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5 α -reductase inhibition and hair growth promotion of some Thai plants traditionally used for hair treatment

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ABSTRACT

Ethnopharmacological relevance: Many Thai traditional herbs have been used for hundreds of years for hair treatment and nourishment, including hair loss. However, scientific evidence about their mechanisms of action has not yet been elucidated.

Aims of the study: The purpose of this research is to define the possible mechanisms involved in hair loss treatment of the selected plants by determining the 5 α -reductase enzyme inhibition and hair growth promoting activities, and the relationship between these two activities.

Materials and methods: Seventeen Thai plants traditionally used for hair treatment were selected. The plants were dried, ground and extracted by maceration with ethyl alcohol. These extracts were further tested for 5 α -reductase inhibition using enzymes from rat livers. Hair growth promoting activity was tested in C57BL/6 mice.

Results: *Carthamus tinctorius* L. was the most potent 5 α -reductase inhibitor, with a finasteride equivalent 5 α -reductase inhibitory activity (FEA) value of 24.30 ± 1.64 mg finasteride equivalent per 1 g crude extract. *Phyllanthus emblica* L. was the second most potent inhibitor, with FEA of 18.99 ± 0.40 . *Rhinacanthus nasutus* (L.) Kurz. was the least potent 5 α -reductase inhibitor (FEA 10.69 ± 0.96). *Carthamus tinctorius* also was the most potent hair growth promoter in C57BL/6 mice. There were strong relationships between 5 α -reductase inhibitory activity and hair growth promoting activity ($r=0.719$), and between 5 α -reductase inhibitory activity and hair follicle count ($r=0.766$).

Conclusions: Ethanolic extract of *Carthamus tinctorius* was the most potent 5 α -reductase inhibitor and hair growth promoter. This discovery may lead to the development of new alternative medicines for hair loss prevention and treatment.

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1. Introduction

Although hair loss may not be a life-threatening disorder, it has a great impact on a person's self-respect, mental health, and overall quality of life. Within the disorder, androgenic alopecia (androgenetic alopecia or AGA) is the most common type of hair loss, which affects large numbers of both men and women (Sinclair, 2004). AGA can occur as early as the teen years, but usually begins in the later years of life. It affects at least half of all men by the age of 50, and up to 70% of 70-year-old men (Trüeb, 2002). AGA is an androgen-

dependent and genetically acquired disorder, caused by excessive activity of the 5 α -reductase enzyme in hair follicles (Sawaya, 1998). It is usually observed that the hair follicles of AGA patients are smaller than those in normal adults, which is a direct occurrence of the hair miniaturization process caused by overactivity of dihydrotestosterone (Sinclair, 2004).

At present, there are some medicines that are used to treat AGA. For example, 5 α -reductase inhibitors, finasteride and dutasteride, are used to treat androgen-related disorders (Robinson et al., 2003). But these medicines have several undesirable side effects: for example, impotence (erectile dysfunction), abnormal ejaculation, decreased ejaculatory volume, abnormal sexual function, gynecomastia, testicular pain, impairment of muscle growth, and severe myopathy (Lacy et al., 2008). Another medicine for

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treating AGA is a topical minoxidil lotion. Minoxidil was first used as a vasodilator to treat cardiovascular disorders, but the unexpected side effect of hirsutism led to its topical use as a hair-growth stimulator. The mechanisms involved in AGA treatment are still unclear. It seems to open potassium channels and increase the proliferation and differentiation of epithelial cells in the hair shaft. However, local irritation, itching, dryness and erythema may occur when minoxidil is topically used, as well as systemic side effects such as dizziness and tachycardia. Serious side effects, such as an increase in left ventricular end-diastolic volume, cardiac output, and left ventricular mass, have been reported with the use of 2% minoxidil solution. Unfortunately, another potential drawback of minoxidil therapy is the loss of newly grown hair within one to three months after discontinuation of the medicine (Abramowicz, 1998).

The 5 α -reductase enzyme (5 α R, EC 1.3.99.5; Δ^4 -3-oxo-steroid 5 α -oxidoreductase) is a microsomal enzyme that is responsible for the reduction of 3-oxo- Δ^4 steroidal compounds such as testosterone, progesterone and corticosterone. In humans, 5 α R plays a major role in the reduction of testosterone into a more potent androgen, dihydrotestosterone (DHT), which is necessary for normal male growth. However, high expression of DHT causes androgen-related disorders such as acne, hirsutism, androgenic alopecia, benign prostatic hyperplasia (BPH), and prostate cancer (McGuire et al., 1960; Bruchovsky and Wilson, 1968).

