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Anti−hairloss efficacy of coffee berry extract

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Abstract

An important enzyme causing hair loss in human is 5−alpha−reductase by catalyzing the conversion of testosterone to dihydrotestosterone (DHT) which induced miniaturization of the hair follicle. Coffee berry is a natural source of caffeine and chlorogenic acid which shows inhibition of the enzyme activity. The objective of this research was to determine the total phenolic content, DPPH radical scavenging and inhibition of 5−alpha reductase activities of coffee berry extract and evaluate anti−hair loss efficacy of tonic containing the extract on 47 volunteers. Coffee berry extract showed high quantity of caffeine 0.43 mg/mL by HPLC technique. The total phenolic content was found to be 0.73 ± 0.02 mg GAE/mL extract. The DPPH radical scavenging of the extract showed the IC_{50} value of 4.10 ± 0.29 mg/mL. The cytotoxicity and 5−alpha−reductase inhibition of the extracts were investigated on prostate cancer cell lines (DU−145). The cell viability of coffee berry extract at concentration 0.1, 1, 10, 100 and 1,000 µg/mL showed 102.8, 97.6, 91.8, 97.7 and 88.8%, respectively, which indicating that the extract has not cytotoxic on the cell lines. The coffee berry extract at 1,000 µg/mL was selected to evaluate 5−alpha−reductase inhibition. The extract showed slightly lower inhibition of 5−alpha−reductase activity with 16.5% than the standards, finasteride (32.5%) and dutasteride (20.1%). In clinical trial, 13 male and 34 female volunteers who have hair loss problem were selected and divided randomly into two groups. First group applied placebo spray tonic (C) and the second group applied spray tonic containing 10% coffee berry extract (CB) everyday for 12 weeks. The volunteers were assessed for hair loss reduction by one−minute combing test every month. The CB group showed significant reduction in the mean number of hairs loss after combing test from 11.5 to 2.4, 1.9 and 1.4 ($P<0.01$) for 1, 2 and 3 months, respectively. The CB group showed better efficacy for hair loss reducing than placebo. In conclusion, the results indicated that coffee berry can be used as anti-hair loss ingredient in hair care cosmetics.

Keywords: Coffee berry, caffeine, hair loss, 5−alpha−reductase, spray tonic

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1. Introduction

Androgenetic alopecia (AGA.), also known as female pattern hair loss (FPHL) and male pattern hair loss (MPHL), is a common dermatological problem affecting both men and women with significant negative impact on their social and psychological wellbeing. It commonly begins between ages 12 and 40 years and continues to progress with increasing age. AGA is induced by dihydrotestosterone (DHT) in genetically susceptible women and men. DHT is formed by the peripheral conversion of testosterone by 5-alpha reductase (5α-R) and response to the transformation of large follicles to small follicles brings forth progressive thinning of the scalp hair and hair loss. Therefore, 5α-R inhibitors such as finasteride and dutasteride are used in the treatments of AGA. (Price, 2003; Ghanaat, 2010).

Coffee is an important crop that assures a sustainable economy for many developing countries. Coffee crops were represented by three botanical species (*Coffea Arabica* or Arabica, *Coffea anaphora* or Robusta and *Coffea liberica*), which Arabica makes up approximately 70% of the world's coffee production (Poltronieri and Rossi, 2016). Coffee berries are fruits of coffee plants, which are ripe round characteristics with red and purple colors. Coffee is a rich source of antioxidant of the hidroxycinnamic acid derivatives; caffeic, chlorogenic, coumaric, ferulic and sinapic acids. Moreover, caffeine is a well-known substance for stimulating hair growth by possessing two pathways, including inhibition of 5α-R and stimulating hair growth parameters. 5α-R inhibitor prevents the shortening of the anagen phase (growth phase of the hair cycle), resulting in diminution of the resting phase of hair cycle known as the telogen phase (Farah, 2012). Despite the extant evidence of protective effects of caffeine on the hair, the scientific basis and clinical outcomes of coffee berry are yet to be explored. Therefore, the objectives of this research were to investigate the anti-hair loss potential of coffee berry extract by evaluating caffeine content, phenolic content, antioxidant activity, cytotoxicity, 5α-R inhibition, and clinical trial investigation.

