Studies on the Skin Defense Mechanism of Kaki Calyx Fractionated with n-BuOH

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Abstract

This study was conducted to evaluate the inhibitory effects of Kaki Calyx extracted with 70% acetone(KCA) on the production of nitric oxide(NO) and pro-inflammatory cytokines in LPS-activated RAW 264.7 cells. KCA was fractionated with $CHCl_3$(KCC), EtOAc(KCE), n-BuOH(KCB), H_2O(KCH) again. The experiments were carried out by using the 100 μg/mL not affecting the cell viability. Inhibitory Effect on NO production of KCB was the higher (45% at 100 μg/mL) than KCC (7% at 100 μg/mL), KCE (29% at 100 μg/mL) and KCH (27% at 100 μg/mL) fractions. Expressions of iNOS and COX-2 were suppressed by treatment KCB, and inhibited the production of inflammatory cytokines such as IL-1β and IL-6. These results indicate that the n-BuOH extract of Kaki Calyx warrant further development as an anti-inflammatory agent in cosmeceutical.

Materials & Methods

1. Cell viability
 : measured by Thiazolyl Blue Tetrazolium Bromide (MTT)[1)] method.
2. Nitric Oxide Production
3. ELISA Kit
 : PGE2, IL-1β, IL-6, IL-8
4. Western Blot
 : iNOS, COX-2, GAPDH
5. RT-PCR (Reverse Transcriptase PCR)
 : iNOS, COX-2, GAPDH

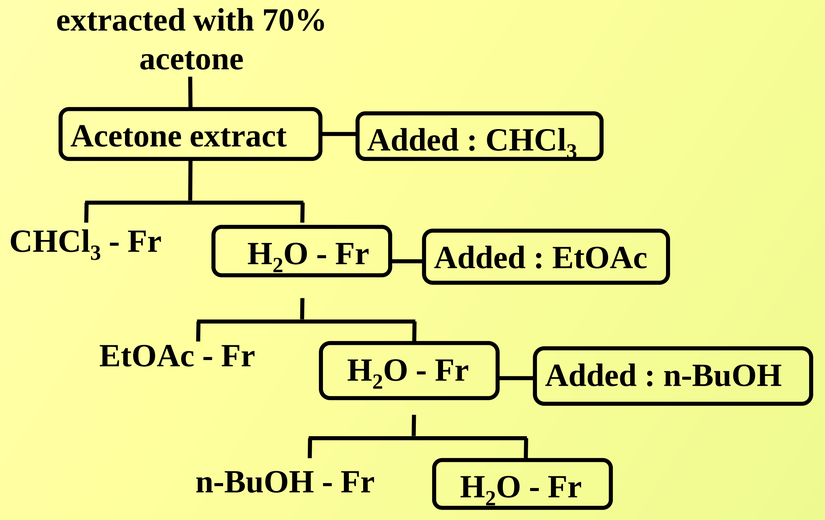


Fig. 1. The procedure of solvent fractions from Kaki Calyx extracts.

Results

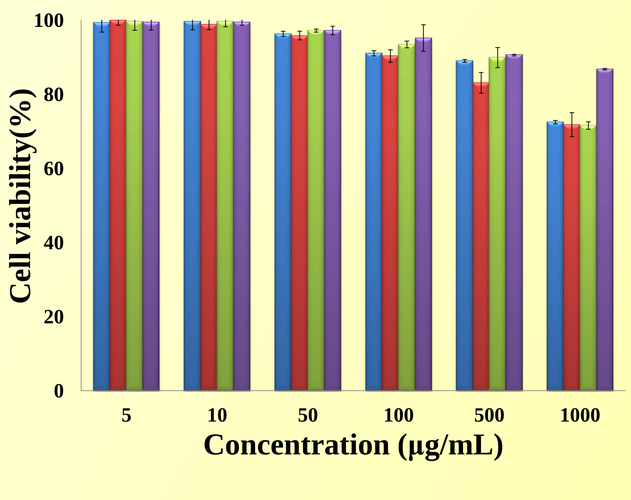


Fig. 2. Cell viability of solvent fraction from Kaki Calyx extract on macrophage cell(RAW 264.7).

- KCC : LPS + $CHCl_3$ layer of Kaki Calyx extracted with acetone.
- KCE : LPS + EtOAc layer of Kaki Calyx extracted with acetone.
- KCB : LPS + n-BuOH layer of Kaki Calyx extracted with acetone.
- KCH : LPS + H_2O layer of Kaki Calyx extracted with acetone.

Results are ± means S.D. of triplicate date.