Many studies in Europe and the US have indicated that several plants have the potential to inhibit 5 α R: for example, the American dwarf palm (*Serenoa repens* or *Sabal serrutala*, saw palmetto) which is rich in free fatty acids such as oleic, lauric, myristic and linoleic acids, can inhibit 5 α R (Niederprüm et al., 1994). This plant is extracted and developed into a variety of health supplements, and is widely used in both Europe and the USA. The most popular brand is Permixon[®], a standardized saw palmetto extract whose effect has been proven both in vitro, *in vivo* and in human clinical trials (Di Silverio et al., 1998; Paubert-Braquet et al., 1998; Bayne et al., 1999; Raynuad et al., 2002; Habib, 2009). Lingzhi mushroom (*Ganoderma lucidum*) extract is also able to inhibit the 5 α R enzyme (Fujita et al., 2005). Its triterpenoids may be responsible for this action (Liu et al., 2006).

Some other plants with reported 5 α R inhibition activity are *Piper nigrum* (Hirata et al., 2007), *Alpinia officinarum* (Kim et al., 2003), *Lygodium japonicum* (Matsuda et al., 2002), *Pleurotus ostreatus*, and *Lentinula edodes* (shiitake) (Fujita et al., 2005).

Additionally, many reports have indicated that plants or substances with anti-5 α R activity can promote hair growth as well. For example, *Myrica rubra* (red bayberry) bark (Matsuda et al., 2001), *Thuja orientalis* (white cedar) seed (Park et al., 2003), *Piper nigrum* (black pepper) leaf (Hirata et al., 2007), *Boehmeria nipononivea* (Shimizu et al., 2000), and epigallocatechin-3-gallate (EGCG) found in green tea (Kwon et al., 2007) are all able to promote hair growth as well as inhibit the 5 α R enzyme.

In Thailand, several varieties of plants have been used for preventing or treating hair loss, for hair nourishment, and for improving the esthetic properties of hair (Boonyaprapas and Chokchajareonporn, 1996). These plants, the parts used, and their method of uses are shown in Table 1. However, the possible mechanisms involved in their treatment of hair loss have not yet been elucidated.

This work aims to define the possible mechanisms of Thai traditional plants which have been used as herbal remedies or cosmetics to treat or prevent hair loss, to promote hair growth, to nourish hair, or that have been used as ingredients in natural cosmetics. This research will determine the 5 α R inhibitory and hair growth promoting activity of these plants. The relationship between 5 α R inhibition and the hair growth promoting activity of these plants will also be investigated.

2. Materials and methods

2.1. Plant materials and extraction

Traditional plants, as shown in Table 1, were purchased from local markets in Chiang Mai, Thailand. Since the plants were purchased from local market as a fresh form, they were confirmed by comparing with herbarium specimens at Faculty of Pharmacy, Chiang Mai University, to ensure that the plants used in this experiment were correct materials. Ethanol was used as an extraction solvent due to the semipolar property of this solvent, which soluble various phytochemical groups more than the use of polar solvents like water or non-polar solvents. Moreover, using ethanolic extract as an active ingredient in pharmaceutical and cosmetic products provided more safety and compatibility than other organic solvent. To extract the plants, they were separately dried at 45 °C in a hot-air oven. Next, they were ground by using an electric grinder and extracted by maceration with 95% ethyl alcohol. The extracts were then evaporated to dryness under controlled pressure and temperature using a rotary evaporator (Eyela, Tokyo, Japan).

2.2. Animals

Six-week-old male Sprague Dawley (SD) rats and seven-week-old male C56BL/6J mice were obtained from the National Laboratory Animal Center, Bangkok, Thailand, and housed under a 12 h light/dark cycle with free access to food and water. The protocol of this study was approved by the Animal Research Ethics Committee of the Faculty of Pharmacy, Ubon Ratchathani University, Ubon Ratchathani, Thailand.

2.3. Chemicals

Dithiothreitol, sucrose, testosterone, finasteride and NADPH (all of analytical grade) were purchased from Sigma Chemical (St. Louis, MO). Methanol, dichloromethane and ethanol were purchased from Fisher Scientific (Fair Lawn, NJ). Safflower yellow was purchased from Tokyo Chemical Industry (Tokyo, Japan). Other chemical compounds were purchased from Wako Pure Chemical Industry (Osaka, Japan).

2.4. Method for determining the 5 α -reductase inhibitory activity

Rat microsomal suspension was prepared using the method described in our previous paper (Kumar et al., 2011). Briefly, excised SD rat livers were minced with scissors and homogenized in a solution composed of 0.32 M sucrose and 1 mM dithiothreitol in 0.02 M phosphate buffer at pH 6.5. The liver homogenate was further centrifuged twice at 4500 \times g at 0 °C for 30 min each time. All of the supernatants were collected and kept at -50 °C until used as an enzyme source.

