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생육시기에 따른 양마 잎의 항산화 활성

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Antioxidant Activity of *Hibiscus cannabinus* L. Leaves in Different Growth Time

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ABSTRACT : The results on the useful functional compounds of kenaf (*Hibiscus cannabinus* L.) leaves cultivated in reclaimed lands and the biological activity effects of extracts were as follows. On 98 days after sowing (DAS) Tainung-2 showed the highest total chlorophyll content (1.68 ㎎/g), and on 141 DAS Dowling showed higher chlorophyll content (1.50 ㎎/g) than the other two did. In all cultivars the total chlorophyll content was higher on 141 DAS than on 98 DAS. Total polyphenol and total flavonoid contents were the highest in Tainung-2 (30.50 ㎎/g and 57.03 ㎎/g, respectively), and total polyphenol and total flavonoid contents (30.50 ㎎/g and 57.03 ㎎/g, respectively) were the highest in 30% ethanol extraction. Ascorbic acid contents were higher on 141 DAS than on 98 DAS in three cultivars. SOD activities of kenaf leaf extract were generally over 90%. DPPH radical scavenging activity of Tainung-2 was higher than others.

Key Words **:** Kenaf, Total polyphenol, Total flavonoids, Antioxidant Activity

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) has been used as a industrial plant for use as fiber and fodder (Webber, 1993; White *et al*., 1994), 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). There are many substances in plants and animals as well as artificial compounds known to have such functions, and most natural antioxidants are found in plants such as trees, stems, roots, leaves and flowers and are known to be polyphenol compounds(Choi *et al*., 2008; Lee *et al*., 2007; Hong *et al*., 2007; Lee *et al*., 2005). In Kenaf essential oil are detected 58 elements including major components such as (*E*)-phytol (28.16%), (*Z*)-phytol (8.02%), *n*-nonanal (5.70%), benzene acetaldehyde (4.39%), (*E*)-2.-hexanl (3.10%) and 5-methylfurfural (3.00%). The essential oil is reported to be antibiotic against *Colletotrichum fragariae, Colletotrichum gloeosporioides* and *Colletotrichum accutatum* but have little or no algaecidal activity (Mozaina *et al*., 2001). Recently there are increasing demands for extracting specific elements from natural materials like medical herbs and develop natural food preservatives and natural antibiotics harmless to the human body, and increasing interest in bioactives found among secondary metabolic products of organisms (Eom *et al*., 2007, 2008; Lee *et al*., 2007; Villar *et al*., 1987). Among substances with bioactivity, those with antibiotic activity against microorganisms causing the decomposition and spoilage of food are known to be secondary metabolic products such as alkaloid, terpenoid, phenol and essential oil or their derivatives (Tabanca *et al*., 2001). Phenolic compounds are part of secondary metabolic products distributed widely throughout the vegetable kingdom. They are diverse in structure and molecular weight, and because they have phenolic hydroxyl groups they has the nature to

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be combined with macromolecules such as protein. Furthermore, they contain bioactive functions such as antioxidant effect. Natural polyphenol compounds include flavonoid, lignans, lignins and tannin. These polyphenol compounds are contained a lot in tea leaves and show various bioactivities. It is reported that polyphenol contained in green tea prevents gout, suppresses lipid peroxide, delays aging, and suppresses the production of neutral lipid, which in turns prevents obesity and improves the resistance of capillaries (Park *et al*., 1997). Kenaf leaves contain a lot of vegetable calcium, protein, iron and vitamins, so have high potential as functional food. Thus, the present study purposed to analyze useful substances contained in kenaf leaves and their activities and look for their possibilities as the materials of industrial and medicine.

MATERIALS AND METHODS

1. 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 × 20 ㎝ on a reclaimed land at Gyehwa-myeon, Buan-gun, Jollabuk-do, Korea and was identified by Prof. James S. Han. (USDA Madison. WI. USA). And Collected kenaf leaves on the 98^{th,} 127th and 141st days from sowing on May 24, 2005.

2. Specimen preparation

Kenaf leaves collected by different growth stage were dried in shade and ground, and 80% methanol was applied to 1g of ground specimen 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℃, 100ml of the solution was measured, and filtered.

3. Total polyphenol content

The total polyphenol content was measured using a slightly 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.

4. 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.

5. 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 *et al*., 2001).

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

A: Absorbance of specimen, B: Absorbance of control

6. 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

1. Differences in the total polyphenol content of kenaf leaves by variety and growth time

Phenol compounds, which are secondary metabolic products distributed widely in the vegetable kingdom, are known to give a special color to plants, function as a substrate in oxidation and reduction, and protect plants from microorganisms.

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2. Differences in the total flavonoid content of kenaf leaves by variety and growth time

Flavonoids are nearly ubiquitous in plants and are recognized as the pigments responsible for the colors of leaves, especially in autumn. They are rich in seeds, citrus fruits, olive oil, tea and red win. They are low molecular weight compounds composed of a three-ring structure with various substitutions(Middleton *et al*., 2000). In general, flavonoid is composed of anthocyanidins, flavonols, flavones, cathechins and flavanones and it is reported that specific types of flavonoid have antioxidative and antibiotic effects depending on the structure (Lam *et al*., 1994; Middleton and Kandaswami, 1994).

