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Biochemical conversion and bioactivity in kenaf extract after irradiation of far infrared

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실험목적 (Objectives)

The purpose of this study was to evaluate the biochemical transformation, isolation of the compounds, and also to monitor the change in biological activity (antityrosinase and antioxidant) in kenaf leaf extract exposed to FIR.

재료 및 방법 (Materials and Methods)

1. Preparation of kenaf extract by expose to FIR
2. Detection and Isolation of compounds using HPLC, LC/MS and NMR
3. Antioxidant activity (DPPH free radical scavenging activity, Lipid peroxidation, Nitric oxide scavenging activity)
4. Antityrosinase activity.
5. Cell line RAW 264.7 macrophase cell culture. 6. Cytotoxicity evaluation by MTT assay.

실험결과 (Results)

The ethanolic extract of keanf (*Hibiscus cannabinus* L) leaf was subjected to far infrared (FIR) irradiation and evaluated it for bioconversion change and monitor its biological (antityrosianse and antioxidant) activity. The main component of the extract was analyzed as kaempferitrin (kaempferol-3,7-0-a-dirhamnoside) in untreated sample. However, in the FIR treated sample, there causes biotrasformation and derhamnosylation products (kaempferol 15.2 mg/g; afzelin 2.4 mg/g and a-rhamnoisorobin, 5.7 mg/g) were detected. In an antioxidant assay, the IC50 values for the inhibition of lipid peroxidation were 409.02μM for kaempferol, 298.00μM for rhamnoisorobin and 311.43μM for kaemferitrin. Similarly, kaemferol and a-rhamnoisorobin exhibited high radical scavenging property with IC_{50} value of 96.74 and 92.51μM respectively. Likewise, the antityrosinase activity was higher (IC50=3500ppm) in FIR treated sample that could be due to the existence of derhamnosylation products. Furthermore, in LPS activated macrophage cells, the kaempferol and a-rhamnoisorobin scavenge nitric oxide production in a dose dependent manner with the IC_{50} value of 15.38 and 37.76 respectively without cytotoxic effect. This study demonstrated that FIR irradiation causes bioconversion in kenaf leaf and produce different functional compounds and also this plant can be used in medicinal, food and cosmetic industry.

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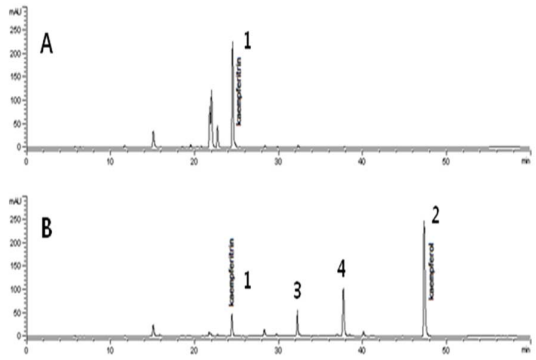

Fig.1. HPLC Chromatogram of untreated (A) and FIR 60°C (1hr)treated (B) Kenaf leaf extract.

1- kaempferitrin 2- kaempferol 3- afzelin 4- α-rhamnoisorobin

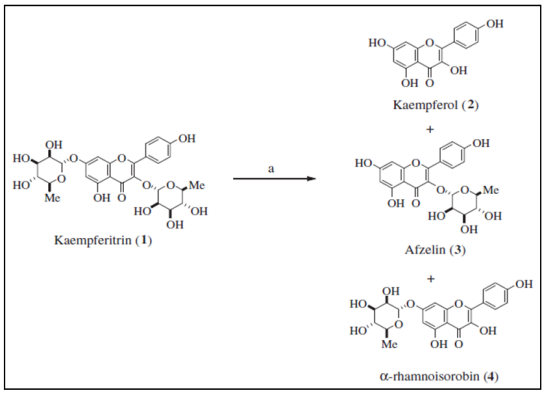

Fig. 2 . Reaction conditions; (a) FIR irradiation (1 hr 60 °C)

Table 1. Contents of compounds 1, 2, 3, and 4 in FIR treated and untreated kenaf leaf extract. The values are expressed in mg/g.

Compounds	Non-FIR (mg/g)	FIR (mg/g)
1. (Kaempferitrin)	29.3	3.1
2. (Kaempferol)	ND	15.2
3. (Afzelin)	ND	2.4
4. (Alfa-rhamnoisorobin)	ND	5.7

Table 3. DPPH free radical and lipid peroxidation inhibitory activities of Kaempferol, Kaempferitrin, Afzelin and a-rhamnoisorobin. Quercetin and BHA was used as a positive control.

Compounds	Inhibitory activity [IC_{50} [a], (μM)]	
	DPPH	Lipid peroxidation
BHA (std)	ND	133.24
Quercetin (std)	97.48	ND
1 (Kaempferol)	96.84	409.017
2 (Kaempferitrin)	>500	311.43
3 (Afzelin)	>500	>500
4 (α-Rhamnoisorobin)	92.51	298.001

Table 4. NO inhibitory activities of kaempferol and its glycosides (kaempferitrin, afzelin and a-rhamnoisorobin). Pentoxifyllin was used as a positive control.

Compounds	Inhibitory activity [IC_{50} [a], (μM)]	
	NO	Cytotoxicity
1 (Kaempferol)	15.38 ± 0.25	> 100
2 (Kaempferitrin)	>100	> 100
3 (Afzelin)	>100	> 100
4 (a-Rhamnoisorobin)	37.76 ± 2.06	> 100
Pentoxifyllin	446 ± 0.00	> 1000

Table 2. Anti-tyrosinase activities of untreated (control) kenaf extract and FIR treated kenaf extract. The samples were used in different concentration. Arbutin was used as a positive control for comparison

Compounds	Concentration (ppm)	*Inhibition of Tyrosinase Activity (%)*	IC_{50} [a]
Kenaf extract	100	3.9 (± 2.9)	>10,000 ppm
	1,000	10.2 (± 4.6)	
	5,000	17.7 (± 1.4)	
	10,000	30.1 (± 3.8)	
FIR treated Kenaf extract	100	16.2 (± 3.9)	3,500 ppm
	1,000	36.9 (± 4.2)	
	5,000	58.8 (± 0.9)	
	10,000	68.9 (± 3.5)	
Arbutin	2,300	50.0 (± 2.1)	2,300 ppm