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Changes in flavonoid content and tyrosinase inhibitory activity in kenaf leaf extract after far-infrared treatment

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ABSTRACT

The tyrosinase inhibitory activity of ethanolic extract of kenaf (*Hibiscus cannabinus* L.) leaf was evaluated before and after subjecting it to far-infrared (FIR) irradiation. The main component of the extract was analyzed as kaempferitrin (kaempferol-3,7-*O*-α-dirhamnoside). Prior to FIR irradiation, no inhibitory activity of the extract was detected in a tyrosinase assay. However, after FIR irradiation for 1 h at 60 °C, significant tyrosinase inhibitory activity (IC_{50} = 3500 ppm) was observed in it. In HPLC analysis, derhamnosylation products (kaempferol, afzelin, and α-rhamnoisorobin) were detected. The inhibitory activity may be due to the existence of derhamnosylation products. This study demonstrated that FIR irradiation can be used as a convenient tool for deglycosylation of flavonoid glycoside.

Melanin, which is the major pigment of skin, plays an important role in protecting the skin against the harmful effects of UV-irradiation. However, an increase in the levels of epidermal melanin synthesis and its uneven distribution can cause esthetic problems[1] such as freckles, melasma, and aging spots. Melanin synthesis is controlled by a cascade of enzymatic reactions. Tyrosinase,[2] which is the rate-limiting enzyme in the melanin synthesis, converts tyrosine to DOPA and oxidizes DOPA to dopaquinone. Therefore, the inhibition of tyrosinase to treat pigmentation disorders has been a recent subject of many studies. Recent research efforts on investigating tyrosinase inhibitors have been focused on natural extracts. Kenaf (*Hibiscus cannabinus* L.) is a member of the malvaceae family and has been prescribed in traditional folk medicine in Africa and India. It exhibited a broad spectrum of biological activities such as hepatoprotective activity,[3] anti-oxidative activity,[4] and haematinic activity.[5] Recently, an immunomodulatory effect of kenaf extract has been elucidated.[6] However, the depigmenting activity of kenaf extract has not been evaluated. In the present study, the tyrosinase inhibitory effect of kenaf leaf extract was investigated before and after subjecting the extract to infrared (FIR) irradiation. Several Letters have shown the potential of FIR treatments for enhancing the activity levels and number of antioxidants in order to increase the content of functional components in food products.[7] FIR irradiation involves electromagnetic waves with wavelengths ranging from 4 to 15 μM. It has been hypothesized that FIR treatment during extraction of polyphenols from plant cells stimulates exudation of chemical components in cells without destroying the cells by radiant heat and breaks covalent bonds of polymerized polyphenols, resulting in the release of active, natural antioxidants with a low molecular weight. The purpose of this study was to evaluate not only the changes in anti-tyrosinase activity but also the chemical transformation of kenaf extract exposed to FIR (Scheme 1).

FIR treatment was applied to the powder of kenaf leaf for 1 h at 60 °C in an FIR radiation chamber emitting wavelengths of 3–10,000 μM. After FIR treatment, kenaf leaf powder was extracted with EtOH.[8] Prepared samples were evaluated for mushroom tyrosinase activity.[9] The results are listed in Table 1.

Tyrosinase inhibitory activities were tested in a range from 100 ppm to 10,000 ppm. Kenaf leaf extract exhibited no inhibitory activity at tested concentrations (IC_{50} >10,000 ppm). However, the FIR-treated kenaf leaf extract exhibited inhibitory activity in a dose-dependent manner (IC_{50} = 3500 ppm). These results indicated that FIR irradiation onto kenaf leaf extract could change the composition of its contents and the changes resulted in tyrosinase inhibitory activities. The changes of contents are shown in Figure 1.