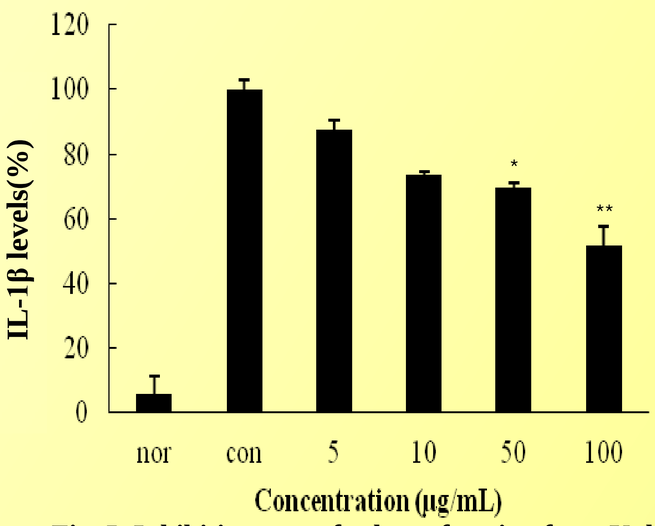


Fig. 5. Inhibition rate of solvent fraction from Kaki Calyx extracts on IL-1β. RAW 264.7 cells were incubated with various concentrations (5, 10, 50 and 100 μg/mL) of solvent fractions from Kaki Calyx extracts for 1h and then treated with 1 μg/mL of LPS for 24h. Data are represented as means ±SEM. (significant as compared to control *p<0.05,**p<0.01)

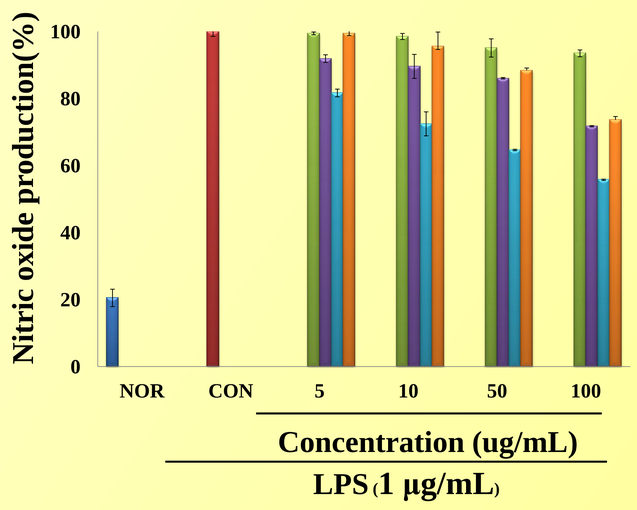


Fig. 3. The Inhibition rate of solvent fractions from Kaki Calyx extracts on nitro oxide.

- NOR : Control
- CON : Lipopolysaccharide (LPS)
- KCC : LPS + $CHCl_3$ layer of Kaki Calyx extracted with acetone.
- KCE : LPS + EtOAc layer of Kaki Calyx extracted with acetone.
- KCB : LPS + n-BuOH layer of Kaki Calyx extracted with acetone.
- KCH : LPS + H_2O layer of Kaki Calyx extracted with acetone.

Results are ± means S.D. of triplicate date.

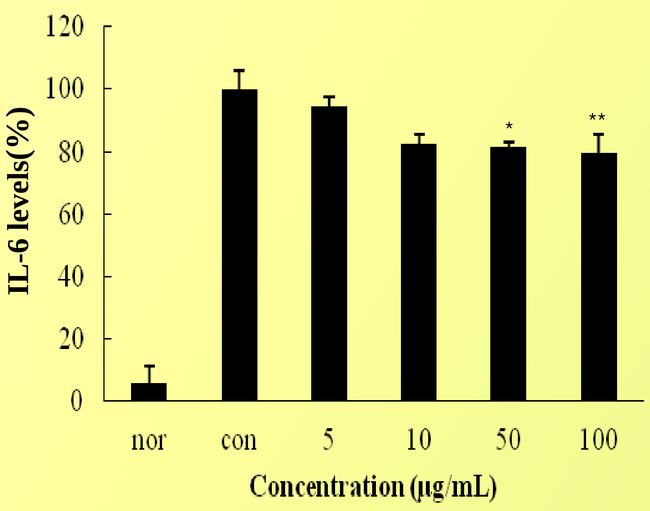


Fig. 6. Inhibition rate of solvent fraction from Kaki Calyx extracts on IL-6. RAW 264.7 cells were incubated with various concentrations (5, 10, 50 and 100 μg/mL) of solvent fractions from Kaki Calyx extracts for 1h and then treated with 1 μg/mL of LPS for 24h. Data are represented as means ±SEM. (significant as compared to control *p<0.05,**p<0.01)

