Verification Efficacy for Solvent Fraction from n-Buthyl Alchol Extract of Kaki Calyx as a Cosmeceutical Material

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Abstract

This study investigated about the whitening activity of the solvent fraction from 70% acetone extract of Kaki Calyx. Fraction layer using four solvents which are chloroform (KCC), ethyl acetate (KCE), n-buthyl alchol (KCB) and water (KCH) were separated. The cell viability of KCB fraction identified each over 90% from 5 μg/mL to 100 μg/mL concentration. The cell tyrosinase activity of KCB fraction was reduced from 95% to 84% as the concentration increase. The melanin contents of KCE, KCB, KCH fractions demonstrated less than 50% at a concentration of 100 μg/mL. Inhibition cAMP levels of KCB fraction was significantly reduced from 50 μg/mL concentration. Western Blotting and PCR experiments of whitening effect reduced in a dose-dependently. The immunofluor-escence were identified as to be effective in whitening.

Materials & Methods

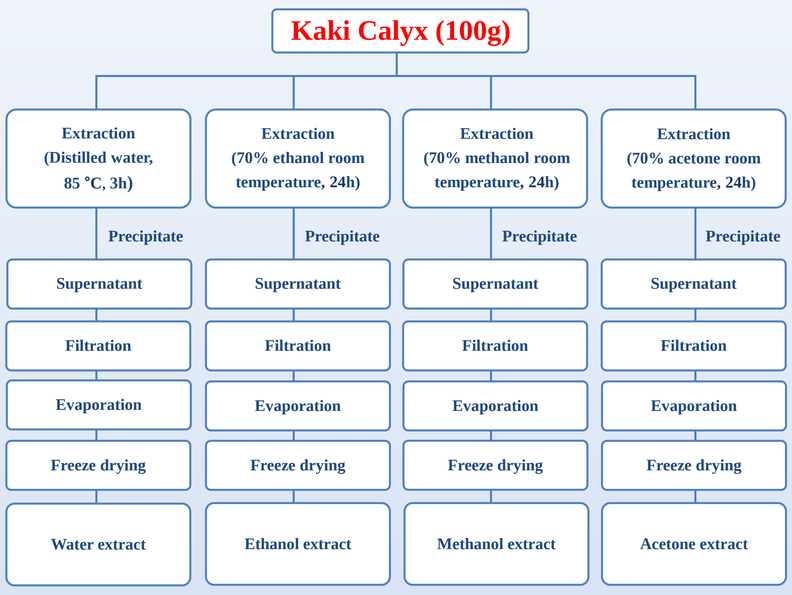

Fig. 1. The procedure for extraction from Kaki Calyx.

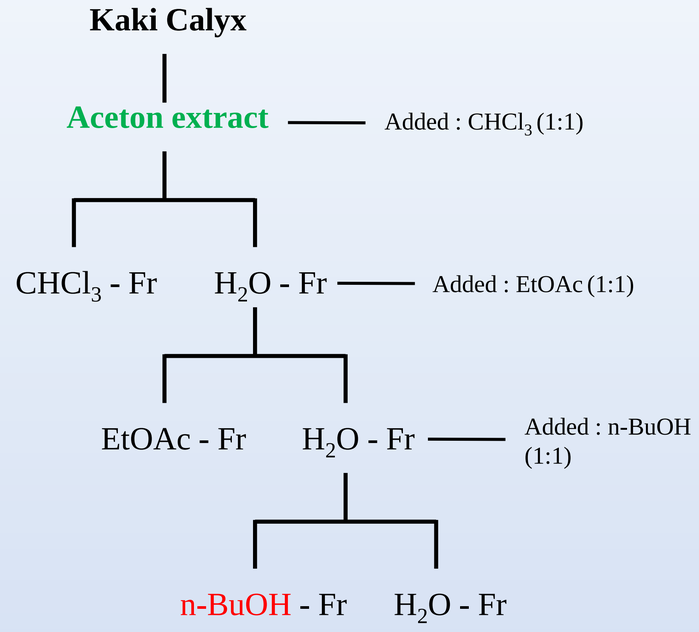

Fig. 2. The procedure for fraction from Kaki Calyx extracted with 70% acetone.