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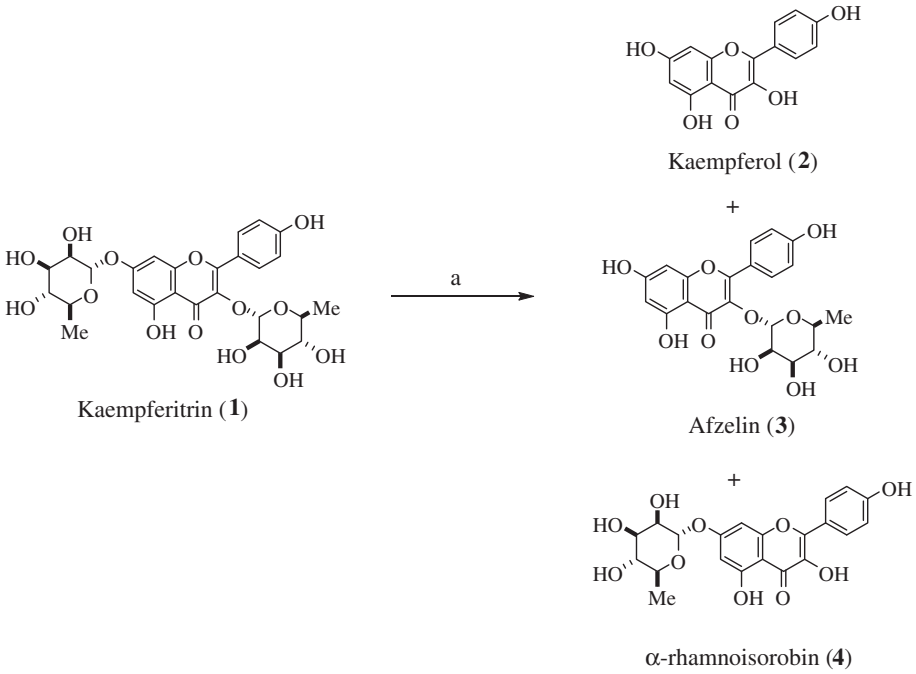

Scheme 1. Reagent and conditions: (a) FIR irradiation, 1 h, 60 °C.

Table 1
Anti-tyrosinase activities of kenaf leaf extract and FIR-treated kenaf leaf extract

Compounds	Concentration (ppm)	Inhibition of tyrosinase activity (%)	IC_{50}[a]
Kenaf leaf extract	100	3.9 (±2.9)	>10,000 ppm
	1000	10.2 (±4.6)	
	5000	17.7 (±1.4)	
	10,000	30.1 (±3.8)	
FIR-treated kenaf leaf extract	100	16.2 (±3.9)	3500 ppm
	1000	36.9 (±4.2)	
	5000	58.8 (±0.9)	
	10,000	68.9 (±3.5)	
Arbutin	2300	50.0 (±2.1)	2300 ppm

[a] Values were determined from logarithmic concentration–inhibition curves and are given as means of three experiments.

We purified and identified the compounds present in the kenaf leaf extract and the FIR-treated kenaf leaf extract. The HPLC profile of the kenaf leaf extract gives compound **1** as the major component (Fig. 1A). The kenaf leaf extract was further purified by MPLC for structure analysis. In order to determine the structure of compound **1**, acid hydrolysis, NMR, and MS analyzes were conducted. On the basis of the analysis data, compound **1**[10] was identified as kaempferol-3,7-*O*-α-dirhamnoside (kaempferitrin). After the FIR treatment, the kaempferitrin content significantly decreased; however, compounds **2**, **3**, and **4** were found (Fig. 1B). Compound **2**, **3**, and **4** were identified as kaempferol[11] (4′,5,7-trihydroxyflavonol), afzelin[12] (keamperol 3-*O*-α-L-rhamnopyranoside), and α-rhamnoisorobin[13] (keamperol 7-*O*-α-L-rhamnopyranoside), respectively. Contents of compounds **2**, **3**, and **4** were significantly increased by the FIR treatment (Table 2).