As for the total flavonoid content of dry kenaf leaves by variety, the content on day 98 from sowing was highest (55.44 ㎎/g) in Tainung-2, and 48.12 ㎎/g and 50.55 ㎎/g, respectively, in Dowling and Everglade-41, not much different between each variety. On day 127, it was as high as 54.23 ㎎/g and 52.38 ㎎/g, respectively, in Everglade-41 and Tainung-2, and on day 141, 56.18 ㎎/g and 54.01 ㎎/g, respectively, in Dowling and Tainung-2, showing slightly higher content in Tainung-2 but not much different among the varieties. In general, Tainung-2 showed a higher content than the other two. By growth time, the total flavonoid content on day 141 from sowing was higher than that on day 98, which was in the middle period of growth, in all of the three varieties (Table 1). The composition of flavonoid compounds in plants is known to vary significantly according to season (Kang *et al*., 1993). In addition, the medical contents of herb materials are quite different depending on the environment of growth, growth year, harvest season, part and the method of drying (Lee *et al*., 1999).

3. Difference in SOD activity of leaf extract by variety and growth time

Superoxide radical (O_2^- ·), which is generated from

Table 1. Changes in total polyphenol, total flavonoid and Superoxide dismutase activity of kenaf varieties leaves.

Date	Cultivars	Total polyphenol (㎎/g Dry weight)	Total flavonoid (㎎/g Dry weight)	Superoxide dismutase activity (%) (10 min.)
98 DAS*	Dowling	22.56±0.12^{a†}	48.12±0.98^{a}	90.68±0.00^{a}
	Everglade-41	23.32±0.28^{b}	50.55±2.31^{b}	92.68±0.46^{b}
	Tainung-2	27.14±0.21^{c}	55.44±1.38^{c}	92.55±0.11^{b}
127 DAS	Dowling	23.84±1.60^{a}	47.39±2.87^{a}	89.22±1.67^{a}
	Everglade-41	25.34±1.13^{b}	54.23±1.36^{b}	90.11±1.11^{b}
	Tainung-2	26.64±0.23^{c}	52.38±2.42^{c}	91.00±0.65^{b}
141 DAS	Dowling	31.12±0.26^{a}	56.18±0.83^{a}	89.49±0.56^{a}
	Everglade-41	27.69±0.68^{b}	54.01±1.51^{b}	91.55±0.93^{b}
	Tainung-2	30.50±0.03^{c}	57.03±0.66^{a}	92.55±0.86^{b}

*DAS; Days after sowing

†Means followed by the same letter are not significantly different at 0.05 probability level according to Duncan's multiple range test.

metabolic process related to aging in living organs, is known to be highly reactive to electronic reduction and cause diseases through its toxic effect on cells and tissues. It may accelerate tumors and cause cancers. In order to remove O_2^- · , the body secrets SOD (superoxide dismutase) that converts superoxide radical into hydrogen peroxide and normal oxygen.

We analyzed SOD enzyme activity related to the scavenging of superoxide anion radical (· O_2^-) through NBT reduction. As for the SOD activity of leaf extract by variety, the activity was 92.6%, 91.0% and 92.6%, respectively, in Tainung-2 on day 98, 127 and 141 from sowing, slightly higher than those in the other two varieties but not significantly different. The activity was slightly higher in the middle period of growth than in the harvest period, but not much different according to harvest time (Table 1).

Active oxygen has strong oxidizing power, so it decomposes membrane and protein, oxidizes lipid and suppresses the synthesis of DNA. Natural antioxidants and antioxidant enzymes are useful substances of high added value, and play important roles in understanding the defensive mechanism against environmental stress (Ceruti, 1985; Halliwell and Gutteridge, 1984).

4. Differences in the DPPH radical scavenging activity of leaf extract by variety and growth time

As for the DPPH radical scavenging activity of leaf extract by variety, the activity was 80.87% and 80.71%, respectively, in Tainung-2 on day 98 (30th of August) and day 127 (28th of September), slightly higher than the other two varieties. On day 141 from sowing (12th of October), the activity was 84.95%, 84.80% and 84.88%, respectively, in Dowling, Everglade-41 and Tainung-2, showing no difference among the varieties. In general, according to variety, DPPH radical scavenging activity was highest in Tainung-2 among the varieties, and according to harvest time, it was high (86.40%, 86.82% and 87.7%, respectively, in Dowling, Everglade-41 and Tainung-2) on day 127 (28th of September) compared to that on the other two harvest times (Fig. 1).

It was reported that electron donating ability is an indicator to the antioxidant effect of phenolic acids, flavonoids and other phenolic substances, and these substances have high electron donating ability when they have high reducing ability (Kang *et al*., 1996). N addition, electron donating ability is an ability to donate hydrogen or electrons to ROO-, R-, RO-, etc. generated from the spontaneous oxidation of oils and fats, and is closely connected to reducing ability but the general effects of antioxidants cannot be explained with electron donating ability alone, and DPPH is known as a convenient method of measuring the electron donating ability of antioxidants.

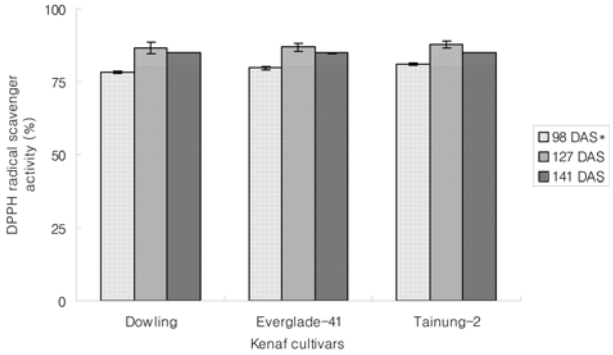

Fig. 1. Changes in DPPH radical scavenger activity of kenaf varieties leaves.
*DAS; Days after sowing.

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