

Potency of Leaf Extracts of Cocor Bebek (*Kalanchoe pinnata*) as Hair-Growth Promoting Agent

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Abstract: Plant extracts of cocor bebek, *Kalanchoe pinnata* (Lam.) Pers., containing various types of bioactive compound expected to have cosmetic benefits, however studies on its uses in haircare is lacking. Current study is a part of our effort in extending seeks of plant species growing in Indonesia that are potent to be use as hair-growth promoting agents. Four healthy, adult, male New Zealand rabbits, aged 4-5 months, weighing 1.5 kg – 2 kg were used as test animals. The dorsal aspect of each rabbit were shaved and divided into six areas, with a size of 2 cm x 2 cm each. Each area of shaved skin were then administered with CMC gel containing substances set for treatment namely: CMC gel without cocor bebek extract (as negative control), 2% minoxidil (as positive control), and leaf extracts of cocor bebek of four levels of concentration i.e. 25% (v/v), 50% (v/v), 75% (v/v) and 100% (v/v) respectively. Topical administration of extract was done twice a day (morning and afternoon), for 21 days. Hair length was assessed using calliper on day 7, 14 and 21 by taking randomly 10 hairs from each treatment area, while the hair mass was measured using microbalance on day 22 by harvesting all hairs on each treatment area. The result showed CMC gel containing leaf extracts of *K. pinnata* remarkably increased hairs length of rabbits in a concentration-related manner. At the highest concentration, the effects even surpassed minoxidil. However, no treatment found to affect hair mass of the animals. It is inferred that plant extract of cocor bebek is potential to be use as a hair growth promoting agent.

Keywords: cocor bebek, *Kalanchoe pinnata*, hair-growth promotion, anti hair loss, minoxidil.

INTRODUCTION

It cannot be denied that for most people, either men or women, hair disorders especially hair loss or baldness is something that is very disturbing. That is why many people are willing to spend a lot of money to care for and maintain the beauty of their hair [1]. So far, the standard drugs that have been proven to be most effective in preventing hair loss are minoxidil and finasteride [2]. However the side effects of these drugs were known to limit their pharmacological benefits hence it is a necessity to replace this synthetic drug with that of botanical ingredient [3]. Currently, there are already dozens of herbs / plants from South Asia that indicated to have anti-hair loss effects, such as *Emblica officinalis* (Euphorbiaceae), *Centella asiatica* (Umbelliferae), *Eclipta alba* Linn. (Asteraceae), *Cocos nucifera* Linn (Palmae), *Eucalyptus globulus* (Myrtaceae), *Lawsonia inermis* (Lythraceae), *Azadirachta indica* (Meliaceae), *Hibiscus rosa sinensis* (Malvaceae), *Nardostachys jatamansi* (Valerianaceae), *Trigonella foenum-graecum* (Leguminosae), *Juniperus virginiana*, *Rosmarinus officinale* Linn.(Labiatae), *Acacia concinna* (Mimosaceae), *Prunus dulcis* (Rosaceae), *Ginkgo biloba* (Ginkgoaceae), *Santalum*

album (Santalaceae), *Sesamum indicum* (Pedaliaceae), *Cassia angustifolia* (Leguminosae), *Citrus limonum* (Rutaceae), *Rosa damascena* (Rosaceae), *Salvia officinalis* Linn.(Labiatae), *Arnica montana* (Apiaceae), *Simmondsia chinensis* (Simmondiaceae), *Trigonella foenum-graecum* L (Fabaceae), and *Ocimum sanctum* (Labiatae) [4].

Research reports from Indonesia revealed that in addition to dozens of plant species above, anti-hair loss activities also shown by pisang kepok (*Musa balbisiana*) and suruhan (*Peperomia pellucida*). Topical gel containing corm extract of *M. balbisiana* as well as plant extract of *P. pellucida* were proven to increase hair length and mass in rabbits [5, 6]. Among thousands of plant found in Indonesia, cocor bebek, *Kalanchoe pinnata* (Lam.) Pers. (Synonyms: *Bryophyllum calycinum*, *Bryophyllum pinnatum*), is one species that has long been known in folk medicine system in many tropical and subtropical countries. Medicinal values of this plant include wound-healing, antioxidant, anticancerous, antiproliferative, antimicrobial, antiviral, antiprotozoal, antileishmanial, anthelmintic, insecticidal, anti-allergic, analgesic, antinociceptive,

anti-oedematogenic, anti-inflammatory, muscle-relaxant, antipyretic, anticonvulsant, antidepressant, sedative, antilithiatic, hepatoprotective, gastroprotective, antidiabetic, nephroprotective, haemoprotective, antihistamine, antihypertensive and immunosuppressant [7].

Phytochemical analysis of plant extracts of *K. pinnata* revealed that this succulent plant containing various types of bioactive compound expected to have anti hair loss activity, such as malic acid, sitosterols and saponins [8]. However, until now there has been no research that reveals the influence of cocor bebek plant ingredients on hair care remedies. Current study is a part of our effort in extending seeks of plant species growing in Indonesia that are potent to be use as hair-growth promoting agents.

