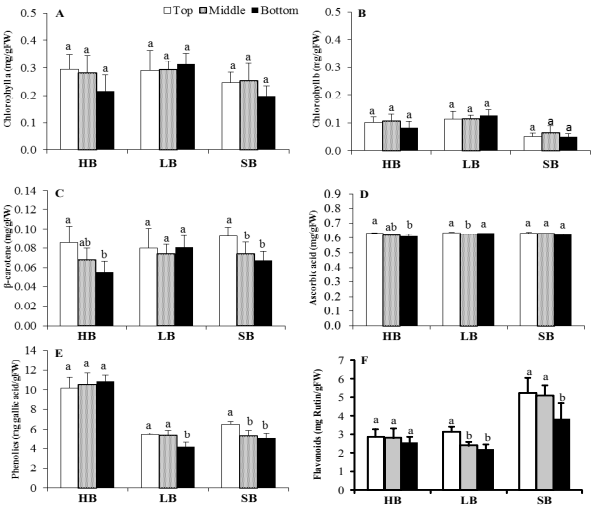

Figure 2. Chlorophyll a (A), chlorophyll b (B), β-carotene (C), ascorbic acid (D), phenolics (E) and flavonoids (F) contents in leaves of holy basil (HB), lemon basil (LB) and sweet basil (SB). Data are means±SD of 5 replications and those with the same letter over each bar from the same plant species are not significantly different ($P < 0.05$).

Antioxidative components

In each of the three basils studied, the levels of chlorophyll a or chlorophyll b were similar in three different positions of the same plant (Figures 2A and B). There were higher levels of β-carotene and ascorbic acid in holy basil leaves from the top region than the bottom part (Figures 2C and D). The phenolics and flavonoids contents were not significantly different in leaves from the different positions (Figures 2E and F). The β-carotene content was similar in three different positions of the lemon basil leaves (Figure 2C) while the ascorbic acid content was the lowest in those from the middle (Figure 2D). The leaves from the top compared to those from the bottom part had higher levels of phenolics and flavonoids (Figure 2 E and F). In sweet basil, the β-carotene and phenolics contents were the greatest in leaves from the top (Figures 2C and E). However, the ascorbic acid content was found similiar in all the different positions (Figure 2D). On the other hand, the flavonoid content from the top and middle was greater than those from the bottom (Figure 2F).

Antioxidant activities

Leaves of the three basils from the top and middle had similiar level of reducing power capability which was higher than those from the bottom (Figure 3A). Lemon basil had about 2-fold and 6-fold more ferrous ion chelating activity in all leaf positions than holy basil and sweet basil, respectively (Figure 3B). In holy basil, ferrous ion chelating and DPPH free radical scavenging activities were higher in the leaves from the top than from the middle and the bottom (Figures 3B and C). On the contrary, DPPH free radical scavenging capability was the lowest in the lemon basil leaves from the top (Figure 3C) while ferrous ion chelating activity in those from the middle or bottom was higher than those from the top (Figure 3B). In sweet basil, ferrous ion chelating activity was higher at the top and middle than from the bottom (Figure 3B) while DPPH free radical scavenging capacity was the lowest in leaves from the bottom (Figure 3C).

Discussion

The present research on the contents of chlorophyll a, chlorophyll b, β-carotene, ascorbic acid, phenolics and flavonoids and antioxidant activities in various leaf positions of three basils added to the limited research into the influence of leaf position on antioxidant contents and activities. Cano and Arnao (2005) reported that total phenol content in Romaine lettuce increased from stem to outermost leaves correlating with a sharp increase in lipophilic antioxidant activity from stem to outermost leaves

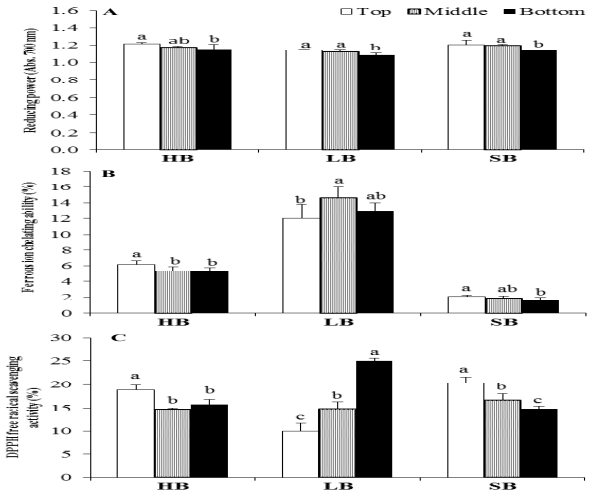

Figure 3. Reducing power, ferrous ion chelating and DPPH free radical scavenging activities in leaves of holy basil (HB), lemon basil (LB) and sweet basil (SB). Data are means ± SD of 5 replications and those with the same letter over each bar from the same plant species are not significantly different (P<0.05).