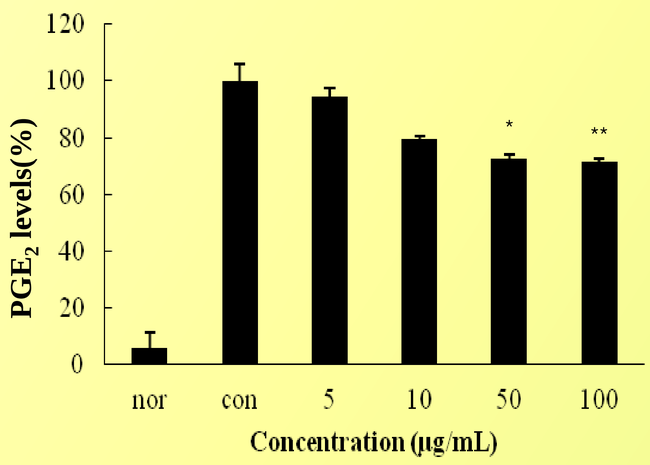


Fig. 4. Inhibition rate of solvent fraction from Kaki Calyx extracts on PGE_2. RAW 264.7 cells were incubated with various concentrations (5, 10, 50 and 100 μg/mL) of solvent fractions from Kaki Calyx extracts for 1h and then treated with 1 μg/mL of LPS for 24h. Data are represented as means ±SEM. (significant as compared to control *p<0.05,**p<0.01)

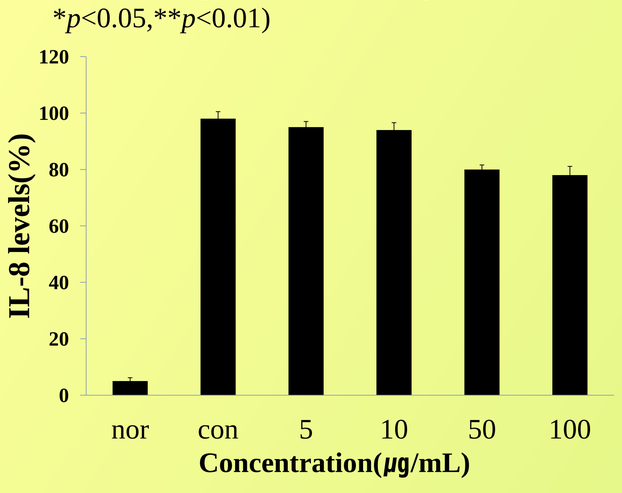
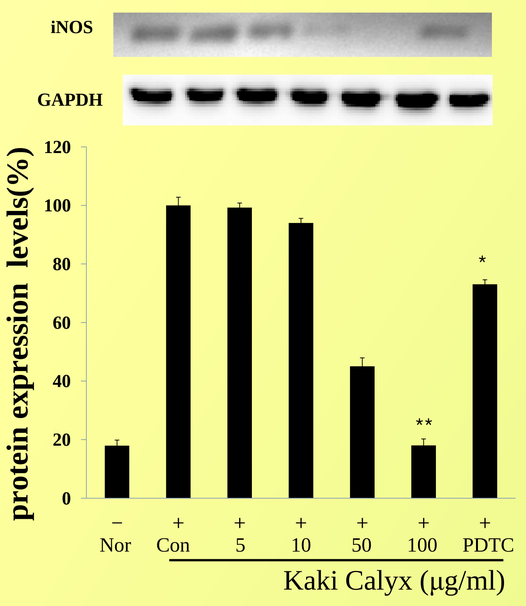


Fig. 7. Inhibition rate of solvent fraction from Kaki Calyx extracts on IL-8. RAW 264.7 cells were incubated with various concentrations (5, 10, 50 and 100 μg/mL) of solvent fractions from Kaki Calyx extracts for 1h and then treated with 1 μg/mL of LPS for 24h. Data are represented as means ±SEM. (significant as compared to control *p<0.05 ,**p<0.01)



