academicJournals

Vol. 7(17), pp. 1121-1128, 3 May, 2013
DOI 10.5897/JMPR11.1431
ISSN 1996-0875 © 2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plant Research

Full Length Research Paper

Far infrared assisted kenaf leaf tea preparation and its effect on phenolic compounds, antioxidant and ACE inhibitory activity

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Accepted 14 November, 2011

The kenaf (*Hibiscus cannabinus* L.) leaf tea was prepared by subjecting kenaf leaf in far infrared roaster at different time and temperature. Further, the effect of far infrared (FIR) irradiation on the total polyphenol, total flavonoid, antioxidant activity and angiotensin I-converting enzyme (ACE) inhibition ability were investigated in kenaf leaf tea. The data revealed that the FIR irradiation at 60°C increased the total polyphenol (TP) contents and total flavonoids (TF) content as the treatment time increased, and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity and lipid peroxidation inhibition activity were also increased by FIR irradiation. The ACE inhibition activity also altered after FIR treatment. Likewise, the quantification analysis of kaempferitrin by use of HPLC showed an increase in the content in time and temperature dependent manner. Overall, this research showed that kenaf leaf treated with FIR at 60°C for 30 min is an optimal condition in processing to make a functional healthy kenaf leaf tea with higher phenolic content and higher biological (antioxidant and ACE inhibitory) activity.

Key words: Angiotensin I-converting enzyme (ACE) inhibition, antioxidant, far infrared, kaempferitrin, kenaf.

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is a fiber plant native to east central Africa where it has been grown for several thousand years for food, vegetables and fiber (Lemahieu et al., 2003; Agbor et al., 2005a). This plant is widely prescribed in traditional folk medicine in Africa and India (Agbor et al., 2005a; Kobaisy et al., 2001). The plant is reported to cure anaemia and fatigue and also have antioxidant and anti-inflammatory property (Lee et al., 2007; Agbor et al., 2005b).

The plant contains large amounts of compounds including polyphenols, tannins, saponins, alkaloids, steroids, essential oils etc (Kobaisy et al., 2001). Kaempferitrin (3,7-diglycosylflavone), is the main compound present in the kenaf leaf. The compound was found to have an acute lowering effect on blood glucose level in diabetic rats (Cazarolli et al., 2006; Jorge et al., 2004). In other reports, kaempferitrin compound showed high reactivity with 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals and also decreased lipid peroxidation (De Sausa et al., 2004). Likewise, there are also reports of antimicrobial (Abdel-Ghani et al., 2001) and anti-inflammatory effect in macrophage cells (Fang et al., 2005).

Far infrared (FIR) ray has wavelength of around 3.0 ~ 1,000 μm, and is used in the heated and unheated mode.

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It is applied in ripening food, improving the taste and flavor of food and so on. FIR rays transfer heat to the center of materials evenly without degrading the constituent molecules of the surface. FIR may be capable of cleaving covalent bonds and releasing antioxidants, such as flavonoids, carotenes, tannin, flavoprotein and polyphenols, from repeating polymers (Niwa and Miyachi, 1986). Compared with hot air drying, FIR heating offers many advantages such as greater energy efficiency, higher heat transfer rate, and higher heat flux, which result in fewer drying time and higher drying rate (Shi et al., 2008). In some previous study, FIR irradiation greatly increased the polyphenolic contents and antioxidant activities in rice hulls (Lee et al., 2003), green tea leaves (Kim et al., 2006) and citrus peels (Jeong et al., 2004).

Thus, the present study purposed to make a research on the manufacturing condition of functional healthy kenaf leaf tea by changing far infrared application time and temperature in processing of leaves, and setting the optimal condition. Also, we monitor the alteration in phenolics and biological (antioxidant and ACE inhibitory) activity of kenaf leaf tea in a given temperature and time.

MATERIALS AND METHODS

Tannic acid, Hippuryl-L-histidyl-L-leucine and 1,1-diphenyl-2-picrylhydrazyl purchased from Sigma Chemical Co. (St. Louis, Mo). Quercetin and Folin-Ciocalteu was purchased from Wako pure chemicals, Japan. All other chemicals and reagents used were of the highest commercially available purity. The pure compounds like kaempferitin, afzelin, α-rhamnoisorobin and kaempferol were previously isolated in our laboratory (Rho et al., 2010) and used in the HPLC as an external standard.