2. Materials and Methods

2.1 Materials

Coffee berry was collected in Chiang Rai province, Thailand. Ethanol, caffeine, folin-ciocaltue reagent, sodium bicarbonate, gallic acid, ascorbic acid, 1,1-diphenyl 1-2-picrylhydrazyl (DPPH), finasteride, and dutasteride were purchased from Sigma-Aldich Co., USA. Prostate cancer cell line (DU-145, ATCC® HTB-81™) was purchased from Biomedia (Thailand) company. Fetal bovine serum, phosphate buffered saline, penicillin streptomycin, and RPMI-1640 medium was purchased from Gibthai Co., Ltd, Thailand.

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2.2 Methods

2.2.1 Extraction of coffee berry

The fresh coffee berries were crushed with the blender. Then, 100 g of crushed coffee berries were soaked in 500 mL of ethanol for 24 h at room temperature. The extract was filtered using a buchner funnel through filter paper (whatman® no.1). Then, the extract was evaporated by a rotary evaporator (Buchi R-114 Rotary Vap System) at 40ºC. The coffee berry extract was stored at 4ºC until analysis.

2.2.2 Identification of caffeine using HPLC

Caffeine was determined using HPLC (1260 Infinity II LC System, Agilent Technologies) according to the method was modified from Kiattisin *et al*. (2016). Coffee berry extract was filtered through a 0.45 µm regenerated cellulose syringe filters (Whatman Spartan). Then, the extract (10 µL) was injected into a C18 column (Poroshell 120 EC-C18). The mobile phase consisted of acetonitrile and 1.5% acetic acid with a ratio of 15:85 v/v at flow rate 1 mL/min. HPLC chromatograms were detected using a photodiode array UV detector at 280 nm according to the absorption maxima of caffeine. Caffeine was identified by its retention time with standards under the same conditions. The quantification of the samples was done by measurement of the integrated peak area, and the content was calculated using the calibration curve by plotting peak area again the concentration of respective standard caffeine (CFN, > 99 % HPLC, c0750, Sigma-Aldich).

2.2.3 Determination of total phenolic content (TPC)

The total phenolic content was analyzed using the Folin-Ciocaltue assay (Vichit and Saewan, 2016). The extract (20 µL) was mixed with 50 µL of deionized water, 20 µL of Folin-Ciocalteu reagent and 125 µL of 7% sodium bicarbonate. The mixture was incubated for 90 min at room temperature. Then, the absorbance was measured at 750 nm using a microplate reader (BMG LABTECH/SPECTRO star Nano). The result was reported as gallic acid equivalent (mg GAE/mL extract).

2.2.4 DPPH radical scavenging activity assay (DPPH)

The scavenging activity of 1,1-diphenyl 1-2-picrylhydrazyl (DPPH) radical method was modified from Vichit and Saewan (2015). The various concentration of extract (1.0-5.0 mg/mL, 5 µL) and 0.1 mM of DPPH solution (195 µL) were mixed and reacted at room temperature for 30 min. Then, the absorbance of each mixture was measured at 515 nm using a microplate reader. The scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where, $A_{control}$ is the absorbance of the control without extract and A_{sample} is the absorbance of the tested sample.

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Then, the DPPH scavenging activity of coffee berry extract was express as 50% inhibition concentration (IC_{50}) and ascorbic acid was used as positive standard.

2.2.5 Cytotoxicity

The human prostate cancer cell lines (DU–145) were cultured under the standard conditions in the complete culture medium containing Roswell Park Memorial Institute medium (RPMI–1640 medium) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin streptomycin solution. Cells were maintained in 5% CO_2 at 37ºC in the incubator. The cytotoxic assessment was performed by SRB assay (Ruksiriwanich *et al*., 2011). The standard finasteride and dutasteride at 0.1, 1, 10, 100 and 1,000 µg/mL were used as positive control. Cells were placed in 96–well plates, left overnight for cell attachment on the plate, and kept at 5% CO_2 at 37ºC in the incubator. Then, cells were treated with five serial concentrations of the extracts (0.1–1,000 µg/mL) and incubated for 24 h. After incubation, cells were washed and dyed with SRB solution. The bound dye was solubilized and measured the absorbance at 540 nm by a microplate reader. The percentage of cells proliferation was calculated as follow:

$$\% \text{ Cell viability} = (A_{sample} / A_{control}) / A_{control}) \times 100$$

Where, $A_{control}$ is the absorbance of the control without extract and A_{sample} is the absorbance of the tested sample.

