Korean J. Medicinal Crop Sci. 14(5) : 307 - 310 (2006)

Changes in Total Polyphenol, Total Flavonoid Contents and Antioxidant Activities of *Hibiscus cannabinus* L.

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ABSTRACT : This study purposed to look for its uses by analyzing useful substances in kenaf (*Hibiscus cannabinus* L.) leaves based on the total polyphenol and flavonoid contents in the leaves, DPPH radical scavenging activity of leaf extract, SOD activity, etc. by different growth stage and variety. According to the results of experiment, the total polyphenol content was highest in Everglade-41 (27.9 ㎎/g dw.), the total flavonoid content was highest in Tainung-2 (42.3 ㎎/g dw.), and SOD activity was highest in Dowling (96.1%). However, DPPH radical scavenging activity was similar among the three varieties.

Key words : kenaf, total polyphenol, total flavonoid, antioxidant activity

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) has long been used as a medicial plant for anemia in Africa, and there is a report that kenaf leaf extract is used for protecting and activating liver cells, activating the antilipidperoxidative effect, blood formation, etc. (Agbor *et al.*, 2001, 2002). The calcium content of Kenaf is 4 times higher than milk, and the plant also contains a lot of protein, vitamin and iron as well as antioxidant substance in its leaves. Thus, the plant can be used against cancer, virus, microorganism, bacteria, etc. and these effects suggest its usability in developing medicine. Phenolic compounds are part of products of secondary metabolism distributed widely in the plant kingdom, having various structures and molecular weights. Because they have phenolic hydroxyl radical, they couple with giant molecules such as protein, and are reported to have functions of physiological activation such as antioxidant effect (Lee *et al.*, 2005). Polyphenol compounds in buckwheat such as proanghocyanidine, rutin and lignan are known to be antioxidant, antibiotic and anticancer, and extract from germinated buckwheat is reported to have an anticancer effect against Calu-6 the cell strain of lung cancer and SNU-601 the cell strain of liver cancer (Hwang *et al.*, 2006). In addition, polyphenol in green tea is reported to prevent gout, suppress lipid peroxide, delay aging and suppress the generation of neutral lipid, which in turns prevents obesity and increases the resistance of capillary vessels (Park *et al.*, 1997). The present experiment cultivates kenaf, a subtropical crop, in Cheolwon, a northern region in Korea and analyze substances in kenaf leaves and physiologically activating elements in order to measure its usability as the material of medicinal herb and to examine its possibility as a new additive to the functional foods.

MATERIALS AND METHODS

Kenaf varieties and sowing

We sowed the seeds of three varieties of kenaf, namely, Dowling, Everglade-41 and Tainung-2 at planting space of 20 × 10 ㎝ on a farmland in Cheolwon-gun, Gangwon-do on May 20 2005, and collected plants on the 53 rd, 84 th and 115 th days from sowing.

Specimen preparation

Kenaf leaves collected by different growth stage were dried in shade and ground, and 80% methanol was applied to 1 g of ground specimen was and extraction was made three times at 80 ℃ for 2 hours using the reflux cooling method. The extract was concentrated under reduced pressure at 40 ℃, 100 ㎖ of the solution was measured, and filtered.

Total polyphenol content

The total polyphenol content was measured using a slightly

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Received August 10, 2006 / Accepted September 28, 2006
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modified Folin-Denis method, which uses the phenomenon that a phenolic substance turns blue by reaction with phosphomolybdate. That is, 0.2 ㎖ of the extract above was taken into a test tube and 1.8 ㎖ of distilled water was added, preparing 2 ㎖ of solution. Then, 0.2 ㎖ of Folin-ciocalteau phenol reagent was added, and the mixture was stirred well and left at room temperature for 3 minutes. After reaction for exactly 3 minutes, 0.4 ㎖ of solution saturated with Na_2CO_3 was added and mixed, and 1.4 ㎖ of distilled water was added so that the solution became 4 ㎖. The solution was left at room temperature for an hour, and the absorbance of the supernatant was measured at 725 ㎚. Here, the total polyphenol content was measured from the standard curve using tannic acid. The standard curve using tannic acid was prepared by melting 1 g of tannic acid in 1 ㎖ of 80% methanol and taking the solution so that the final concentration became 0, 50, 100, 150, 200, 300 and 500 ppm, and the absorbance was measured at 725 ㎚ using the method above (Hagerman *et al.*, 2000).