Preparation of the samples

The leaves of *H. cannabinus* were harvested from the Gyehwa-myeon, Buan-gun, Jollabuk-province of Korea, and dried in the shade. The dried kenaf leaves were subjected to infrared ray for various times (10, 20, 30 and 45 min) at 60 and 80°C, respectively using a far infrared grain roaster (MK-2, Korean Energy Co., Seoul, Korea). The FIR radiator emits thermal radiation in the wavelength range of 4 to 15 µm. Irradiated leaves (1.0 g) were dissolved in 50 mL of boiling water for 3 min to make kenaf leaf tea, and filtrated on Mixed Cellulose Ester filter (0.45 µm, Advantec).

Determination of total polyphenol content

The total polyphenol (TP) content was measured using Folin-Ciocalteu method (Kim et al., 2007), which uses the phenomenon that a phenolic substance turns blue by reaction with phosphomolybdate. Briefly, 0.2 mL of the kenaf leaf tea was diluted 10 times by distilled water to prepare 2 mL of test solution in a test tube. Then, 0.2 mL of Folin-Ciocalteau phenol reagent was added, and the mixture was stirred well and left at room temperature for 3 min. After reaction for exactly 3 min, 0.4 mL of solution saturated with Na_2CO_3 was added and mixed, and 1.4 mL of distilled water was added to make the volume 4 mL. The solution was left at room temperature for an hour, and the absorbance of the supernatant was measured using spectrophotometer (U-2001, Hitachi, Japan) at 725 nm. The total polyphenol content was measured from the standard curve (concentrations used were 100, 150, 200, 300 and 500 ppm) prepared by melting 1 mg of tannic acid in 1 mL of 80% methanol and expressed in mg/L tannic acid equivalent.

Determination of total flavonoid content

Total flavonoid (TF) content was measured using the method described by Park et al. (1997). An aliquot of 0.5 mL of the kenaf leaf tea was added to test tubes containing 0.1 mL of 10% aluminium chloride hexahydrate, 0.1 mL of 1 M potassium acetate, 2.8 mL of deionized water and 1.5 mL 95% ethanol. After 40 min at room temperature, the absorbance was determined using spectrophotometer (U-2001, Hitachi, Japan) at 415 nm. The total flavonoid content was measured from the standard curve (concentrations used were 100, 150, 200, 300 and 500 ppm) prepared by melting 1 mg of quercetin in 1 mL of 80% methanol and expressed in mg/L quercetin equivalent.

HPLC analysis

Kenaf leaf tea were analyzed by a HPLC system (CBM-20A, Shimadzu Co. Ltd., Japan) with two gradient pump systems (LC-20AT, Shimadzu), a UV-detector (SPD-10A, Shimadzu), an auto sample injector (SIL-20A, Shimadzu) and a column oven (CTO-20A, Shimadzu).

Separation was achieved on a Gemini C_{18} column (4.6 × 100 mm, 3 µm, Phenomenex. Inc., Torrance, CA, USA) using a linear gradient elution program with a mobile phase containing solvent A (0.4%, v/v, formic acid in distilled deionized water) and solvent B (acetonitrile). Initially started with a gradient of 18% B changing to 32% in 15 min and finally to 50% in 40 min followed by washing for 25 min with a flow rate of 1.0 mL/min. Sample injection volume was 10 µL. Peaks were monitored at 280 nm. Identification of kaempferitrin and other compounds (afzelin, α-rhamnoisorobin and kaempferol) were accomplished by comparing the retention time and absorption spectra of peaks to the external standard compounds.

DPPH radical scavenging activity

DPPH free radical scavenging activity was measured according to the protocol of Braca et al. (2001) with slight modification. Briefly, 1 mL of the kenaf leaf tea (20 times diluted) were mixed with 4 mL of 0.15 mM DPPH methanol solution and left at room temperature for 30 min and measured at 517 nm using spectrophotometer (U-2001, Hitachi, Japan). The radical scavenging activity (%) was calculated with the following formula.

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

Where, A=Absorbance of sample, B=Absorbance of control (without sample).

