Study of Some Medicinal Plants on Dermal Papilla Cells

Vanuchawan Wisuitiprot¹, Kornkanok Ingkaninan², Wudthichai Wisuitiprot³, Neti Waranuch¹

¹ Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Thailand, 65000
² Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Thailand, 65000
³ Department of Thai traditional medicine, Sirindhorn College of Public Health, Thailand, 65130

Corresponding author. E-mail: Netiw@nu.ac.th

Abstract

Alopecia is a serious disturbing skin disorder in people having baldness. Miniaturization of hair follicles was evidenced on balding scalp. Topical minoxidil and oral finasteride are approved medications for the treatment of hair loss. However, these synthetic substances sometime come with undesirable adverse effects such as itching or skin rash, loss of erectile function and libido, etc. Therefore, a lot of medicinal plants have been investigated for anti-hair loss activity. Most of them were usually from traditionally use. The purpose of this study was to determine the potential effect on anti-hair loss activity and the possible mechanisms involved in hair loss treatment of ten selected Thai plants namely, Acanthus ebracteatus, Acacia concinna, Bridelia ovata, Cleome viscosa, Cocos nucifera, Hibiscus subdariffa, Oryza sativa, Terminalia chebula and Tinospora crispa. Toxicity and an effect on dermal papilla cell cycle were also investigated. These plants have been traditionally used for the treatment of hair loss. The plants were dried, and extracted by maceration with ethyl alcohol. These extracts were further studied for...
the effect on human dermal papilla cell proliferation and aggregation. Cell toxicity of each extract was also measured. Muse cell analyzer was used for determining the effect of plant extract on dermal papilla cell cycle. The results of this study revealed that most of plant extract did not present cytotoxic effect, except Acacia concinna, Bridelia ovata and Cleome viscosa. Interestingly, both Acanthus ebracteatus and Cocos nucifera obviously induced proliferation of dermal papilla via MTT assay. However, only Acanthus ebracteatus increased S and G2/M stages of cell cycle. On the contrary, Cocos nucifera extract did not increase the number of cells in every stage of cell cycle, moreover cells damaged was evident. Therefore, the ethanolic extract of Acanthus ebracteatus is the promising candidate for hair loss prevention and anti–hair loss treatment.

**Keywords:** Androgenic alopecia, Acanthus ebracteatus, Dermal papilla, Cell cycle

**Introduction**

Hair loss is the one of symptoms influencing human everyday life. It impairs human confidence. Actually, hair loss in each day about 70–100 hairs particularly during shampooing and combing [1]. If hair falls out more than grows, hairstyle becomes thinner and even baldness may occur. Androgen hormone particularly testosterone plays the crucial role in men baldness or androgenic alopecia. Three percent of testosterone distributing in blood circulation permeated through dermal papilla cell. 5α-reductase in cell cytosol metabolized testosterone to dihydrotestosterone (DHT) [2]. DHT bind with androgenic receptors locating on nuclease membrane. DHT-androgenic receptor complex is established and permeated into nuclease. The complex compounds bind with DNA strains and induce mRNA expression that influences the production of hair follicular protein damage [2, 3]. Finally, dermal papilla cell apoptosis and miniaturization are evident [4].

Since androgenic alopecia involves testosterone metabolism a long with dermal papilla apoptosis induction, a lot of studies attempted to develop the treatment for androgenic alopecia. Finasteride and dutasteride, the medications have been involved the metabolism of testosterone such as inhibition of 5α-reductase activity. They can slow the progression of hair loss by reversing miniaturization process of dermal papilla. Because of testosterone inhibition, adverse effect of sexual dysfunctions are reported. The important side effects reported erectile dysfunction, decrease of libido and impairment of ejaculation [3, 5]. In addition to enzyme activity inhibition, the enhancement of hair re–growth also is concerned. Minoxidil is well–known as anti–hypertensive drug possessing vasodilatation activity. It enhanced hair re–growth by increasing local blood supply. Moreover, several reports also revealed that minoxidil increase proliferation of dermal papilla cells [6]. Although minoxidil for anti–hair loss is topical use, adverse effect also has been reported particularly skin irritation and inflammation. Systematically adverse effect of minoxidil also was reported rarely [7].

Due to adverse effects of anti–hair loss medications, a lot of researchers attempted to find the new sources possessing anti–hair loss activity with less of adverse effects. Medicinal plant is the promising sources that has been reported about anti–hair loss activity. Hence, this experiment focuses on Thai
medicinal plants that have been reported about hair nourishment. Those plants were screened their benefit on dermal papilla cells by using MTT assay and cell cycle analysis.

**Methodology**

**Plant extraction**

Traditional plants, as shown in Table 1, were purchased from Thai traditional pharmacy drug store in Phitsanulok, Thailand. All of plants was macerated with absolute ethanol for 3 days. Extract solutions were filtered by Whatman No1 and the filtrates then were evaporated for removing organic solvent by using rotary evaporator. The crude extracts were kept in the cool place and protected from light.