Total flavonoid content

To measure the total flavonoid content, we mixed 1 ㎖ of the extract above with 10 ㎖ of diethylene glycol, added 1 ㎖ of 1N-NaOH solution and mixed well, reacted the solution at 37 ℃ for 30 minutes, and measured the absorbance at 420 ㎚. The standard calibration curve was made using Rutin.

DPPH radical scavenging activity

To measure antioxidant activity by DPPH, we took 1 ㎖ of the extract above, added 4 ㎖ of 0.15 mM DPPH methanol solution, and mixed homogenized through voltexing, left at room temperature for 30 minutes and measured absorbance at 517 ㎚. DPPH radical scavenger activity (%) was represented in the difference (%) of absorbance between the sample containing the specimen and the control (Braca, 2001).

DPPH radical scavenger activity (%) = [(1 − A/B) × 100%]

A: Absorbance of specimen, B: Absorbance of control

SOD activity

(1) Preparing coenzyme solution

We ground kenaf leaves harvested by time with liquid nitrogen. The ground leaves were mixed with 20 mM potassium phosphate buffer (pH 7.0) at a ratio of 1 : 1, and the solution was centrifuged at 12,000 rpm for 20 minutes. The supernatant obtained from the centrifuging was used as a coenzyme solution. Using Bradfor's (1976) method for protein quantification, BSA (bovine serum albumin) was used as standard protein.

(2) Measuring enzyme activity

To measure SOD activity, we followed the method used by Kim *et al.* (1998), adding 0.1 ㎖ of enzyme solution to 2.9 ㎖ of solution containing 50 mM potassium phosphate buffer (pH 7.8), 13 mM Methionine, 150 μmol nitro blue tetrazolium (NBT), 0.1 mM EDTA (Na salt) and 2 μM riboflavin, and reacting at a fixed distance (10 ㎝) for 10 minutes. Here, the solution was kept at 25 ℃. Then, we measured absorbance at 560 ㎚ as the degree that NST caused by superoxide inhibits the formation of insoluble blue formazan generated by photochemical reduction (U-2001 Spectrophotometer). SOD activity was calculated using the equation below.

NBT reduction inhibition rate (%) = [(1 − A/B) × 100%

A: Absorbance of specimen, B: Absorbance of control

RESULTS AND DISCUSSION

As for difference in the total polyphenol content of kenaf leaves by variety, Tainung-2 showed a higher content than the other two varieties on the 53 rd day from sowing, and Everglade-41 showed 20.0 ㎎/g dw. and 27.9 ㎎/g dw., respectively, on the 84 th and 115 th day from sowing, higher than the other two varieties. In general, Everglade-41 was slightly higher and Dowling slightly lower. As for difference in the total polyphenol content by time, the contents on the 115 th day, the latter period of growth, were 1.3, 1.6 and 1.3 times higher, respectively, than those on the 53 rd day, the early period, in Dowling, Everglade-41 and Tainung-2, showing that the content grew higher throughout the process of growth (Table 1).

Flavonoid comprehends phenolic compounds with C_6-C_3-C_6 as basic structure, and is distributed in almost every part of vegetables and vascular plants including flower, fruit, stem, leaf and root (Riccardo *et al.*, 1995). Flavonoid is mainly

Table 1. Changes in total polyphenol and total flavonoid contents of kenaf varieties.

Date	Varieties	Total polyphenol	Total flavonoid
		(㎎/g dw.)	
53 DAS*	Dowling	17.4 ±2.1	31.5 ±2.1
	Everglade-41	17.5 ±1.7	34.8 ±1.3
	Tainung-2	18.3 ±1.5	33.3 ±1.5
84 DAS	Dowling	19.0 ±1.6	41.4 ±2.6
	Everglade-41	20.0 ±1.5	41.1 ±3.1
	Tainung-2	19.8 ±1.3	42.3 ±2.8
115 DAS	Dowling	22.7 ±0.8	41.0 ±2.6
	Everglade-41	28.0 ±1.3	39.4 ±2.1
	Tainung-2	23.0 ±1.3	41.1 ±3.6

*DAS; Days after sowing

composed of anthocyanidins, flavonols, flavones, cathechins and flavanones, and depending on its structure some types of flavonoid are reported to have antioxidant and antibiotic effects (Lam *et al.*, 1994; Miller *et al.*, 1994). Flavonoid known to have anticancer and antimutagenic effects includes quercetin, kaempferol and myricetin in the flavonol line, apigenin and luteolin in the flavone line, and limonin and nomilin (Hertog & Hollman, 1996). As for difference in the total polyphenol content of leaves among the varieties, Everglade-41 showed 34.8 ㎎/g dw. on the 53 rd day from sowing, higher than the other two varieties, and Tainung-2 showed 42.3 ㎎/g dw. and 41.1 ㎎/g dw., respectively, on the 84 th and 115 th day from sowing, higher than the other two varieties. In general, Tainung-2 was slightly higher and Everglade-41 slightly lower but the differences among the varieties were not significant (Table 1). As for difference in the total polyphenol content by time, the content was higher in the latter period of growth than in the early period. The flavonoid content in plants is known to vary according to season, and it is reported that medical element contents in herb materials vary according to the environment of cultivation, the number of cultivation years, harvesting time, plant part and drying method (Lee *et al.*, 1999; Shin *et al.*, 1998).