5 α -reductase assay was performed according to our previous paper (Kumar et al., 2011). Briefly, the reaction solution contained 0.2 ml of various plant extracts in 50% ethanol, 1.0 ml of 0.02 mM phosphate buffer (pH 6.5), 0.3 ml of 500 ppm testosterone in 50% ethanol, and 1.0 ml of rat microsomal suspension. Reactions were then initiated by the addition of 0.5 ml of 0.77 mg/ml NADPH in phosphate buffer, followed by incubation in a water bath at 37 °C for 30 min. The reactions were then stopped by adding 5.0 ml dichloromethane, and then adding 0.5 ml of 100 ppm propyl *p*-hydroxybenzoate in 50% ethanol (as an internal standard for HPLC). Four millilitres of the organic phase was decanted and evaporated to dryness under controlled pressure. The residues were collected and dissolved in 5.0 ml of methanol. An aliquot of 10 μ l was injected into the HPLC system (Agilent 1100 series, using a Hypersil[®]-ODS column, 250 mm \times 4.6 mm, 5 μ M). The

Table 1
Thai ethno-medicinal plants used in this experiment, their usage and method of preparation.

Botanical name	Family	Part used, method of preparation, and ethno-medicinal uses
<i>Acacia concinna</i> Wall.	Leguminosae	Dried pods are crushed and decocted with water; the juice is used as an anti-dandruff shampoo and for hair nourishment
<i>Alpinia galanga</i> Willd.	Zingiberaceae	Fire-grilled rhizomes are crushed and filtered through cloth; the juice is applied on the scalp to kill fungi and to promote hair growth
<i>Andrographis paniculata</i> Nees	Acanthaceae	Plants are boiled in water and filtered through cloth; the juice is applied on the hair as a hair rinse for hair growth promotion and hair loss prevention
<i>Averrhoa carambola</i> L.	Oxalidaceae	Fruit juice is used as an anti-dandruff shampoo and for hair nourishment
<i>Carthamus tinctorius</i> L.	Asteraceae	Flowers are decocted in water and used as a hair rinse to enhance hair color
<i>Cassia siamea</i> Lam.	Cesalpiniaceae	Leaves are decocted in water and used as an anti-dandruff shampoo, and for oil control and hair nourishment.
<i>Citrus hystrix</i> DC.	Rutaceae	Fresh fruits are squeezed and mixed with coconut (<i>Cocos nucifera</i> L.) juice, and used as a hair conditioner and to promote hair growth
<i>Clitoria ternatea</i> L.	Fabaceae	Fresh flowers are crushed and filtered through cloth; the juice is applied on the scalp to promote hair growth.
<i>Cymbopogon citratus</i> Stapf.	Poaceae	Whole plants are boiled in water and used as a hair rinse for oil control
<i>Ipomoea aquatica</i> Forssk.	Convolvulaceae	Leaves and stems are boiled in water and used as a hair rinse for hair loss treatment and hair conditioning
<i>Lawsonia inermis</i> L.	Lythraceae	Sun-dried leaves are soaked in water and applied to the hair as a hair-coloring agent
<i>Phyllanthus emblica</i> L.	Euphorbiaceae	Dried fruits are fried in the sesame (<i>Sesamum indicum</i> L.) oil, and then applied to the hair to promote hair growth
<i>Rhinacanthus nasutus</i> Kuntze	Acanthaceae	Whole plants are boiled and filtered through cloth; the juice is applied on the scalp to promote hair growth
<i>Sapindus rarak</i> DC.	Sapindaceae	Dried fruits are crushed and soaked in water, the filtered through cloth and used as a shampoo
<i>Tinospora rumphii</i> Boerl.	Menispermaceae	Vines are boiled and filtered through cloth; their juice is applied on the scalp to promote hair growth
<i>Trichosanthes cucumerina</i> L.	Curcubitaceae	Fruits are peeled and chopped into small pieces, then applied throughout the scalp, left for a while, and rinse off with water to promote hair growth
<i>Zingiber officinale</i> Roscoe	Zingiberaceae	Fire-grilled rhizomes are crushed and filtered through cloth; their juice is applied on the scalp to control scalp oil release and to promote hair growth

mobile phase was a mixture of methanol and deionized water (65:35) with a flow rate of 1.0 ml/min. A UV detector at 245 nm was used to collect data. Finasteride was used as a standard enzyme inhibitor. All of the results were expressed as finasteride equivalent 5 α -reductase inhibitory activity (FEA) value (units of mg finasteride equivalent per 1 g extract).