Methods

1. Cell viability
: measured by Carmichael[1)]

2. Protein expression rate
: measured by Western blot

3. mRNA expression rate
: measured by RT-PCR

4. cAMP levels
: measured by cAMP immunoassay kit (Cayman, Ann Arbor, MI, USA)

Results

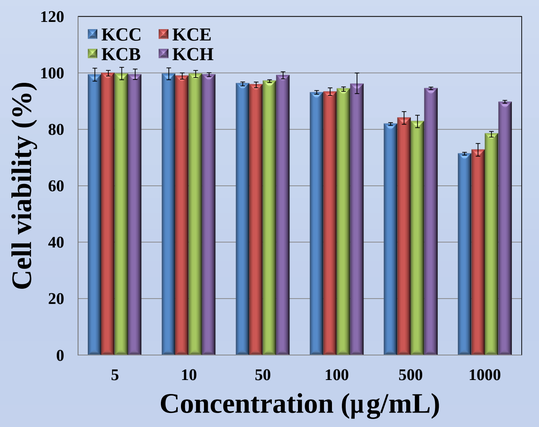

Fig. 3. Cell viability of solvent fraction from Kaki Calyx extracts on melanoma cell (B16F10).

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

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

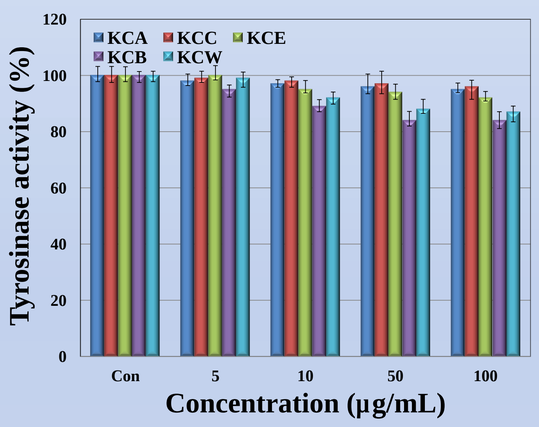

Fig. 4. Inhibition rate of solvent fraction from Kaki Calyx extracts on melanoma cell (B16F10) originated tyrosinase.

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

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

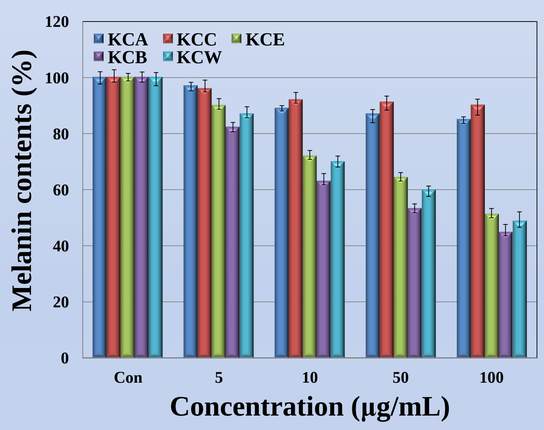

Fig. 5. Inhibition melanin synthesis of solvent fraction from Kaki Calyx extracts on melanoma cell (B16F10).

