

Anti-Phototoxicity of Phenolic Compounds from *Entada phaseoloides* Leaves in Human Epidermal Keratinocytes

ミットラパブ, ヤニサ

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Name : MITTRAPHAB YANISA

Title : Anti-Phototoxicity of Phenolic Compounds from *Entada phaseoloides* Leaves in Human Epidermal Keratinocytes
(ヒト表皮ケラチノサイトにおける *Entada phaseoloides* の葉からのフェノール化合物の抗光毒性)

Category : Kou

Thesis Summary

Entada phaseoloides (Family: Fabaceae) is a huge evergreen liana that grows high in the tropical forests and is native in Africa, Asia, and Australia's lowland coastal forests. In Southeast Asia and tropical regions, *E. phaseoloides* is a well-known traditional medicinal plant and the components and biological activities (such as anti-inflammatory activity and antioxidant) of the seeds and stems have been investigated in many studies. In 1989, the first report, *E. phaseoloides* leaves yielded entadamide C, a sulphur-containing amide that was isolated and described. In other studies, the isolated compounds from *E. phaseoloides* seeds and stems such as quercetin, luteolin, apigenin, and dihydrokaempferol have been reported. However, few data are available on compounds isolated from *E. phaseoloides* leaves.

The human skin is the body's most exposed organ, and it protects the body from a range of environmental afflictions. The sun's ultraviolet (UV) radiation is a form of high-energy electromagnetic radiation that is considered phototoxic to all organisms. UV-B (280-315 nm) is a large part of the solar UV. While it is ineffective in penetrating into the deep skin layer, it particularly influences the epidermis (the skin's superficial layer which mostly comprises of keratinocytes). UV-B irradiation has been linked to skin inflammation due to oxidative stress caused by reactive oxygen species (ROS). The oxidative processes induced by ROS results in lipid peroxidation, DNA mutations and damage of membrane protein that play a key role in skin aging. Over the past years, the development of natural products and flavonoids have drawn attention as protective agents for preventing against UVB-induced skin damage *via* scavenging ROS. Thus, this study focused on isolation and identification of active compounds from acetone extract of *E. phaseoloides* leaves (AE) and investigation of their anti-phototoxicities in keratinocytes (HaCaT cells).

In preliminary screening, the several biological activities of *E. phaseoloides* extracts (70% EtOH, EtOH, 70% MeOH, MeOH, acetone, and ethyl acetate, respectively) on HaCaT cell line have been investigated (*e.g.*, circadian rhythm, cytotoxicity). The results showed that acetone extract significantly increased the expression of clock genes, *Per1* in HaCaT cells after addition of acetone extract at 12 h, while *Bmal1* was weakly expressed. The other extracts were less active on the expression of *Per1* and *Bmal1* genes.

The antioxidant and protective effect of AE on UV-B irradiated HaCaT cells was investigated. AE showed antioxidant activity in DPPH and ABTS assay with IC₅₀ values of 125.4±35.7 and 704.6±56.0 µg/mL, respectively. AE at a concentration of 40 µg/mL increased cell viability of the UV-B damaged cells. Thus, AE was selected for further isolation and identification of the active compounds.

The characteristic profile of AE was investigated with LC-DAD-MS. Eleven compounds were identified or

proposed in AE by based on their retention times, UV, and the accurate mass values, as well as comparisons with the reference standard compounds. Among of these compounds, gallic acid (**1**), protocatechuic acid (**2**), 4-hydroxybenzoic acid (**3**), (-)-epicatechin (**4**), luteolin-7-*O*- β -D-glucoside (**5**), luteolin (**7**), quercetin (**8**), (+)-dihydrokaempferol (**9**), apigenin (**10**) and kaempferol (**11**) were confirmed by comparison with authentic standards. On the other hand, the AE was also separated by various chromatographic techniques such as open column chromatography, MPLC and preparative TLC, to obtain six compounds. The chemical structure of isolated compounds, protocatechuic acid (**2**), 4-hydroxybenzoic acid (**3**), luteolin-7-*O*- β -D-glucoside (**5**), cirsimaritin (**6**), (+)-dihydrokaempferol (**9**), and apigenin (**10**) were confirmed by NMR and MS data.

The cytotoxicity of isolated/standard compounds on normal HaCaT cells were evaluated by using MTT assay. The results showed that compounds **7** and **10** (100 μ M) have toxicity to HaCaT cells while all the other compounds showed no toxicity. The phototoxicity of UV-B irradiation was evaluated with 30 mJ/cm². The protective effect against UV-B irradiation of isolated/standard compounds were determined at different concentrations of 25, 50, and 100 μ M. The cell viability pre-treated with isolated/standard compounds were increased when compared with UV-B irradiation group, which indicated that pre-treatment with compounds **2**, **4**, and **11** protect HaCaT cells from UV-B irradiation damage. The effect of compounds **2**, **4**, and **11** on keratinocyte migration was assessed using an *in vitro* HaCaT cell scratch assay. After wounding and UV-B irradiation, at 0 and 24 h the wound area surrounded by the edges of the wound monolayer was measured. The findings revealed that compounds **2**, and **4** helped to reverse the effects of UV-B on wound healing compared with non-treated group. UV-B irradiation increased the expression of *COX-2* and *iNOS* markers in HaCaT cells in the current investigation. *COX-2* and *iNOS* expression were investigated using RT-qPCR. Meanwhile, pre-treatment by 100 μ M of compounds **2**, **4**, and **11** reduce expression of *COX-2* and *iNOS* in HaCaT cells. UV-B irradiation increased *iNOS* expression and generation of its reactive product in HaCaT cells, which were successfully protected by pre-treatment with compounds **2**, **4**, and **11** at 100 μ M.

In conclusion, the anti-phototoxicity effect of isolated/standard compounds from AE on HaCaT cells were evaluated using MTT assay. Protocatechuic acid (**2**), (-)-epicatechin (**4**), and kaempferol (**11**) from AE showed that the inhibition of UV-B induced inflammation, which includes the improved expression of *COX-2*, and *iNOS* gene. Moreover, protocatechuic acid (**2**), and (-)-epicatechin (**4**) could potentially enhanced cell migration during wound closure. The results suggest that acetone extract of *E. phaseoloides* leaves and their active compounds, protocatechuic acid (**2**), (-)-epicatechin (**4**), and kaempferol (**11**) are potential agents for treating photoaging.