2.5. Method for determining hair growth promoting activity

Hair growth promoting activity of the extract was determined by the method reported by Roh et al. (2002), with some modification. Briefly, 25 seven-week-old mice were randomly divided into five groups for five treatments, as follows: vehicle control group, positive control group, extract1 group, extract2 group, and extract3 group. Hair was removed from the 2 cm \times 3 cm dorsal area of these mice by using a depilatory cream. On the next day, 100 μ l of the test solution in a vehicle composed of propylene glycol: water: ethyl alcohol in a ratio of 5:3:2 was applied. Minoxidil (2%) was used as a positive control. A concentration of 1% w/w of each plant extract was used. The hair growth promoting activity of the substances was checked by the darkening of the dorsal skin, which indicated the anagen phase of the hair follicles. Hair growth was measured at days 1, 7, 14, 21 and 28 by assigning a hair growth score, as follows: score 0 = no growth observed; 1 = up to 20% growth; 2 = 20–40% growth; 3 = 40–60% growth; 4 = 60–80% growth; and 5 = 80% to full growth observed.

Digital images of total hair growth on day 28 were obtained using a Coscam[®] USB-225 (Seoul, South Korea) with a 40 \times magnification lens.

2.6. Histological determination of hair follicles

After day 28, all of the mice were sacrificed. Their dorsal skins were removed and then sectioned into two different patterns: transverse sections for determination of hair follicle count,

and longitudinal sections for overall histological assessment under a light microscope (Olympus, Melville, NY).

2.7. Statistical analysis

All samples that tested for 5 α -reductase inhibition were analyzed in triplicate. All values were expressed as mean \pm SD. To compare several groups, analysis of variance was used. Significant differences between means were determined by Duncan's multiple range test. Pearson's correlation coefficient was used to predict the relationship between 5 α -reductase inhibitory activity and hair growth promoting activity. A probability value of $p < 0.05$ was adopted as the criteria for significant differences.

3. Results

3.1. 5 α -reductase inhibitory activity of the extract

Extraction yield of each plant was shown in Table 2. The microsomal suspension was prepared using the provided method, and was assessed for soluble protein by the Lowry method (Lowry et al., 1951). Soluble protein was found to be 4.69 mg/ml.

The IC₅₀ of finasteride, a well-known 5 α -reductase inhibitor, was 0.39 μ M. The inhibitory equation of finasteride was expressed as: $y = 166.78x - 15.285$ ($R^2 = 0.999$) with y representing % inhibition and x representing concentration of finasteride in μ M. This equation was used to calculate the results and was expressed as finasteride equivalent inhibitory activity (FEA) value: the higher the FEA value, the stronger the inhibitory activity of the extract. The inhibitory activity of each plant extract is shown in Table 2.

FEA values of the extracts ranged from 10.69 to 24.30 mg FEA per g extract (Table 2). The 5 α R inhibitory activity of each extract can be arranged from higher to lower, as follows: *Carthamus tinctorius* L., *Phyllanthus emblica* L., *Cymbopogon citratus* (DC.) Stapf., *Alpinia galanga* Willd., *Zingiber officinale* Roscoe., *Clitoria ternatea* L. (CT),

Table 2
Extraction yield and 5 α -reductase inhibition activity of each plant extract reported as FEA (finasteride equivalent 5 α -reductase inhibition activity) value.

Plants	Extraction yield (%)	Finasteride equivalent 5 α -reductase inhibition ability: FEA value (mg finasteride/1 g crude extract) ^a
<i>Carthamus tinctorius</i> L.	19.25	24.30 \pm 1.64a
<i>Phyllanthus emblica</i> L.	21.63	18.99 \pm 0.40b
<i>Cymbopogon citratus</i> (DC.) Stapf.	4.00	18.55 \pm 0.78b
<i>Alpinia galanga</i> Swartz.	5.88	18.54 \pm 0.85b
<i>Zingiber officinale</i> Roscoe.	8.44	18.32 \pm 0.82b
<i>Clitorea ternatea</i> L. (CT)	18.61	15.39 \pm 0.67c
<i>Citrus hystrix</i> DC.	8.24	13.72 \pm 0.79d
<i>Trichosanthes cucumerina</i> L.	2.75	13.37 \pm 0.84d
<i>Tinospora rumphii</i> Boerl.	3.88	13.33 \pm 0.30d
<i>Ipomoea aquatica</i> Forssk.	1.90	13.16 \pm 0.43d
<i>Averrhoa carambola</i> L.	4.65	13.12 \pm 0.87d
<i>Andrographis paniculata</i> Nees	2.94	13.01 \pm 0.81d
<i>Cassia siamea</i> Lam.	2.18	12.87 \pm 1.12d
<i>Acacia concinna</i> Wall.	15.23	12.78 \pm 0.87d
<i>Sapindus rarak</i> DC.	2.67	12.81 \pm 0.84d
<i>Lawsonia inermis</i> Linn.	16.60	12.58 \pm 0.45d
<i>Rhinacanthus nasutus</i> (L.) Kurz.	4.60	10.69 \pm 0.96e

^a Values in table expressed as mean \pm SD of triplicate experiments. Means in column with different letters are significantly different ($p < 0.05$).