Lipid peroxidation assay

Following the protocol of Dasgupta et al. (2004), lipid peroxidation assay was performed. Briefly, egg homogenate (0.5 mL of 10% v/v) and 0.1 mL of sample (concentration 200, 100 and 50 ppm) were added to a test tube and made up to 1 mL with distilled water; 0.05 mL of $FeSO_4$ (0.07M) was added to induce lipid peroxidation and the mixture incubated for 30 min.

Then, 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of 0.8% (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulphate were added and the resulting mixture was vortexed and boiled for 60

Table 1. Changes in total polyphenol (TP) and total flavonoid (TF) contents of kenaf leaf tea after far infrared irradiation in different temperature and time.

Temperature (℃)	Far infrared irradiation (min)	(mg/L)	
		Total polyphenol	Total flavonoid
60	0	474.13±34.45[a]	426.35±21.06[a]
	10	630.60±19.27[b]	558.29±21.79[b]
	20	757.97±23.36[c]	664.71±29.28[c]
	30	828.97±29.45[d]	786.56±37.31[d]
	45	848.25±13.09[d]	794.35±11.47[d]
80℃	0	474.13±34.45[a]	426.35±21.06[a]
	10	792.88±23.73[d]	575.72±16.50[c]
	20	685.15±12.55[c]	511.15±24.44[b]
	30	581.78±25.19[b]	479.53±21.87[b]
	45	553.62±12.25[b]	463.85±18.26[b]

Data are the mean ± SD of three independent experiments. Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different ($p<0.05$).

min.

After cooling, 5.0 mL of butan-1-ol were added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the upper layer was monitored spectrophotometrically (Lambda UV-vis, Perkin-Elmer, USA) at 532 nm. Inhibition of lipid peroxidation (%) by the sample was calculated thus:

[(1-E/C) x100%]

Where "C" is the absorbance value of the fully oxidized control and "E" is the absorbance in presence of sample.

Determination of the angiotensin I-converting enzyme (ACE) inhibition activity

The ACE inhibition activity was assayed as reported by Nakamura et al. (1995). The different concentrations of extracts were added to 5 mM hippuryl-L-histidyl-L-leucine (HHL), and pre-incubated for 3 min at 37℃. The reaction was then initiated by adding 0.1 U/ml ACE from rabbit lung, prepared in the 100 mM borate buffer (pH 8.3) containing 300 mM NaCl and incubated for 30 min at 37℃. The enzyme reaction was stopped by the addition of 0.1 M HCl. The released hippuric acid (HA) was extracted by the addition of 1.7 mL of ethyl acetate. After vortex for 15 s, 1 mL of the upper layer supernatant was transferred into a glass tube and evaporated at 90℃ for 15 min. The released HA was dissolved in 1 mL of distilled water, and the absorbance was measured at 228 nm, using a spectrophotometer (Lambda UV-vis, Perkin-Elmer, USA). The average value from three determinations at each concentration was used to calculate the ACE inhibition rate as follows:

ACE inhibition (%) = (B-A)/(B-C)×100%

where A is the absorbance of HA generated in the presence of ACE inhibitor component, B is the absorbance of HA generated without ACE inhibitors and C is the absorbance of HA generated without ACE (corresponding to HHL autolysis in the course of enzymatic assay).

Statistical analysis

The data were analyzed using SPSS program (ver. 10; SPSS Com, Chicago, IL, USA). The data between non FIR treated (control) and FIR treated group were analyzed by one-way analysis of variance (ANOVA) and differences among experimental groups were evaluated using Duncan's multiple range tests at the $p<0.05$ significant level.

RESULTS

Total polyphenol (TP) and total flavonoid (TF) contents

The TP contents of kenaf leaf tea after the treatment with FIR for different times and temperatures are shown in Table 1. Compared to the control, the TP and TF contents were increased in a time-dependent manner in 60 and 80℃ treated groups. The highest TP (828.97 mg/L) and TF (786.56 mg/L) contents were observed after treatment with FIR for 30 min in 60℃ (Table 1). However, more the temperature increased, the TP and TF contents decreased. In the 80℃ treated group, the decrease in the TP content was probably related with the increase in treatment time where the phenolic compounds exposed to high temperature for long time became unstable.