2.2.6 Inhibition of 5α–R activity

The inhibition of 5α–R activity was followed protocol from Ruksiriwanich *et al*. (2011). The extract and 2 positive standards, finasteride and dutasteride, were prepared at a concentration of 1,000 µg/mL. The samples were filtered through membrane filter 0.2 micron. Then, cultivated cells were determined the inhibition of 5α–R activity in DU–145. The agarose gel electrophoresis was performed and the bands of 5α–R DNA were observed by gel documentation (Bio–Rad Laboratories, UK). The inhibition of 5α–R was calculated by followed equation:

$$\% \text{ Inhibition of } 5\alpha\text{–R} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where, $A_{control}$ is the absorbance of the control without extract and A_{sample} is the absorbance of the tested sample.

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2.2.7 Skin irritation test

The study of skin irritation was performed according to patch testing (Schnuch *et al*., 2008). All volunteers signed written informed consent before participating in the clinical study. The inclusion criteria include volunteers have hair loss problem and suffering from their hair thinning and loss. The volunteers were acknowledged about the type of study, the procedures to be followed, the general nature of materials being tested and any known or anticipated adverse reaction that might result from participation. Forty−seven volunteers (13 male and 34 female) who have hair loss problems (age 25 to 61 years) were subjected to a closed single patch test by patch testing method. The four samples were included a positive control (0.2% sodium laureth sulfate), negative control (distilled water), spray tonic base, and spray tonic with coffee berry extract. All samples (0.015 mL) were soaked in a circular filter paper. Then, each sample was applied on the Finn Chambers® and placed on the upper arm for 24 h. The grades of skin irritation, erythema and oedema, were evaluated using score criteria for dermal reactions after removing patch for 30 min and 24 h. Data collection was calculated the cumulative irritation index (C.I.I) for each subject to the following formula:

$$C.I.I = \sum \text{of the grade (erythema + oedema)} / \text{number of reading}$$

This index is then divided by the number of volunteers to obtain the mean cumulative irritation index (M.C.I.I) by the following formula:

$$M.C.I.I = C.I.I / \text{number of volunteers}$$

The M.C.I.I value was reported by classifying irritation; non-irritate (M.C.I.I value < 0.25), very slightly irritate ($0.25 \leq$ M.C.I.I < 0.50), slightly irritate ($0.50 \leq$ M.C.I.I < 1), moderately irritate ($1 \leq$ M.C.I.I < 2) and irritate (M.C.I.I ≥ 2). The protocol was approved by the Ethics Committee of Mae Fah Luang University (REH−62046).

2.2.8 One-minute combing test

In this study, the volunteers were divided into two groups by randomization. The first group (22 volunteers) applied placebo spray tonic (C), and the second group (25 volunteers) applied spray tonic containing 10% coffee berry extract (CB). The changing of associated symptoms (dryness, oily, and normal scalp) were compared between baseline and during experiments. The volunteers applied products twice a day on the morning and night by massaging for 5 min without rinse off to increase the absorption of the scalp. Then, they were evaluated for their hair loss by a one−minute combing test of dry hair according to Madan

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method (Madan *et al*., 2014). The number of hair falls after combing for 1 min (50 times) of each volunteer was counted, and the experiment was performed by the same technician. The teeth of the comb were 15 cm long, which separated by 1 mm on one half of the comb, and 2 mm on the other haft. The combing test was performed every month. The mean number of hair loss was compared before, during, and after using the products.

2.2.9 Statistical analysis

The obtained data was statistically analyzed using the SPSS program version 23 for window (SPSS Inc, Chicago, IL, USA). Cytotoxicity and inhibition of 5α−R activity on DU-145 cell line were analyzed by using One Way Analysis Variance (ANOVA). The reduction of number of hair loss after using the product at 1, 2 and 3 mounts was used Paired Sample T−Test. The significant difference was considered when $P<0.01$.