Citrus hystrix DC., *Trichosanthes cucumerina* L., *Tinospora rumphii* Boerl., *Ipomoea aquatica* Forssk., *Averrhoa carambola* L., *Andrographis paniculata* Nees, *Cassia siamea* Lam., *Acacia concinna* Wall., *Sapindus rarak* DC., *Lawsonia inermis* Linn., and *Rhinacanthus nasutus* (L.) Kurz., respectively. In this experiment, *Carthamus tinctorius* was the strongest 5 α R inhibitor, and *Rhinacanthus nasutus* was the weakest 5 α R inhibitor. There were no significant differences in 5 α R inhibitory activity in *Phyllanthus emblica*, *Cymbopogon citratus*, *Alpinia galanga*, and *Zingiber officinale*, and between *Citrus hystrix*, *Trichosanthes cucumerina*, *Tinospora rumphii*, *Ipomoea aquatica*, *Averrhoa carambola*, *Andrographis paniculata*, *Cassia siamea*, *Acacia concinna*, *Sapindus rarak* and *Lawsonia inermis*.

For confirmation of the enzyme inhibitory activity of *Carthamus tinctorius*, safflower yellow, a major compound found in the florets, was further tested for enzyme inhibitory activity by IC₅₀ determination; the IC₅₀ of safflower yellow was 119.9 ppm (FEA value of 12.74).

3.2. Hair growth promoting activity of the extracts

The three plants with the highest 5 α R inhibitory activity, *Carthamus tinctorius*, *Phyllanthus emblica* and *Clitorea ternatea*, were further tested for hair growth promoting activity (Fig. 1). At day 28, it was found that *Carthamus tinctorius* demonstrated the highest hair growth promoting activity, followed by *Clitorea ternatea* and *Phyllanthus emblica*. As shown in Fig. 1, the normal hair growth rate of the mice was seen in the vehicle curve. In minoxidil-treated mice, it was found that minoxidil constantly promote hair growth of the mice. Plant extracts can promote the hair growth during the first 14 days of the experiment, while during the last 14 days the hair growth rates were constant. Among these extracts, *Carthamus tinctorius* had the highest hair growth promoting activity. Additionally, *Phyllanthus emblica* and *Clitorea ternatea* did not show any difference in hair growth rate increment in the first 14 days, but over the last 14 days *Clitorea ternatea* tended to increase the hair growth rate more than *Phyllanthus emblica*. There was a strong correlation between FEA value and hair growth promoting activity ($r = 0.719$) at day 14 of the treatment.

Total hair growth of the mice is shown in Fig. 2. Fig. 2A shows normal hair growth of mice receiving the vehicle, while Fig. 2B shows the increased hair growth from minoxidil. All three plants extracts were able to promote hair growth better than minoxidil (Fig. 2C-E).

3.3. Histological determination of hair follicles

The mean hair follicle count, obtained from a transverse section of the dorsal skin area, is shown in Table 3. In the vehicle control mice, the mean active hair follicle count was 24.2 \pm 2.8 hair follicles per selected area under 100 \times magnification by light microscope. The mice receiving minoxidil, *Carthamus tinctorius*, *Phyllanthus emblica* and *Clitorea ternatea* had 36.3 \pm 4.1, 69.5 \pm 7.6, 46.4 \pm 3.0, and 52.5 \pm 6.1 hair follicles per area, respectively. It was found that mice that received *Carthamus tinctorius* had the highest number of active hair follicles in their skin.

The morphological structure of the skin, obtained from a longitudinal section of the dorsal skin, is shown in Fig. 3. Mice receiving *Carthamus tinctorius* (Fig. 3C) had more hair follicles than mice receiving *Clitorea ternatea* (Fig. 3E), *Phyllanthus emblica* (Fig. 3D), minoxidil (Fig. 3B) and the vehicle (Fig. 3A).

There was a strong relationship between 5 α -reductase inhibitory activity (as FEA value) and hair follicle number ($r = 0.766$).