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

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

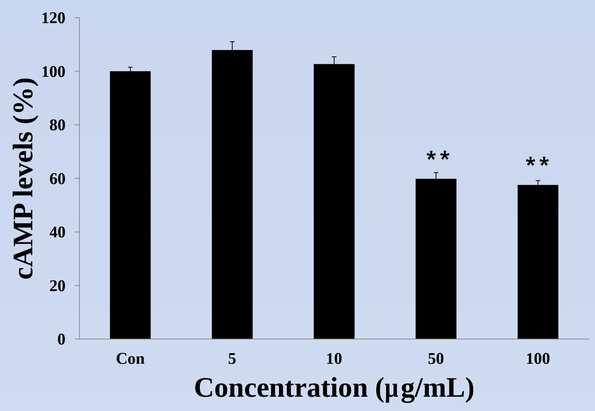

Fig. 6. Inhibition cAMP levels of solvent fraction from Kaki Calyx extracts on melanoma cell (B16F10).

KCB : n-BuOH extracted from Kaki Calyx, After B16F10 cells ($1X10^6$ cells) were started in serum free medium for 1 h the cells were treated with 5, 10, 50 and 100 μg/mL of n-BuOH extracted of Kaki Calyx for 48h. One-way ANOVA was used for comparisons of multiple group means followed by t-test(significant as compared to control. **p*<0.05, ***p*<0.01).

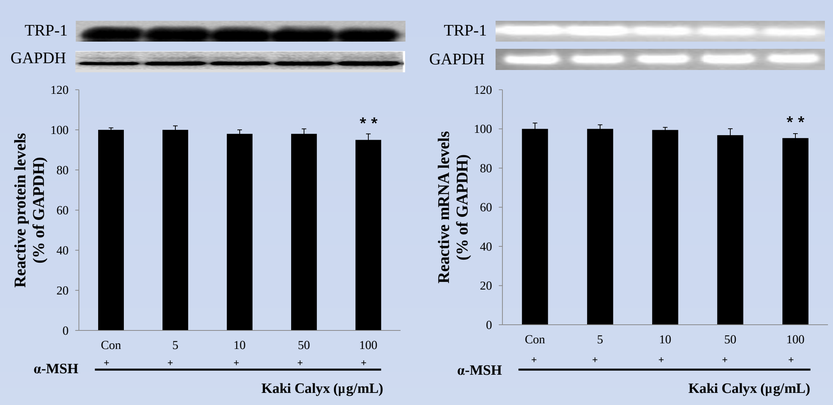

Fig. 7. TRP-1 protein and mRNA expression rate of solvent fraction from Kaki Calyx extracts on melanoma cell (B16F10).

B16F10 were treated Kaki Calyx n-BuOH layer for 24hrs. Histogram show the densitometric of TRP-1 protein normalized to GAPDH. One-way ANOVA was used for comparisons of multiple group means followed by t-test (significant as compared to control. **p*<0.05, ***p*<0.01).

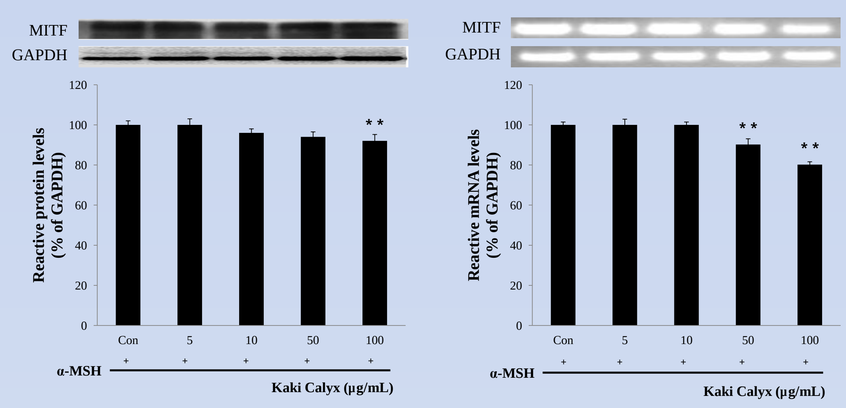

Fig. 8. MITF protein and mRNA expression rate of solvent fraction from Kaki Calyx extracts on melanoma cell (B16F10).