3. Results and discussion

3.1 Extraction of coffee berry

The grinded coffee berry was extracted with ethanol by the maceration and obtained as a light brown solution. Then, the extract was evaporated to remove the solvent. The dark brown viscous liquid was obtained with a percentage yield of 8.86 %. Fig 1 showed the appearance of coffee berry, coffee berry extract, and crude coffee berry extract. The pH of coffee berry extract was 5.7, which suitable for application in hair and skin cosmetic products.

A B C

Fig 1 Coffee berry; fruit (A), extract (B) and crude extract (C)

3.2 Identification of caffeine using HPLC

The coffee berry extract was evaluated by HPLC using caffeine as a reference standard. The method was modified from Kiattisin *et al*. (2016). The chromatogram of coffee berry extract showed the peak of caffeine at retention time of 5.53 min (Fig 2). The caffeine content of coffee berry extract was 0.43 mg/mL.

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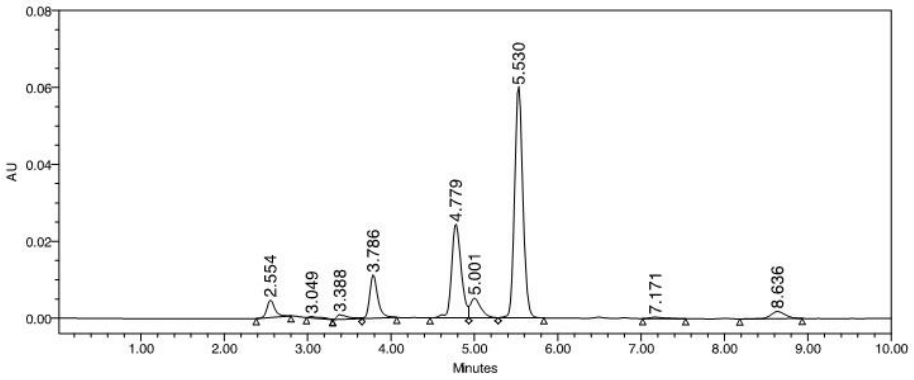

Fig 2 HPLC chromatogram of coffee berry extract

3.3 Total phenolic content

The phenolic compounds provide antioxidant which eliminates the cause of skin aging and skin damage, including wrinkle, wound, and burn. Furthermore, phenolic compounds prevent the progression of certain skin disorders such as acne (Dzialo *et al*., 2016). In this study, the phenolic compound in coffee berry extract was determined by Folin−Ciocaltue assay and the result was expressed as gallic acid equivalents. The coffee berry extract contained the total phenolic content of 0.73 ± 0.02 mg GAE/mL extract.

3.4 Antioxidant activity by DPPH method

Many research papers presented that the phenolic compounds were good free radical scavenger. It related to the efficacy of DPPH radical scavenging, which is one of the most common used methods for evaluating the expertness of samples to scavenger free radical. This method determines the electron donor ability of samples resulting in the reduction of a purple DPPH radical to become a stable molecule (Huang *et al*., 2005). The coffee berry extract showed inhibitory activity against DPPH radicals with IC_{50} values of 4.10 ± 0.29 mg/mL. Although it is less efficient than ascorbic acid (IC_{50} 0.14 ± 0.02 mg/ml), the prevention of free radicals from oxidative stresses could be decreased hair loss (Trüeb, 2011).

3.5 Cytotoxicity on DU−145 cell line

The coffee berry extract and the standards, finasteride and dutasteride, at 0.1, 1, 10, 100 and 1,000 µg/mL were showed no cytotoxicity to DU−145 by SRB assay (Fig 3). The coffee berry extract at the lowest concentration (0.1 µg/mL) showed the highest cell viability (102.8%) and more than standards, finasteride (98.3%) and dutasteride (94.9%). While, the higher concentration (1, 10, 100 and 1,000 µg/mL) of extract showed lower cell viability 97.6, 91.8, 97.7 and 88.8%, respectively. However, all concentration of the tested samples showed cell viability more than 80%, which were classified as low toxicity to cells. Therefore, the highest concentration was selected for further experiment, 5α−R inhibition assay.