4. Discussion

For determination of 5 α -reductase inhibitory activity, radioimmunoassay (RIA) is the most widely accepted method. However RIA, which uses a radioactive compound, requires many complexes instruments and other equipment. Although immunoassay is a fast and easy method, there is a cross-reactivity of many androgens (Lootens et al., 2008). Matsuda et al. (2001) developed a simple isocratic HPLC method. In our previous paper, we modified the detection wavelength from 254 nm to 245 nm which encounters less interference from the reaction system (Kumar et al., 2011). In this experiment, finasteride has IC₅₀ at 0.39 μ M,

Table 3
Effects of vehicle, minoxidil, *Carthamus tinctorius* L., *Phyllanthus emblica* L., and *Clitorea ternatea* L. (CT) on hair follicle count in C57BL/6 mice.

Test substances	Hair follicles count ^a
Vehicle (propylene glycol:water:ethanol)	24.2 \pm 2.8a
Minoxidil	36.3 \pm 4.1b
<i>Carthamus tinctorius</i> L.	69.5 \pm 7.6c
<i>Phyllanthus emblica</i> L.	46.4 \pm 3.0d
<i>Clitorea ternatea</i> L. (CT)	52.5 \pm 6.1e

^a Values in table expressed as mean \pm SD of five mice. Means in column with different letters are significantly different ($p < 0.05$).

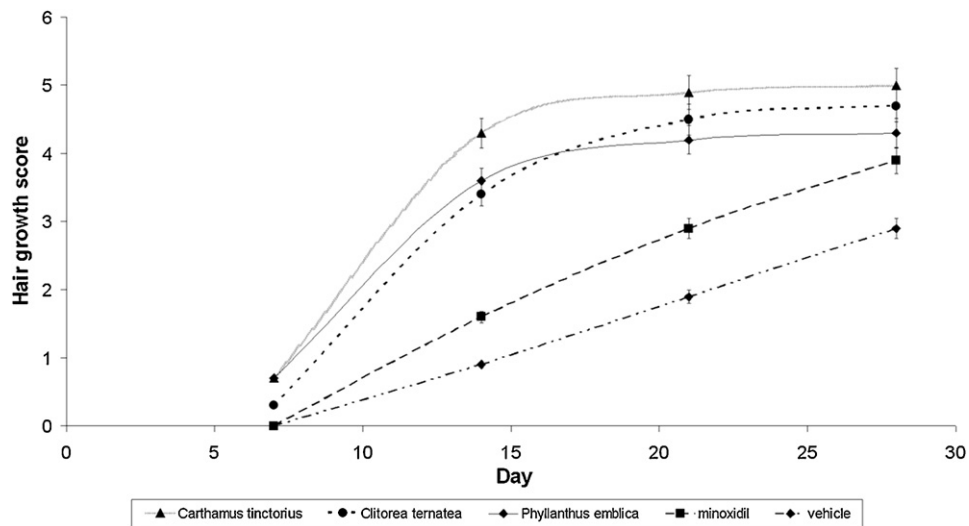


Fig. 1. Hair growth promoting effect of ethanolic extract of *Carthamus tinctorius*, *Clitorea ternatea*, *Phyllanthus emblica* and Minoxidil in C57BL/6Mlac mice model.