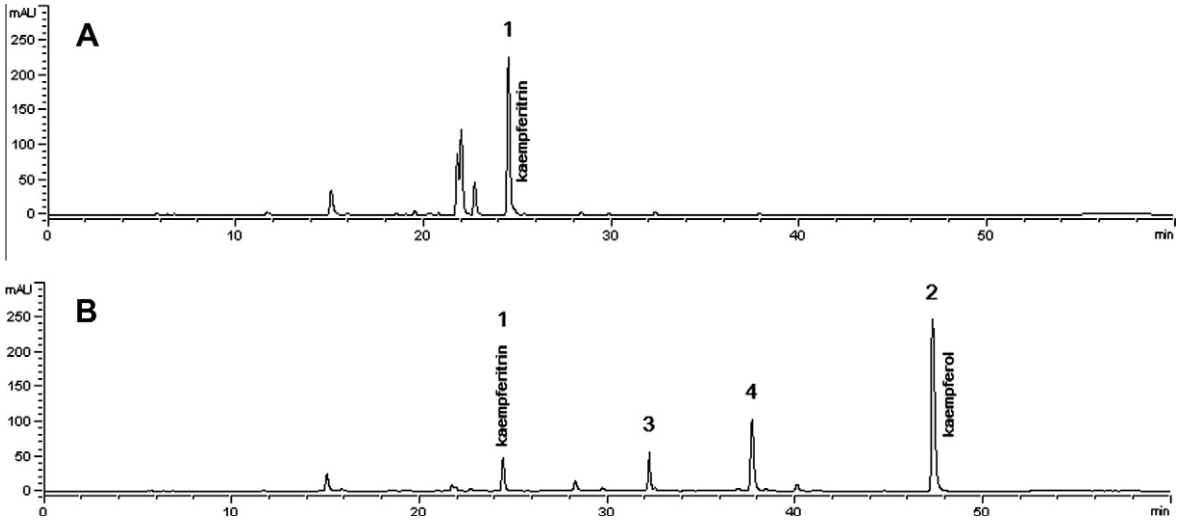

Figure 1. HPLC profiles of (A) kenaf leaf extract and (B) FIR-treated kenaf leaf extract.

Table 2
Contents of compounds **1**, **2**, **3**, and **4** in kenaf leaf extract

Compounds	Contents (mg/g)	
	Non-FIR	FIR
1 (kaempferitrin)	29.3	3.1
2 (kaempferol)	—	15.2
3 (afzelin)	—	2.4
4 (α-rhamnoisorobin)	—	5.7

—: not detected.

Table 3
Anti-tyrosinase activities of compounds **2**, **3**, and **4**

Compounds	IC_{50}[a]
1 (kaempferitrin)	>400 μM
2 (kaempferol)	171.4 μM
3 (afzelin)	>400 μM
4 (α-rhamnoisorobin)	>400 μM (39.1%)[b]

[a] Values were determined from the logarithmic concentration–inhibition curves and are given as the mean values of the results of three experiments.
[b] Inhibitory activity (% of control) at 400 μM.

From HPLC results, we hypothesize that tyrosinase inhibitory activity may have originated from kaempferol[11b] (major product) and other minor products (afzelin **3** and α-rhamnoisorobin **4**) after the FIR treatment of kenaf leaf extract. In order to confirm our hypothesis, anti-tyrosinase activities of deglycosylation products (compounds **2**, **3**, and **4**) were evaluated. The results are summarized in Table 3. Specifically, compound **2** (kaempferol) exhibited inhibitory activities with IC_{50} values of 171.4 μM. However, compound **3** showed no inhibitory activity. The weak inhibitory activity was shown in compound **4** (α-rhamnoisorobin). Compound **4** showed 39.1% inhibition of tyrosinase at 400 μM concentration.

All these data suggested that treatment with FIR irradiation might serve as not only an efficient deglycosylation technique but also a potent tool for enhancing the biological activities of natural extracts.

In conclusion, the present investigation reported for the first time deglycosylation of kaempferol glycoside by FIR irradiation. FIR-treated kenaf leaf extract was more active than kenaf leaf extract against tyrosinase because derhamnosylation of kaempferitrin occurred to give kaempferol and other minor products (afzelin and α-rhamnoisorobin). Further studies on deglycosylation of other natural glycosides are underway in our laboratory.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.082.