**Dermal papilla cell culture**

The dermal papilla cells were purchased from Biomed Diagnostic Thailand. They were thawed from cryotube and re–suspended in Follicle Dermal Papilla Cell Growth Medium (Promocell, USA). The cells were incubated under cell culture condition; 95% RH, 5% CO₂ and 37 °C. Cells were sub–cultured when they grow reach near 80% confluence.

**MTT assay for cytotoxic and cell proliferation testing**

Ten thousand of dermal papilla cells was seeded into each well of 96 wells plate. The cells were incubated for 24 hours and then treated with plant extract solutions with various concentrations. The incubation time of treatment was 24 hours. Five microgram per milliliter of MTT solution was added into each well and incubated for 3 hours. Formazan crystal was dissolved with 100 µl of DMSO. Absorbance was determined at 595 nm. Cell viability was calculated by comparing with control. Plant extracts inducing high cell proliferation were carried out with cell cycle analysis performed by using Muse® cell cycle analyzer (Merck, Thailand)

**Cell cycle analysis**

One hundred thousand of dermal papilla cells was seeded into each well of 6 well plates. The cells were incubated for 24 hours and then treated with plant extract solution with specified concentrations that induced cell proliferation. All cells were collected by trypsinizing and fixed by using 70% of cold ethanol. The fixed cell was kept in −20 °C for 4 hours. The fixed cells were stained with Muse cell cycle reagent and incubated at room temperature for 30 minutes. Cell cycle will be analyzed by using Muse® cell cycle analyzer.
Table 1 Medicinal plants used in this experiment and their usage.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Family</th>
<th>Part of use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia concina</td>
<td>Fabaceae</td>
<td>Pod</td>
</tr>
<tr>
<td>Acanthus ebracteatus</td>
<td>Acanthaceae</td>
<td>Leaf</td>
</tr>
<tr>
<td>Bridelia ovata</td>
<td>Phyllanthaceae</td>
<td>Leaf</td>
</tr>
<tr>
<td>Cleome viscosa</td>
<td>Cleomaceae</td>
<td>Leaf</td>
</tr>
<tr>
<td>Cocos nucifera (meat)</td>
<td>Arecaceae</td>
<td>Meat</td>
</tr>
<tr>
<td>Cocos nucifera (peel)</td>
<td>Arecaceae</td>
<td>Peel</td>
</tr>
<tr>
<td>Hibiscus subdariffa</td>
<td>Malvaceae</td>
<td>Fruit</td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>Poaceae</td>
<td>Seed</td>
</tr>
<tr>
<td>Terminalia chebula</td>
<td>Combretaceae</td>
<td>Fruit</td>
</tr>
<tr>
<td>Tinospora crispa</td>
<td>Menispermaceae</td>
<td>Vine</td>
</tr>
</tbody>
</table>

Result

Dermal papilla cells were grown at 80–90% confluence within 7 days of culture. The cells were sub-cultured for further study with passage less than 10. The cells presented physical appearance as like as fibroblast cells.

Cytotoxicity and cell proliferation testing

This study determined cytotoxicity of plant extract by using MTT assay. The results could be divided into 3 categories. The first was plant extracts presenting cytotoxic effect. They are Acacia concina, Bridelia ovata and Cleome viscosa (Figure 1). Those extracts decreased cell viability when comparing to untreated cells.

![Figure 1](image)

**Figure 1** Cytotoxic effect of *Acacia concina*, *Bridelia ovata* and *Cleome viscosa* on dermal papilla cells
The second was plant extracts that were present non-cytotoxicity on dermal papilla cells. They composed of 5 extracts that were as follows; *Cocos nucifera* (meat), *Hibiscus sabdariffa*, *Oryza sativa*, *Terminalia chebula* and *Tinospora crispa* (Figure 2).

![Figure 2](image_url) Cytotoxic effect of *Cocos nucifera* (meat), *Hibiscus sabdariffa*, *Oryza sativa*, *Terminalia chebula* and *Tinospora crispa* on dermal papilla cells.

The last group was plant extracts presenting cell proliferation inducer activity. They were *Acanthus ebracteatus* and *Cocos nucifera* (peel). Dermal papilla cells were induced to be more proliferated with concentration about 62.5–500 µg/ml (Figure 3).

![Figure 3](image_url) Cytotoxic effect of *Acanthus ebracteatus* and *Cocos nucifera* (peel) on dermal papilla cells.

Since androgenic alopecia cause from dermal papilla cell to be damaged and apoptosis. The decrease of dermal papilla cell number initiated hair follicle miniaturization. Therefore, plant extract that can induce dermal papilla cell proliferation is the promising source for anti-hair loss products. Although *Cocos nucifera* peel extract could induce cell proliferation, the cell damage was also evident (Figure 4).
Figure 4 Dermal papilla cell morphology comparing among control cell (a) and treated cell with 250 µg/ml of Acanthus ebracteatus (b) and Cocos nucifera (peel) (c) extracts.