HPLC analysis

The compound content in the FIR treated kenaf leaf tea was analyzed quantitatively by HPLC. As shown in Table 2, the content of kaempferitrin (major compound) was increased in a time-dependent manner at 60℃. After treatment for 30 min at 60℃, the highest content of kaempferitrin (388.84 mg/L) was obtained which was 2.43 fold higher than that of the control. With the increase of the temperature, the content of kaempferitrin decreased in a time dependent manner at 80℃. However, due to FIR treatment the other minor products (afzelin,

Table 2. Changes in kaempferol glycosides contents in the FIR treated samples at different temperature and time interval.

Temperature (℃)	Far infrared irradiation (min)	(mg/L)			
		Kaempferitrin	Afzelin	α-rhamnoisorobin	Kaempferol
60	0	153.06±5.70^{a}	1.10±0.23^{a}	nd	nd
	10	223.40±8.11^{b}	6.68±0.56^{b}	17.22±1.49^{a}	nd
	20	260.99±8.99^{c}	8.79±0.62^{c}	23.94±1.53^{b}	nd
	30	372.84±10.14^{d}	8.44±0.58^{c}	22.79±1.82^{b}	nd
	45	353.63±9.42^{d}	11.53±0.81^{d}	32.98±2.02^{c}	nd
80	0	153.06±5.70^{b}	1.10±0.23^{a}	nd	nd
	10	200.79±8.29^{d}	11.55±0.42^{b}	30.79±1.62^{a}	2.71±0.21^{a}
	20	175.26±7.24^{c}	11.15±0.66^{b}	43.46±2.33^{b}	7.43±0.68^{b}
	30	125.69±9.74^{a}	13.05±0.86^{c}	46.08±1.26^{b}	14.87±0.84^{c}
	45	111.23±5.23^{a}	13.86±0.77^{c}	47.55±1.13^{b}	15.23±0.76^{c}

Data are the mean ± SD of three independent experiments. Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, ($p<0.05$). nd : not detected.

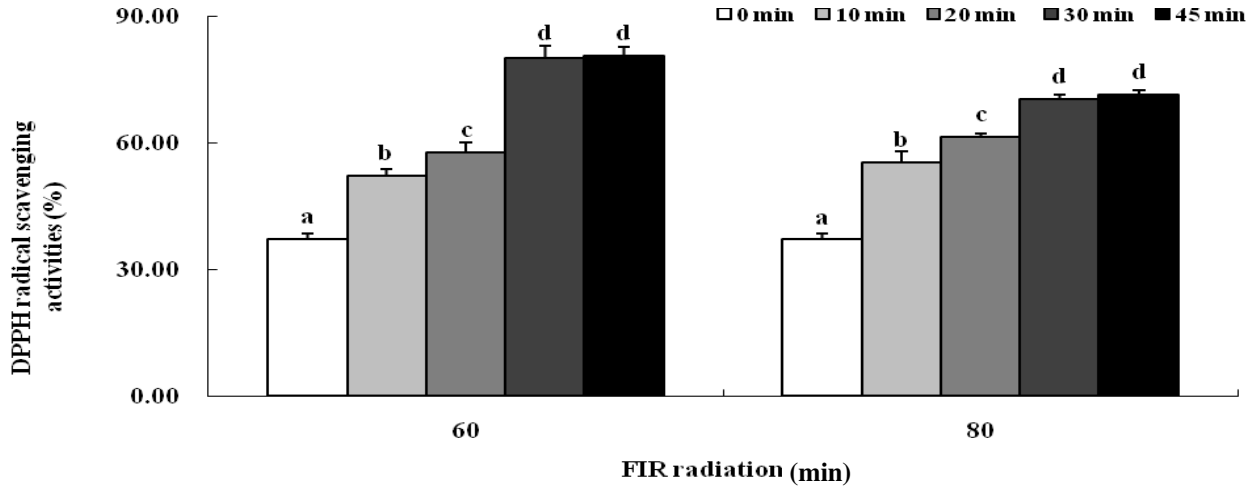

Figure 1. DPPH radical scavenging activities of kenaf leaf tea by far-infrared irradiation.
The result expressed in percentage. Each sample 20 times diluted, data are the mean ± SD of three independent experiments. Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different ($p<0.05$).