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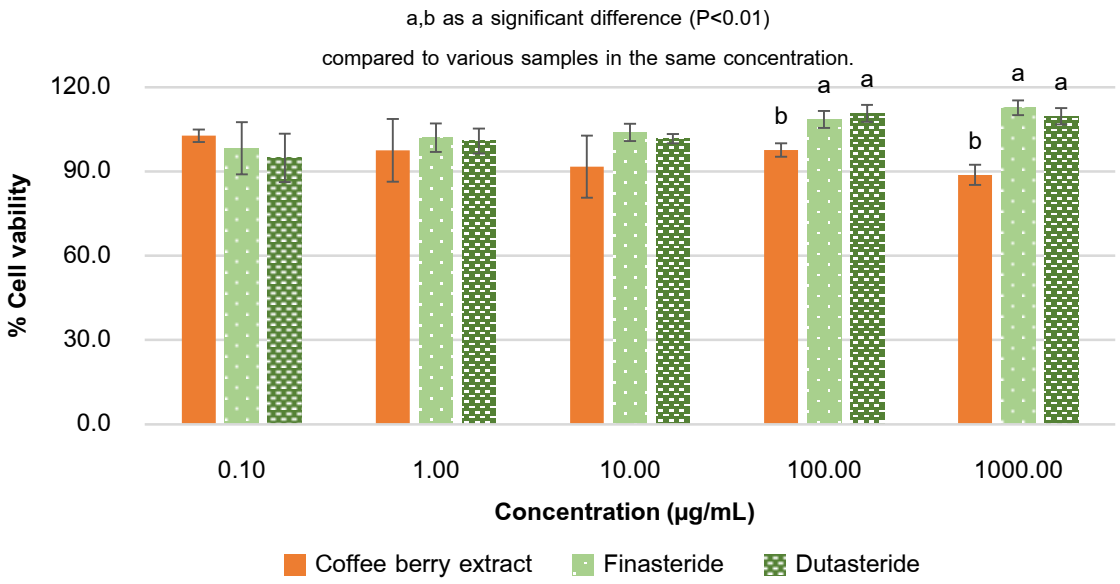

Fig 3 The percentage of cell viability on human prostate cancer cell line (DU–145) of coffee berry

3.6 Inhibition of 5α–R activity

5α–R was the leading cause of hair loss that predominated in the human scalp, especially in the dermal papilla. In several studies, the DU–145 human androgen insensitive prostate adenocarcinoma cell line, which contains the 5α–R, has been used for the 5α–R inhibition assay (Ruksiriwanich *et al*., 2011). Agarose gel electrophoresis was used to investigate the 5α–R inhibition efficiency of extract and standards on DU-145 and the results showed in Fig 4. Coffee berry extract showed 5α–R inhibition activity on cell at 16.5 ± 6.7%, which lower than finasteride (32.5 ± 9.8%) and dutasteride (20.1 ± 4.9%) for 1.9 and 1.2 times, respectively. However, the statistical analysis showed no significant difference (P<0.05) between the extract and standards, finasteride and dutasteride. 5α–R inhibition activity of coffee extract may be effect by the contained caffeine which showed inhibition of the enzyme and has the potential to mitigate androgen–induced hair loss (Daniels *et al*., 2019 and Fischer *et al*., 2007). Then, the coffee berry extract was incorporated in hair spray tonic base for further clinical study.