B16F10 were treated Kaki Calyx n-BuOH layer for 24hrs. Histogram show the densitometric of MITF protein normalized to GAPDH. One-way ANOVA was used for comparisons of multiple group means followed by t-test (significant as compared to control. **p*<0.05, ***p*<0.01).

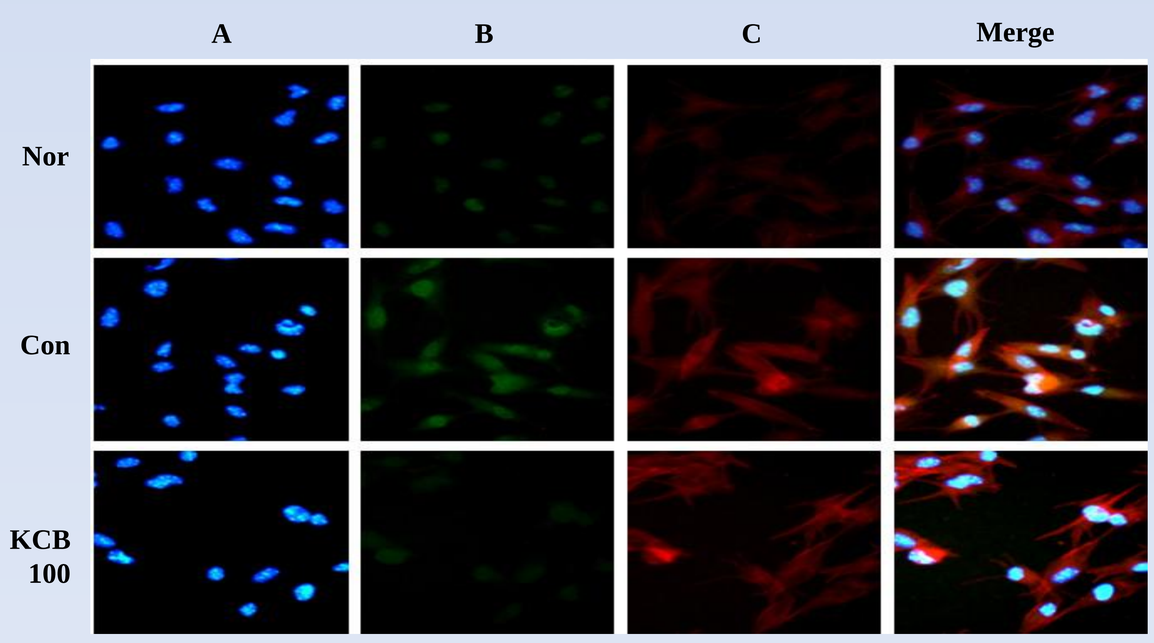

Fig. 9. Indirect immunofluorescence and confocal microscopy analysis demonstrating the effect of n-BuOH extracted from Kaki Calyx on α-MSH-induced sub cellular localization of MITF in B16F10 cells.

A: blue channel (DAPI), B: green channel (FITC), C:red channel and the merge. Results are representatives of three independent experiments.

Conclusion

1. **Whitening activity were evaluated in inhibition of intracellular tyrosinase activity, inhibition of melanin synthesis, inhibition cAMP levels, western blotting, PCR and immunofluorescence. Overall, the whitening activities of n-BuOH fraction showed a higher than those of $CHCl_3$, EtOAc, H_2O fractions.**
2. **Cell tyrosinase activity inhibition rate was reduced to a dose-dependent manner by KCB. Then, KCB fraction showed about 84% at 100 μg/ml.**
3. **Inhibition melanin synthesis of solvent fraction was reduced in all sectors. Melanin contents of KCE, KCB and KCH fractions showed inhibition rate in each of 51%, 44% and 49% at 100 μg/mL.**
4. **Inhibition cAMP levels of KCB fraction investigated about 60% at 50 μg/mL.**
5. **mRNA expression rate of MITF was about 80% at 100 μg/mL.**

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.**