α-rhamnoisorobin and kaempferol) were produced at higher (80℃) temperature (Table 2).

DPPH radical scavenging activity

The DPPH radical scavenging activity of kenaf leaf tea after treatment with FIR for different time and temperature is shown in Figure 1. The result revealed that the FIR treated kenaf tea showed higher DPPH radical scavenging activity than that of the control in time dependent manner. The sample showed the highest DPPH free radical scavenging activity with 79.94 and 80.40% after treatment with FIR at 60℃ for 30 and 45 min, respectively. However, the activity was comparatively decreased with the increase in temperature at 80℃.

Inhibition of lipid peroxidation

Egg yolk lipids undergo rapid non-enzymatic peroxidation when incubated in the presence of ferrous sulphate. The ability of kenaf leaf tea extract to inhibit lipid peroxidation was evaluated by measuring a decrease in the amount of malondialdehyde generation. As shown in Figure 2, all the samples treated with FIR at different temperature displayed time-dependent inhibition of lipid peroxidation *in vitro*. After FIR irradiation for 10 min at 60℃, the lipid

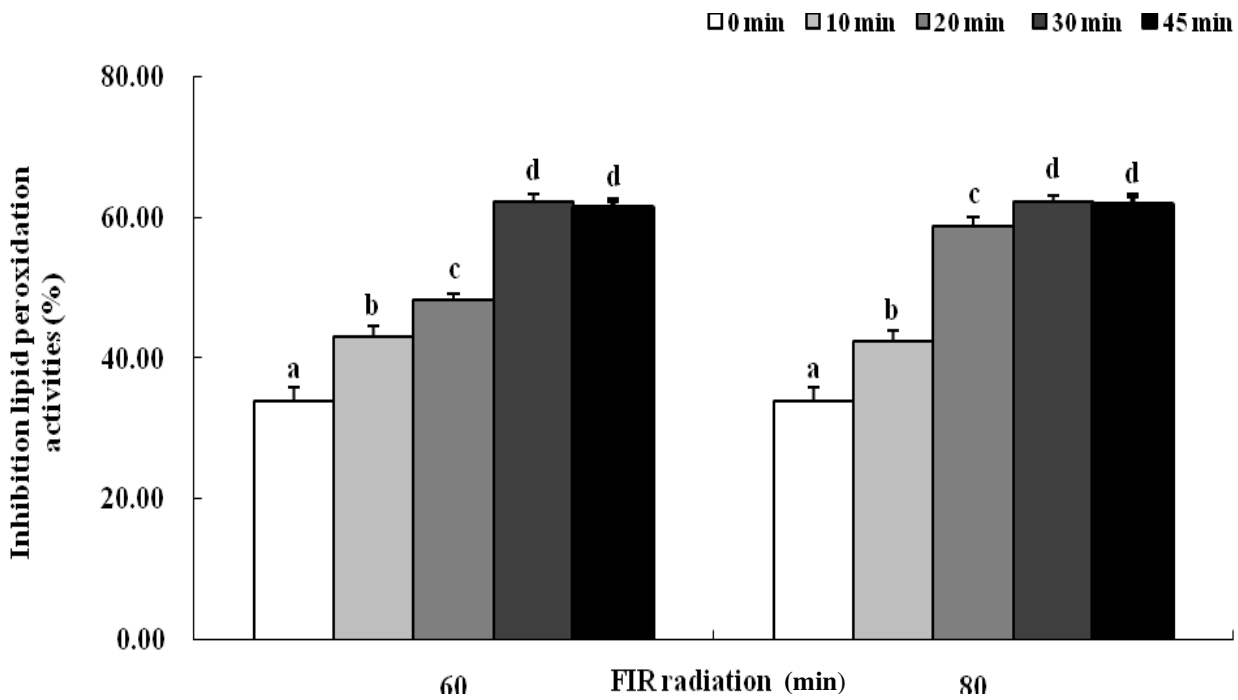

Figure 2. Inhibition of lipid peroxidation of kenaf leaf tea by far-infrared irradiation and expressed in percentage. Each sample 5 times diluted, data are the mean ± SD of three independent experiments. Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different ($p<0.05$).

peroxidation inhibition percent increased from 34 to 44% and reached a plateau after about 30 min of irradiation (62%).