as well. This finding appeared to be related to the chlorophyll contents in leaves from different positions of the plants as well and was in agreement with the study on red and green lettuce (Ozgen and Sekerci, 2011). However, no such relationship was found between variation in chlorophyll contents and that in antioxidant contents in the different leaf positions of the three basils in the present study. Presumably, in basils, unlike lettuce, the leaves in the different leaf positions were relatively well exposed to light and therefore this environmental factor would seem to have little or no effect on chlorophyll contents. It would be possible that the differential maturity of basil leaves from the different leaf positions might not influence chlorophyll contents as well.

Bhakta and Ganjewala (2009) categorized leaf location of Lantana camara from apex to base into I to V positions respectively and found that phenolic level stayed unchanged from I to V leaf positions, while flavonoids sharply increased in I to III leaf positions and noticeably decreased in two younger leaf stages (IV and V). The extracts from leaf positions I to III showed considerably higher antioxidant abilities in terms of reducing and scavenging activity than those from leaf IV to V positions. In contrast, generally the leaves of the three basils from the top region of the plants had higher antioxidant contents and activities than the leaves from the middle and bottom regions of the plants. The observed variation might be due to differential sensitivity of basil leaves in different positions on the plants to light/shade regime as changes in light intensity were found to have an effect on production of total phenolics and total flavonoids in ginger leaves (Ghasemzadeh *et al*., 2010). Among the three basils and apart from the preference for other attributes such as taste, if a greater amount of a particular antioxidant, for example, β-carotene, is desired, there would be a choice between the top leaves of holy and sweet basil (Figure 2C). However, leaf position did not have any influence on the quantity of β-carotene in lemon basil, ascorbic acid in sweet basil and phenolics in holy basil (Figure 2C to E).

The assay for reducing power capacity involving reduction of Fe^{3+} (ferric form) of potassium ferricyanide to Fe^{2+} (ferrous form) revealed the presence of substances such as electron donors with antioxidant activity (Chanda and Dave, 2009; Hue *et al*., 2011) in all leaf positions of the three basils (Figure 3A). The three basils had a very similar level of reducing power capacity although that of the bottom leaf seemed to be the lowest. The observed variation in reducing power capacity in the three basils might be related to that of antioxidants such as ascorbic acid (Figure 2D). Surprisingly, ferrous ion chelating ability based on the disruption of ferrozine-ferrous ions complex formation (Kong *et al.*, 2010; Apetrei *et al*., 2011) was about 2-fold higher in lemon basil leaves than those of the other two basils regardless of leaf position (Figure 3B). This ferrous ion chelation was apparently unrelated to all the antioxidative components studied here (Figure 2). It is possible that other substances such as essential oil which also has antioxidant activity might contribute to this chelation ability (Juliani and Simon, 2002). Based on chelating ability, it would seem particularly beneficial to include lemon basil as part of a healthy diet. In the determination of DPPH scavenging activity in basil leaf extracts, the influence of leaf position on this activity in the three basils was evident (Figure 3C). Interestingly, variation in the influence of leaf position on DPPH scavenging activity in lemon basil was completely opposite to that of the other two basils, particularly sweet basil suggesting that the influence of leaf position on DPPH scavenging activity was related to factors other than leaf age. Possibly flavonoids might be associated with DPPH scavenging activity in sweet basil but not in other two basils (Figure 2F).

Conclusion

Normally, young basil leaves are consumed because they are not too pungent or too fragrant. Although it was confirmed in this study that generally the antioxidant contents of young leaves from the top of basil plants were similar or higher than those from the middle and bottom parts of the plants. It was clear that the amount of antioxidants and their activities in

different basils could also vary depending on leaf position. The relationship between leaf position and antioxidant contents and activities appeared to be complex and might involve more than just variation in leaf age or photosynthetic potential in terms of chlorophyll content.

Acknowledgement

We would like to thank the Graduate College, King Mongkut's University of Technology North Bangkok for the financial support of this research.

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