Cell cycle analysis

According to cell morphology, Acanthus ebracteatus extract presented potential extract for preventing dermal papilla. Acanthus ebracteatus was further studied about the effect on cell cycle. Ten concentrations of Acanthus ebracteatus revealed the different effects on dermal papilla cell cycle; it was presented in Table 2. The result indicated that A. ebracteatus extract obviously affected dermal papilla cell cycle. G0/G1 stage was decreased significantly when dermal papilla cells were treated with A. ebracteatus extract at 250 – 500 µg/ml while S and G2/M stages were significantly increased. On the contrary, C. nucifera peel extract did not show any effect on dermal papilla cell cycle. All of stages in cell cycle was not different from control.

Table 2 Cell cycle analysis of dermal papilla after treated with various concentration of Acanthus ebracteatus

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cell cycle stages (% of DNA content)</th>
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<tbody>
<tr>
<td></td>
<td>G0/G1</td>
<td>S</td>
</tr>
<tr>
<td>Control</td>
<td>80.70 ± 2.26</td>
<td>7.85 ± 5.16</td>
</tr>
<tr>
<td>A. ebracteatus extract (250 µg/ml)</td>
<td>29.70 ± 1.70</td>
<td>24.75 ± 5.16</td>
</tr>
<tr>
<td>A. ebracteatus extract (125 µg/ml)</td>
<td>36.35 ± 5.16</td>
<td>20.70 ± 8.06</td>
</tr>
<tr>
<td>C. nucifera peel extract (250 µg/ml)</td>
<td>85.25 ± 11.25</td>
<td>4.11 ± 0.25</td>
</tr>
<tr>
<td>C. nucifera peel extract (125 µg/ml)</td>
<td>80.36 ± 16.58</td>
<td>6.68 ± 2.05</td>
</tr>
</tbody>
</table>

Figure 5 Cell cycle profile of dermal papilla cell comparing between control and cells treated by A. ebracteatus and C. nucifera extracts.
Figure 5 presented cell cycle profiles of dermal papilla. The graphs were generated by Muse cell analyzer software. Each of graph can be divided in to 3 regions. Blue, violet and green colors represent cell cycle in G0/G1, S and G2/M stages respectively. According to Figure 5, a graph of cell cycle of cell treated by 500 µg/ml of A. ebracteatus extract showed different pattern from control cells while cells treated by C. nucifera peel extract did not.

**Discussion**

Dermal papilla cell is the important component in hair follicle. It plays the crucial role in hair re-growth. There were study revealed that minoxidil induced hair re-growth by increasing dermal papilla cell proliferation [6, 7]. Current study determined cytotoxicity of 10 medicinal plant extracts by using MTT assay. Plant extracts presenting non-cytotoxic effect on dermal papilla cells and inducing cell proliferation were also interested. Seven of plant extracts presented non-cytotoxic effect on dermal papilla cells, particularly A. ebracteatus and C. nucifera extracts increased the number of cell after treatment. Cell viability and cell proliferation are also indirectly determined by measuring intensity of formazan crystal that is produced by mitochondria enzymes [8, 9]. However, the effect of plant extracts on both results might be incorrected if that plant extract induced activity of mitochondria enzymes [8]. Cell cycle analysis was the one method that was used for indicating cell proliferation enhancement or cell growth inhibition. Previous studies revealed that the compounds increased cell proliferation by presenting the decrease of G0/G1 and increase of S and G2/M stages [10]. In the other hands, the compounds that presented cell growth inhibition also showed the increase of G0/G1 and decrease of S and G2/M stages in cell cycle [11, 12]. According to cell cycle analysis, A. ebracteatus extract increased cell proliferation by increasing the number of cell in S and G2/M stages. The result implied that dermal papilla cell increased DNA contents and prepared for cell mitosis [13, 14] after treating with A. ebracteatus extract. On the contrary, C. nucifera extract did not present the positive effect on dermal papilla cell. The result of cell cycle analysis after treated by C. nucifera extract was not different from untreated cell. The results were represented via the percent of DNA content in each of cell cycle stages (Table 1) and cell cycle profiles (Figure 5) those were not different from control. The discrepancy results of cell proliferation between MTT assay and cell cycle analysis caused from the increase of cell metabolism. C. nucifera extract might increase metabolism of mitochondria of dermal papilla cells while A. ebracteatus extract might not. In addition to cell cycle analysis results, this study also observed and compared dermal papilla cell morphology that were compared between control and treated cells. Morphology of cell treated by C. nucifera extract was obviously different from control. Figure 4 showed that C. nucifera extract damage and change dermal papilla cell morphology while A. ebracteatus extract did not show any effect on cell
morphology. The damage of cell might lead cell to increase cell metabolism for recovering cell regulation [15]; it caused the increase of mitochondria metabolism in MTT assay.

According to the results in this study, it can be summarized that the ethanolic extract of *Acanthus ebracteatus* is the promising candidate for hair loss prevention and anti–hair loss treatment.

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**References**