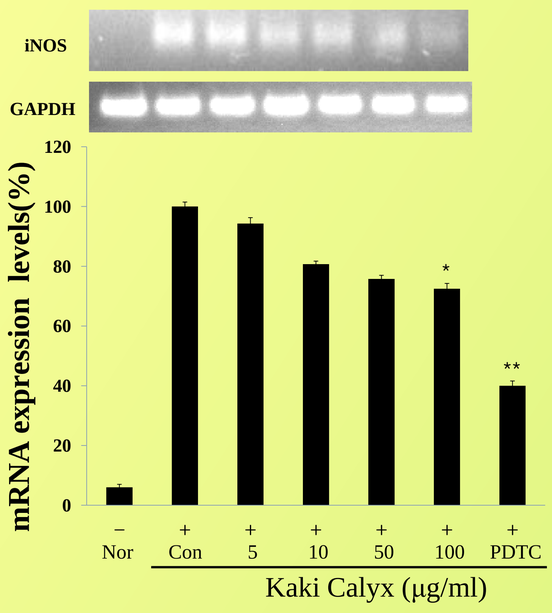
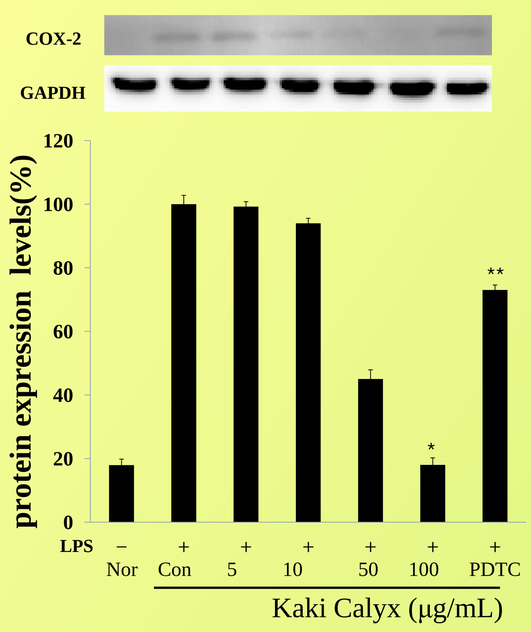


Fig. 8. Effects of Kaki Calyx on the iNOS protein and mRNA expression in RAW264.7 cells. RAW 264.7 were treated Kaki Calyx n-BuOH layer for 24hrs. Histogram show the densitometric of iNOS protein normalized to GAPDH. Data are represented as means ±SEM. (significant as compared to control *p<0.05,**p<0.01)



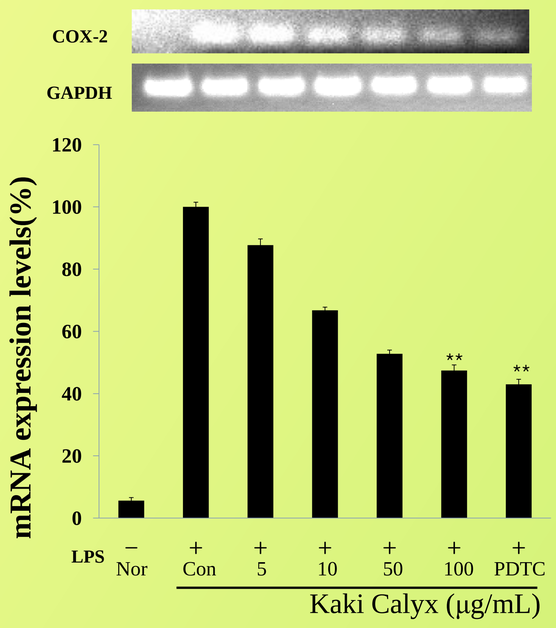


Fig. 8. Effects of Kaki Calyx on the COX-2 protein and mRNA expression in RAW 264.7 cells. RAW 264.7 were treated Kaki Calyx. n-BuOH layer for 24hrs. Histogram show the densitometric of COX-2 protein normalized to GAPDH. Data are represented as means ±SEM. (significant as compared to control *p<0.05,**p<0.01)

Conclusion

1. To the concentration of cell viability 100 μg/mL KCC, KCE, KCB, KCH layer was stable, NO generation rate significantly lower in the KCB layer was used in the experiment.
2. Dependent on the concentration of PGE_2, IL-1β, IL-6 decreased.
3. Treatment with Kaki Calyx suppressed both iNOS and COX-2 protein expression.
4. iNOS and COX-2 mRNA expression were significantly and concentration dependently decreased when co-treatment with Kaki Calyx.

Reference

1) Carmichael J, Dgraff WG, Gazdar AF, Minna JD and Mitchell JB (1987) Evaluation of a tetrazolium based semiautomated colorimetric assay : assessment of chemosensitivity testing. *Cancer Res*. 47(4), 936-942.