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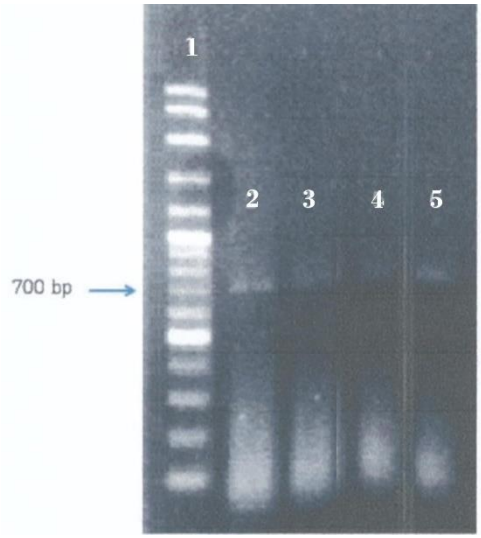

Fig 4 Agarose gel electrophoresis of 5α–R on DU–145 in different lanes (1 = DNA ladder 100 bp, 2 = 5α–R DNA (control), 3 = coffee berry extract (1,000 µg/mL), 4 and 5 = standard finasteride and dutasteride (1,000 µg/mL)

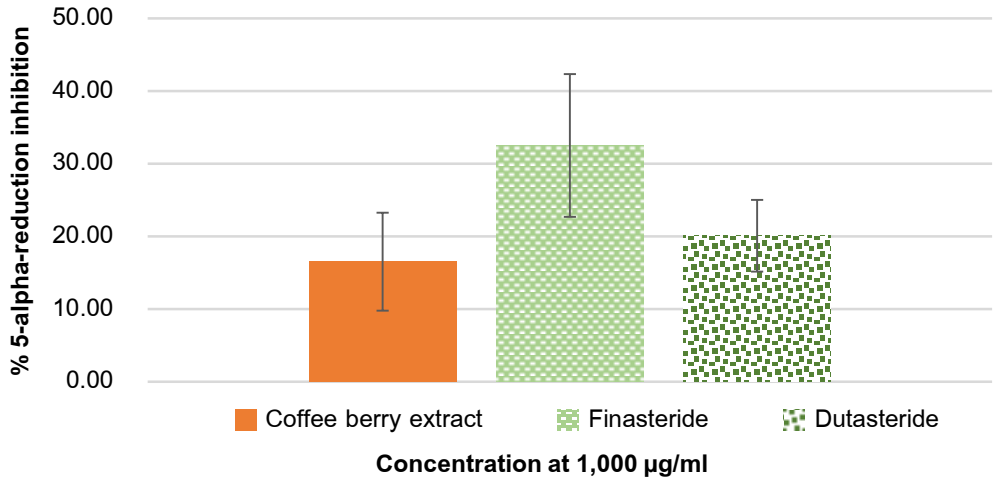

Fig 5 Percentage of Inhibition of 5α–R of coffee berry extract, finasteride and dutasteride at 1,000 µg/mL

3.7 Clinical efficacy of hair loss reduction

3.7.1 Skin irritation test

The skin irritation test was evaluated by using a closed single patch test. The volunteers were assessed for the erythema and oedema on the upper arm after 30 min and 24 h patch removal. The grade of skin irritation was recorded as C.I.I value for each volunteer. Then, the result showed the M.C.I.I value of spray tonic base and spray tonic containing 10 % coffee berry extract was 0.01. It was classified as non–irritation formulas.

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3.7.2 One–minute combing test

The total forty–seven volunteers (25 volunteers in CB group and 22 volunteers in C group) included in the study. The mean age of all volunteers was 40 years old. The associated symptoms (dryness, oily, and normal scalp) were observed. In CB group, 9 volunteers have dry scalp and 16 volunteers have oily scalp. The number of C group volunteers who have dry, oily and normal scalp were 9, 12 and 1, respectively. There was a significant reduction in the associated symptoms (dryness, oily and normal scalp) of application by the end of this experiment. The number of volunteers who has dryness on the scalp was more reduced from 9 to 4 (20.0%) in CB group than C group that reduced from 9 to 8 (4.5%). While, the reduction of oily scalp was found from 16 to 9 (28.0%) in CB group and from 12 to 11 (4.5%) in C group. Besides, the changing of dry and oily scalp to normal skin in CB group was 48.0 % and C group was 9.1%. The severity of hair loss was evaluated by considering of the number of counted hair loss during combing; mild (less than 40), medium (40–100) and severe (more than 100) (Madan *et al*., 2014). In this study, the mean number of hair loss was performed by combing test every month for 3 months. The number of hair falling in all volunteers was less than 40 which was graded as mild. After 1, 2 and 3 months, a significant difference ($P<0.01$) reduction in the mean numbers of hair loss from 11.5 (baseline) to 2.4, 1.9 and 1.4, respectively, was found in CB group. While, C group showed no significant reduction from baseline (6.1 to 4.8, 4.4 and 4.1 for 1, 2 and 3 months, Fig 6). These results were related to sensory evaluation of volunteers which volunteer in CB group provided higher precepted reduction of hair loss (72.7%) than C group (23.1%) as shown in Fig 7. The hair loss reduction efficacy of tonic with coffee berry due to caffeine which is corresponding to the previous finding that using of caffeine shampoo for 3 and 6 months showed significant decreasing the mean number of hair loss ($P<0.01$) 18.6 and 17.4% on AGA's male Bussoleti *et al*. (2010).