However, over time (above 45 min at 60°C) and higher temperature (80°C) exposure caused slight decrease in lipid peroxide inhibition activity.

ACE inhibitory activity

In this study, the ACE inhibitory activity is expressed in percentage inhibition of ACE in an *in vitro* experiment. According to the data (Figure 3), significant (0.05) differences in the percent ACE inhibition were observed in all of the samples after treatment with FIR. The percentage inhibition of ACE was increased in a time dependent manner at 60 and 80°C. Comparing the temperatures, the higher inhibition of ACE was observed in the samples treatment with FIR for 10, 20 and 30 min at 60°C which inhibited ACE by 40.77, 49.58, and 50.44%, respectively.

DISCUSSION

Far infrared grain roaster adopts far infrared ray as its heat source. The high penetration power of FIR helps to stimulate exudation of chemical components without destroying the plant cells (Eom et al., 2009) and thereby causes the alteration of biological activity. According to the data, the total polyphenol (TP) and total flavonoid (TF) content in kenaf leaf tea increased after treatment with FIR in temperature and time dependent manner (Table 1). The increment was about 1.78 and 1.86 fold higher for TP and TF content, respectively than that of the control.

These increases in phenolics due to FIR heat could be due to the transformation of high molecular phenolics to low molecular phenolics that may originate from breakage of covalent bonds of polymerized polyphenols (Eom et al., 2009; Lee et al., 2005; Niwa et al., 1986). This type of increment in phenolic compounds were also observed in previous research of Kim et al. (2006), where they reported that the contents of catechins including epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate, were increased by FIR irradiation during the manufacturing process of green tea. Similarly, the phenolic alteration was also observed in FIR treated defatted soybean and grape berries, which showed 1.9 and 3 folds higher polyphenol content than untreated sample (Rim et al., 2005; Eom et al., 2009).

In our experiment, the major compound kaempferitrin was also gradually increased with the increase in exposure time at 60°C (Table 2). This increment in kaempferitrin compound could be due to the effect of far infrared radiation which causes molecular vibration in tissues/cells and helps to exudates more compound. However, higher temperature causes decrease in the content due to the instability of kaempferitrin when