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8. (a) Preparation of kenaf leaf extract: kenaf (*Hibiscus cannabinus* L.) leaf cultivated in the reclaimed land in Chuncheon, S. Korea, was harvested in the second week of Sept. 2009. The leaves were sun dried and grinded to powder by a grinder machine in the laboratory. The powdered leaf sample (50 g) was suspended in 1 L of 70% ethanol (v/v) and kept overnight in a shaker at room temperature. The extracts were filtered through Advantec 5B Tokyo Roshi Kaisha Ltd, Japan. The ethanol extract was dried using a vacuum rotary evaporator (EYLA N-1000, Tokyo, Japan) in a 40 °C water bath. Dried samples were weighed and kept at 4 °C for further analysis. (b) Preparation of FIR-treated kenaf leaf extract: FIR was applied to powdered leaf sample (2 g dissolve in 10 ml water and acetic acid, pH 3.3 each in a Glass Petri disc, 100 × 20 mm) in an FIR radiation chamber (Korea Energy Co., Seoul, Korea) emitting wavelengths of 3–1000 μm. The sample was exposed at 60 °C for an hour each emitting 8.4 μm wavelengths. After FIR treatment, kenaf powder was extracted with EtOH and the solvent was evaporated to obtain powder.
9. Measurement of mushroom tyrosinase: mushroom tyrosinase, L-tyrosine were purchased from Sigma Chemical. The reaction mixture for mushroom tyrosinase activity consisted of 150 μl of 0.1 M phosphate buffer (pH 6.5), 3 μl of sample solution, 8 μl of mushroom tyrosinase (2100 unit/ml, 0.05 M phosphate buffer at pH 6.5), and 36 μl of 1.5 mM L-tyrosine. Tyrosinase activity was determined by reading the optical density at 490 nm on a microplate reader (Bio-Rad 3550, Richmond, CA, USA) after incubation for 20 min at 37 °C. The inhibitory activity of the sample was expressed as the concentration that inhibits 50% of the enzyme activity (IC_{50}).
10. *Compound* **1**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (d, 2H, J = 9.5 Hz), 6.95 (d, 2H, J = 9.5 Hz), 6.72 (s, 1H), 6.46 (s, 1H), 5.56 (s, 1H), 5.40 (s, 1H), 4.20 (s, 1H), 4.00 (s, 1H), 3.83–3.81 (m, 1H), 3.71–3.70 (m, 1H), 3.60–3.57 (m, 1H), 3.48 (t, 1H, J = 9.2 Hz), 3.34–3.32 (m, 2H), 1.27 (d, 3H, J = 6.5 Hz), 0.93 (d, 3H, d, J = 6.5 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 179.9, 163.5, 163.0, 161.8, 159.8, 158.1, 136.5, 131.9, 122.4, 116.6, 107.6, 103.5, 100.6, 99.9, 95.6, 73.6, 73.2, 72.2, 72.1, 72.0, 71.9, 71.7, 71.3, 18.0, 17.6. FABMS: (m/e) 577 $[M-H]^+$. (a) Urgaonkar, S.; Shaw, J. T. *J. Org. Chem.* **2007**, *72*, 4582. (b) Tzeng, Y.-M.; Chen, K.; Rao, Y. K.; Lee, M.-J. *Eur. J. Pharmacol.* **2009**, *607*, 27.
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12. *Compound* **3**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.77 (d, 2H, J = 8.4 Hz), 6.94 (d, 2H, J = 8.4 Hz), 6.38 (s, 1H), 6.20 (s, 1H), 5.38 (s, 1H), 4.22 (s, 1H), 3.75 (m, 1H), 3.39–3.31 (m, 2H), 0.94 (d, 3H, d, J = 6.5 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 179.6, 165.9, 163.2, 161.6, 159.3, 158.7, 136.2, 131.9, 122.7, 116.5, 106.0, 103.5, 99.8, 94.8, 73.2, 72.2, 72.0, 71.9, 17.6. FABMS: (m/e) 431 $[M-H]^+$. (a) Xu, G.-H.; Ryoo, I.-J.; Kim, Y.-H.; Choo, S.-J.; Yoo, I.-D. *Arch. Pharm. Res.* **2009**, *32*, 275. (b) Lee, S.-Y.; Min, B.-S.; Kim, J.-H.; Lee, J.; Kim, T.-J.; Kim, C.-S.; Kim, Y.-H.; Lee, H.-K. *Phytother. Res.* **2005**, *19*, 273.
13. *Compound* **4**: ^{1}H NMR (500 MHz, CD_3OD) δ 8.02 (d, 2H, J = 8.4 Hz), 6.82 (d, 2H, J = 8.4 Hz), 6.64 (s, 1H), 6.32 (s, 1H), 5.45 (s, 1H), 3.92 (s, 1H), 3.72 (m, 1H), 3.40–3.50 (m, 1H), 3.35–3.42 (m, 1H), 1.17 (d, 3H, d, J = 6.5 Hz). ^{13}C NMR (125 MHz, CD_3OD) δ 177.5, 163.3.9, 162.3, 160.7, 157.7, 148.7, 137.5, 130.8, 123.5, 116.3, 106.2, 99.98, 99.88, 95.3, 73.6, 72.1, 71.7, 71.2, 18.0. FABMS: (m/e) 431 $[M-H]^+$.