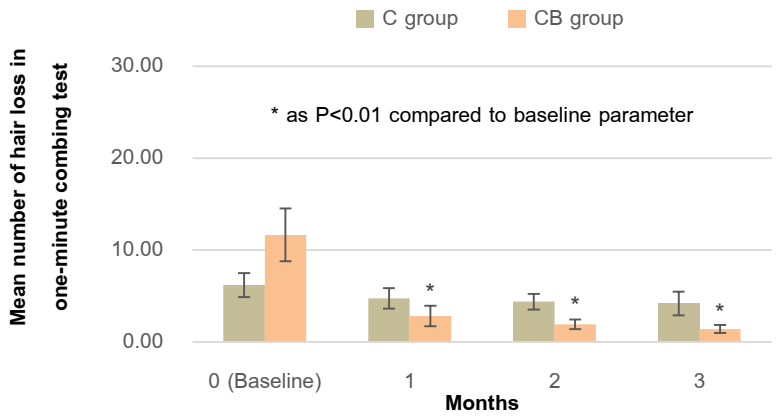

Fig 6 Mean number of hair loss in one–minute combing test

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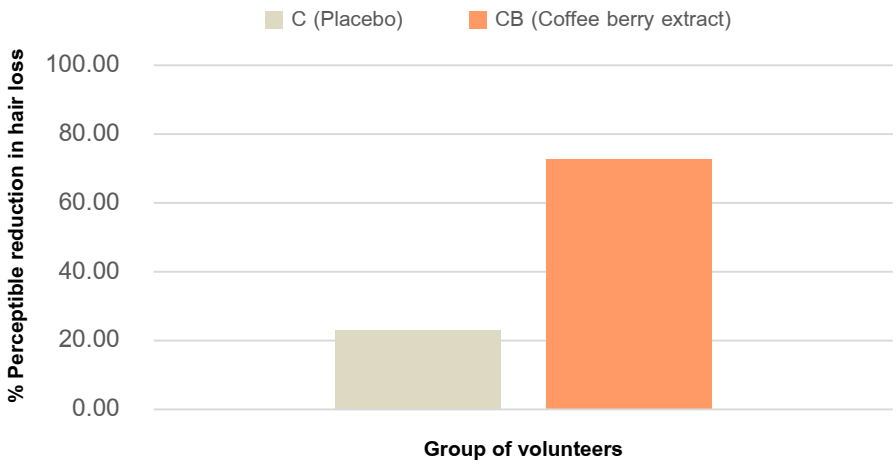

Fig 7 Percentage of perceptible reduction in hair loss of volunteers at the end of experiment (3 months)

4. Conclusion

Coffee berry extract showed caffeine content 0.43 mg/mL by HPLC technique, phenolic content (0.78 ± 0.02 mg GAE/mL extract) and DPPH radical scavenging activity (IC_{50} value of 4.09 ± 0.21 mg/ml). Coffee berry extract showed no significant difference ($P<0.01$) inhibition of 5α–R 16.5 % to the standards, finasteride (32.5%) and dutasteride (20.1%). The spray tonic contained 10 % coffee berry extract provided a significant ($P<0.01$) reduction in the mean numbers of hair loss from 11.5 to 1.4 when compared with the placebo group from 6.1 to 4.1. In conclusion, coffee berry extract was a natural source with potential for use as an active ingredient, anti-hair loss in cosmetics.

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