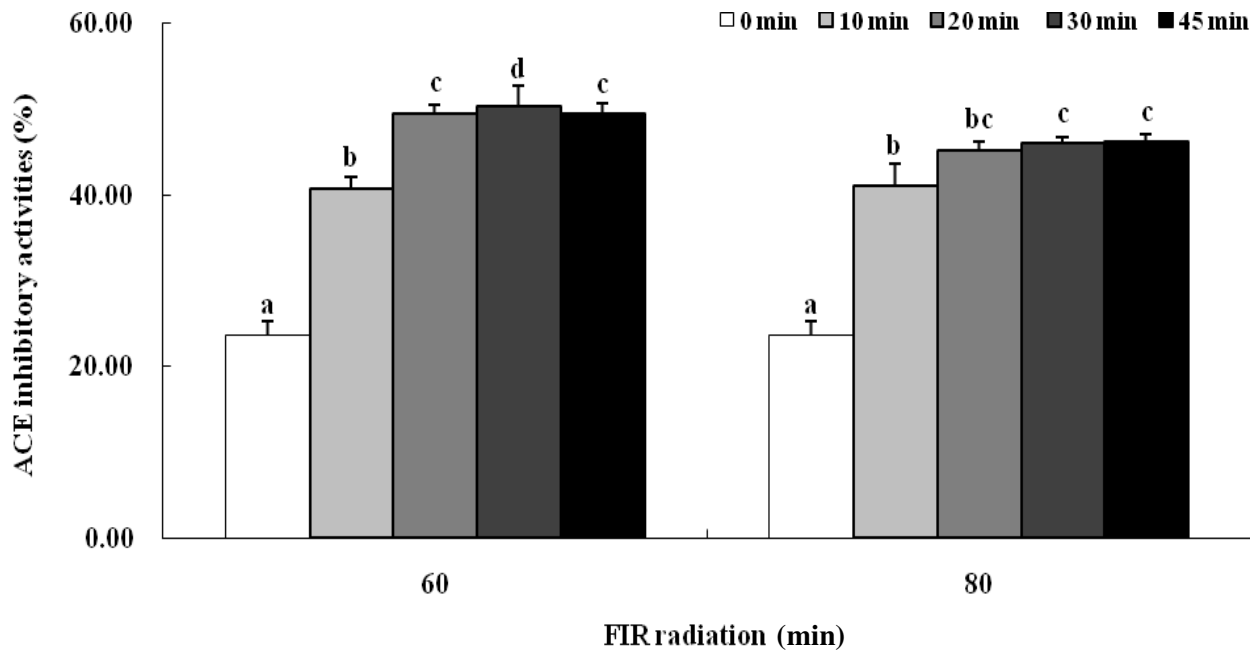

Figure 3. ACE inhibitory activity kenaf leaf tea by far-infrared irradiation treatment and expressed in percentage of inhibition. Each sample 10 times diluted, data are the mean ± SD of three independent experiments. Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different ($p<0.05$).

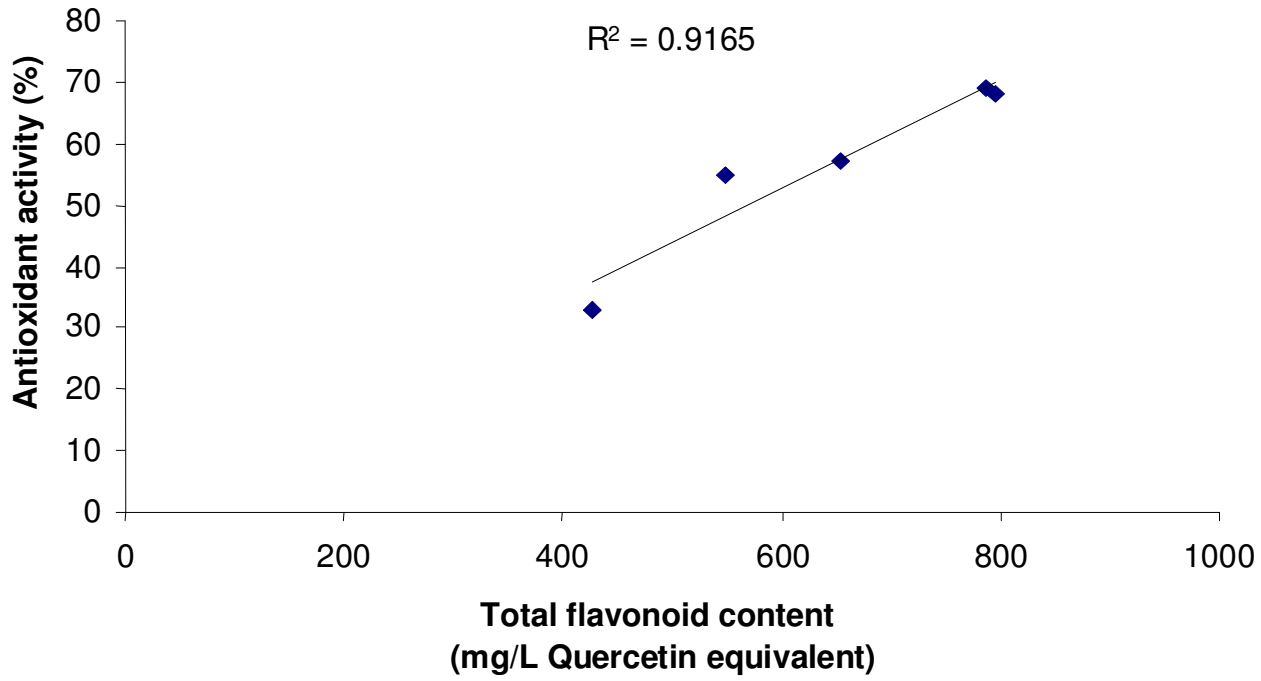

Figure 4. Linear correlation between free DPPH radical scavenging activity (%) at FIR 60°C and total flavonoid contents.

exposed to high temperature (80°C) for a longer time in the far infrared roster.