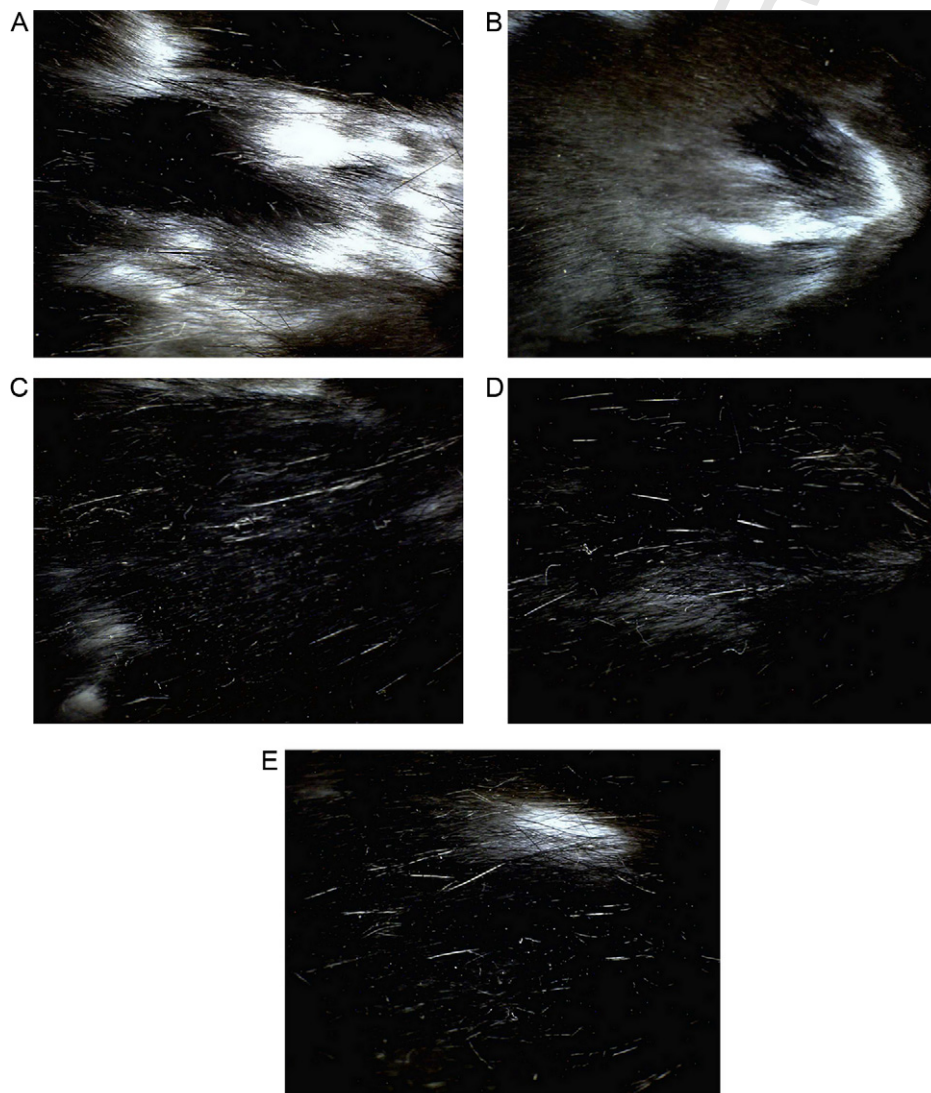


Fig. 2. Digital images obtained from Coscam® with 40× magnification lens showing total hair growth at day 28 of the treatment. (A) C57BL/6 mice received vehicle composed of propylene glycol:water:ethanol at 5:3:2 (B) C57BL/6 mice received 2% w/w minoxidil solution in vehicle. (C) C57BL/6 mice received 1% w/w *Carthamus tinctorius* L. extract in vehicle. (D) C57BL/6 mice received 1% w/w *Phyllanthus emblica* L. extract in vehicle. (E) C57BL/6 mice received 1% w/w *Clitorea ternatea* L. (CT) extract in vehicle.

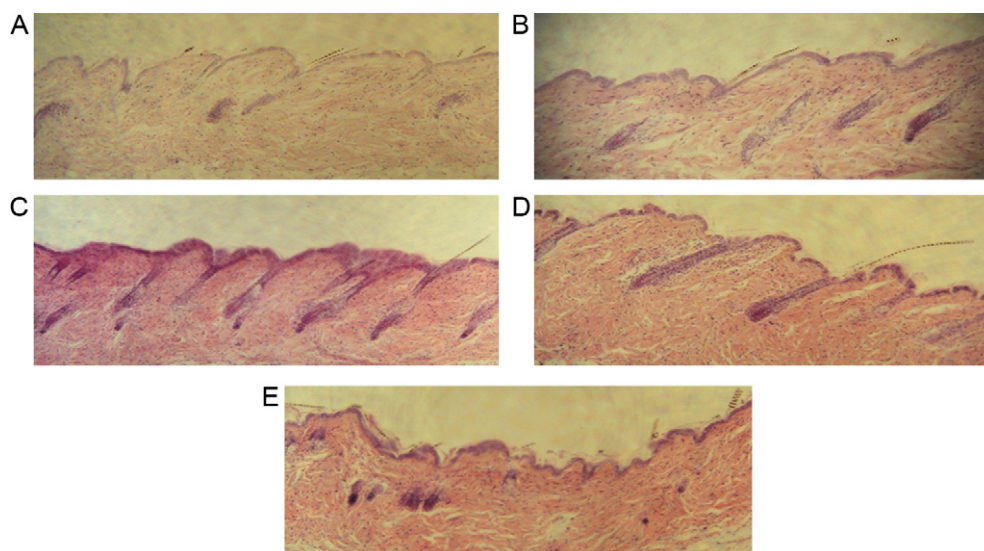


Fig. 3. Effect of vehicle (A), minoxidil (B), *Carthamus tinctorius* L. (C), *Phyllanthus emblica* L. (D), and *Clitorea ternatea* L. (CT) (E) on histological structure of murine hair follicles. Digital images were obtained under the light microscope with 100 \times magnification.