Superoxide radical ($O_2^- \cdot$), which is generated from metabolism related to aging, is known to be highly reactive to electronic reduction and toxic, causing diseases in cells and tissues. It can accelerate tumors, cause cancers and promote the aging of the skin. To remove $O_2^- \cdot$ from the body, superoxide dismutase (SOD) is secreted and transforms superoxide radical to hydrogen peroxide and normal oxygen (Foyer *et al.*, 1997; Levone *et al.*, 1994). As for difference in SOD activity of the leaf extract among the varieties, Dowling showed 96.1% and 92.8%, respectively, on the 53 rd and 115 th days from sowing, significantly higher than the other two varieties. On the 84 th day (12nd of August) from sowing, the activity was lowest in Dowling (87.2%) and highest in Everglade-41 (91.1%). In general, Dowling was higher than the other two varieties. As for difference in the activity according to harvesting time, Dowling showed higher activity during the early period of growth, and Everglade-41 and Tainung-2 during the latter period (Table 2).

Natural antioxidants and antioxidant enzymes are highly value-added useful substances and play critical roles in understanding the defensive mechanism against environmental stress. The antioxidant activity of a specific substance can be measured in several ways, and the DPPH radical scavenging activity method is relatively simple and can measure a large volume. DPPH radical, which is a relatively stable compound among substances with radical, turns blue in methanol solution. However, if it meets a substance with antioxidant activity, the antioxidant substance scavenges DPPH radical and decolors it. This characteristic can be used to measure antioxidant activity and the method is closely linked to actual antioxidant activity. Free radicals generated from the oxidation of fat in the body accelerate the aging of cells and deteriorate the defensive mechanism of cells. Thus, we need to inhibit the generation free radials or protect cells by stabilizing generated free radicals. Electron donation supplies electrons to such active free radicals and inhibits the oxidation of cells(Park & Chang, 2003).

As for difference in the DPPH radical scavenging activity of the leaf extract among the varieties, Tainung-2 showed 87.8% on the 53 rd day from sowing, much higher than the other two cultivars, and Everglade-41 and Dowling showed 85.7% and 89.6%, respectively, on the 84 th and 115 th day, higher than Tainung-2. In general, Tainung-2 was higher, and the activity grew higher throughout the process of growth (Fig. 1). This is the same as the increase of the total polyphenol content over time, suggesting a high correlation between the two factors.

Table 2. Changes superoxide dismutase activity of kenaf varieties.

Date	Varieties	SOD activity (%) (10 min.)
53 DAS*	Dowling	96.1 ±0.6
	Everglade-41	87.8 ±1.1
	Tainung-2	87.8 ±1.1
84 DAS	Dowling	87.2 ±1.7
	Everglade-41	91.1 ±1.1
	Tainung-2	90.0 ±0.0
115 DAS	Dowling	92.8 ±0.6
	Everglade-41	87.8 ±1.1
	Tainung-2	87.1 ±1.1

*DAS; Days after sowing

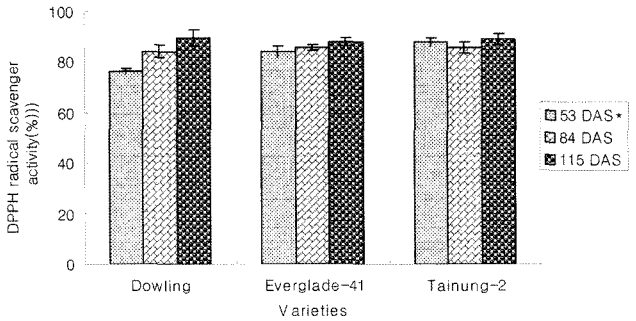

Fig. 1. Changes DPPH radical scanenger activity of kenaf varieties.

ACKNOWLEDGMENTS

This research was conducted by the financial support of the ARPC research fund (204151-3) and by the partial payment of BioHerb research Institute, Kangwon National University.

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