In HPLC data, we also observed the formation of extra chromatogram peaks of different compounds of afzelin, α-rhamnoisorobin and kaempferol at 80°C temperature in different time intervals. These compounds could have formed from the biotransformation of kaempferitrin or other other kaempferol glycosides due to FIR heat (Rho et al.,

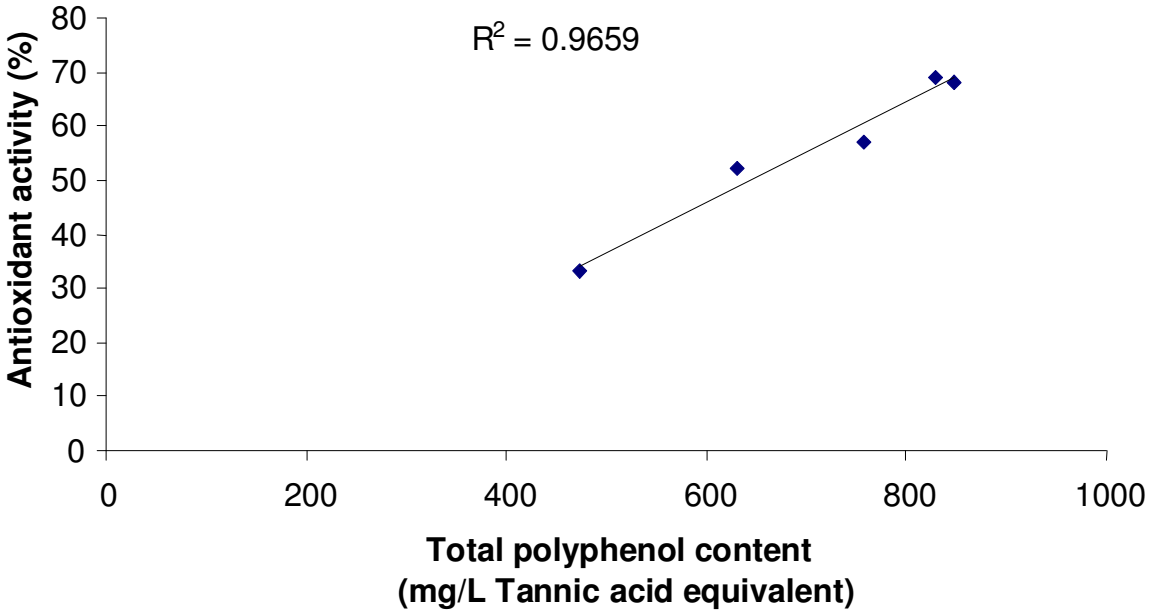

Figure 5. Linear correlation between free DPPH radical scavenging activity (%) at FIR 60℃ and total polyphenol contents.

2010).

In the research, the data also revealed that the DPPH free radical scavenging ability of kenaf leaf tea was increased in time dependent manner at the given temperature and showed good correlation with the TF and TP contents (Figure 4, 5). Flavonoids are known as important compounds in terms of antioxidant activities (Dasgupta and De, 2004; Seyoum et al., 2006; Abraham et al., 2008) and inhibit the ACE (Kang et al., 2003; Kiss et al., 2004; Loizzo et al., 2007). In this study, their content had higher correlation with antiradical activity of kenaf leaf tea. Likewise, the content of phenolics in the extracts also correlates with their antiradical activity, confirming that flavonoids and phenolic compounds are likely to contribute to the radical scavenging activity in kenaf leaf tea. Further, the result showed good inhibition of lipid peroxidation and ACE inhibitory activity, this could be due to the increase in the polyphenol or flavonoid compounds like kaempferitrin and other minor compounds (afzelin, α-rhamnoisorobin and kaempferol) which could play an important role in the inhibition of lipid peroxidation and ACE activity.

Conclusion

Overall, FIR treatment stimulates significantly the content of kaempferitrin (2.43 fold) and increases polyphenol and flavonoid with higher biological antioxidant and ACE inhibitory activity in kenaf leaf. Therefore, we may be able to conclude from this study that the treatment of kenaf leaf with FIR at 60℃ for 30 min is an optimal processing condition to make functional healthy kenaf leaf tea which may help to prevent a wide variety of diseases and contributes to the benefits of health.

ACKNOWLEDGEMENT

Authors appreciate the Institute of Bioscience and Biotechnology of Kangwon National University for supporting research fund.

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