which is comparable to a previous report of 0.34 μ M (Park et al., 2003). The most potent 5 α R inhibitor in this experiment was *Carthamus tinctorius* (safflower), which contains safflower yellow as a major compound in the florets (Duke, 1992; Fan et al., 2009). This suggested that the synergistic interaction of some other phytochemicals in the ethanolic extract of *Carthamus tinctorius*, including flavonoids – for example, carthamin, carthamidin, isocarthamidin, 6-hydroxykaempferol compounds, etc. – may result in the highest inhibitory activity. The other active plants contained different classes and amounts of phytochemicals, which may result in the same inhibitory potency. Besides that, the crude extract of each plant contains many kinds of phytochemicals, some of which may be active against the enzyme inhibition, or even promote the activity of the enzyme. The balance of those two chemicals resulted in the FEA values seen in this experiment. For confirmation, partial or full purification of the extract needs to be performed in order to define which classes of phytochemicals have the great potency. This may lead to the development of new alternative medicines to treat androgen-related disorders, especially androgenic alopecia.

For decades, black C57BL/6 mice have been widely used to evaluate the hair growth promoting activity of many compounds (Datta et al., 2009). Since C57BL/6 mice contain no melanocytes on the skin, the melanogenesis of these mice occurs only in the hair follicles. Melanogenesis in these pigmented mice is strongly related to the hair growth cycle. Melanins are produced only in the anagen phase, and production stops at the beginning of the catagen phase (Slominski et al., 1994). For this reason, C57BL/6 mice are the most useful *in vivo* model for testing of hair growth promoting activity. The conversion of hair follicles into the anagen phase can be easily seen by the blackening of their skin. Moreover, it was found that most hair follicles of C57BL/6 mice at seven weeks of age are in the telogen phase of the hair cycle. Since it is known that melanogenesis in C57BL/6 mice occurs only during the anagen phase of the hair growth cycle, the blackening of the dorsal area indicated that the plant extracts are able to stimulate the anagen phase of the hair growth cycle in these mice. In this experiment, *Carthamus tinctorius* showed the best ability to stimulate hair follicle growth, and hence was the most potent hair growth promoter. Since hair growth is an active process, the vehicle treated group served as a control group for determination of the normal mouse hair growth rate by using the slope of the hair growth curve. Minoxidil, a well-known topical

medicine for treating AGA, increased the hair growth rate in a constant manner throughout the experiment. Surprisingly, the three extracts (*Carthamus tinctorius*, *Phyllanthus emblica* and *Clitorea ternatea*) seemed to increase the rate of hair growth for only the first 14 days of the experiment; during the last 14 days the growth rate appeared to be constant. This is a different result from that obtained in a previous study by Hirata et al. (2007), using methanolic extract of *Piper nigrum* leaf as a test compound, where incremental hair growth was seen throughout the treatment period.

The histological data of hair follicles in each group showed that the mechanism of *Carthamus tinctorius* and other plants in hair growth promoting activity may be due to an increase in an active hair follicle, and as anagen promoter. Since the activity of 5 α R in hair follicles causes hair follicle miniaturization (Sinclair, 2004), this implies that with lower 5 α R activity in hair follicles, larger hair follicles and consequently larger hair shafts are obtained. This may explain the relationship between FEA value and hair growth promoting activity. The Pearson's correlation coefficient between FEA and hair follicle count suggested that the higher the FEA, the higher the number of hair follicles, leading to increased hair growth.

In this experiment, none of the plant extracts applied to mice caused erythema, redness, drying or scaling as was the case with the minoxidil group. This indicated that plant extracts in a suitable vehicle may be useful as an alternative topical medicine to minoxidil therapy. Moreover, some plants with high anti-5 α R activity in this experiment may have the potential for development as herbal supplements for treating androgenic alopecia.

5. Conclusions

In conclusion, ethanolic extract of *Carthamus tinctorius* is the most active 5 α -reductase inhibitor and hair growth promoter, compared to finasteride and minoxidil, respectively. The plant extracts showed strong relationships between 5 α -reductase inhibitory and hair growth promoting activity, and between 5 α -reductase inhibition and the number of hair follicles. This indicates that plant extracts may be beneficial as an alternative medicine. Our group focused on using plant extracts as cosmeceuticals for prevention and treatment of hair loss. To achieve a practical alternative topical treatment for hair loss, *Carthamus tinctorius* is currently being

developed as a suitable hair formulation, using nanoparticles to deliver active substances directly to the hair follicles.

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