METHODS

Plant Sample and Extraction

Plant leaf samples of cocor bebek, *Kalanchoe pinnata* (Lam.) Pers., were collected from suburb of Bandar Lampung, the capital city of Lampung province, Indonesia. For taxonomic verification, the plant samples were brought to the Botany Laboratory, Faculty of Mathematics and Sciences, University of Lampung, Indonesia. The fresh leaves were washed with aquadest, air dried, sliced into small pieces, and then soaked in 96% ethanol for 24 hours. After being macerated for four times, the macerate evaporated using rotary evaporator under low pressure until brownish-viscous extracts formed.

Animals and Experimental Design

Test animals used in the study were four healthy, adult, male New Zealand rabbits, aged 4-5 months, weighing 1.5 kg – 2 kg. During a week of acclimatization and throughout the experiment all rabbits were caged individually in a room with natural light cycle, in a temperature range of 25 °C – 30 °C, and fed with natural diet and water ad libitum.

To make rabbits ready for treatment the dorsal aspects of the animals were divided into six topical administration areas, with a size of 2 cm x 2 cm each. The hairs on each area were shaved using a razor. These areas were then marked imaginatively according to the concentration of extract applied namely: K-, K+, E1,

E2, E3 and E4. Area K- and K+ are the shaved skin treated consecutively with CMC gel without cocor bebek extract (as negative control) and 2% minoxidil (as positive control). Area E1, E2, E3 and E4 are the shaved skin administered with gel containing *K. pinnata* extracts of 25% (v/v), 50% (v/v), 75% (v/v) and 100% (v/v) respectively.

Extract Administrations and Observation

To make ingredients ready to be given topically to the test animals, the ethanolic leaf extracts of *K. pinnata* were suspended in distilled water at concentrations set for treatment, i.e. 25, 50, 75 and 100% respectively, up to a final volume of 20 ml. Into the suspension is then added CMC (carboxymethyl cellulose) little by little while stirring evenly until the gel mixture is formed. Topical administration of extract was done by smearing each shaved skin with 0.1g of the gel, twice a day (morning and afternoon), for 21 days. On day 7, 14, and 21 from each treated area was taken randomly 10 hairs and the length of each hair was measured using caliper. On day 22 all hairs in the treated area were taken and assessed using a digital microbalance.

Statistical Analysis

The data were described as mean \pm standard deviation (SD). One-way ANOVA and Least Significant Difference (LSD) test were used to determine the statistical differences between values of experimental and control groups and the p-values < 0.05 is considered significant.

RESULTS

The descriptive and analytical data indicating the effects of six different treatments on rabbit hairs growth on day 7, 14 and 21 after daily topical administration were consecutively shown in Table 1, 2 and 3. The one-way ANOVA statistics that were applied for the data resulted in the F-values of 34.494, 18.921, and 27.894 with the P-values of 0.0048, 0.00205, and 0.00081 respectively. The LSD test on the mean values between treatments for the data in Table 1, 2 and 3 suggest that CMC gel containing leaf extracts of *K. pinnata* remarkably increased hairs length of rabbits in a concentration-related manner. At the highest concentration, the effects even surpassed minoxidil, the positive control treatment.

Table-1: Hair length of rabbits on day 7 after daily topical administration of leaf extracts of cocor bebek (*Kalanchoe pinnata*)

Treatment	Hair length of rabbits (mm)				Mean ± SD
	1	2	3	4	
K-	2.29	3.09	2.00	4.49	2.963±1.115 ^a
E1	4.67	3.74	4.07	6.22	4.671±1.102 ^b
E2	4.82	4.35	4.80	6.41	5.091±0.903 ^b
E3	5.73	5.54	5.31	6.66	5.806±0.591 ^c
E4	6.89	6.85	5.72	6.98	6.609±0.595 ^d
K+	5.73	6.13	5.65	6.88	6.095±0.560 ^c

K- (negative control) is shaved skin treated with CMC gel without plant extracts; K+ (positive control) is shaved area given 2% minoxidil; whereas E1, E2, E3 and E4 are the shaved skin treated with CMC gel containing suruhan extracts 25%, 50%, 75% and 100% respectively. Mean±SD values followed by different superscripts are significantly different at $\alpha=0.05$.

Table-2: Hair length of rabbits on day 14 after daily topical administration of leaf extracts of cocor bebek (*Kalanchoe pinnata*)

Treatment	Hair length of rabbits (mm)				Mean ± SD
	1	2	3	4	
K-	4.37	5.15	5.86	8.83	6.049±1.948 ^a
E1	6.09	6.19	6.93	11.43	7.660±2.541 ^b
E2	7.18	6.16	7.11	11.86	8.078±2.564 ^b
E3	9.71	9.66	9.42	11.97	10.190±1.193 ^c
E4	10.55	10.78	9.61	11.83	10.689±0.910 ^d
K+	9.84	9.71	9.80	12.63	10.496±1.424 ^{cd}

K- (negative control) is shaved skin treated with CMC gel without plant extracts; K+ (positive control) is shaved area given 2% minoxidil; whereas E1, E2, E3 and E4 are the shaved skin treated with CMC gel containing suruhan extracts 25%, 50%, 75% and 100% respectively. Mean±SD values followed by different superscripts are significantly different at $\alpha=0.05$.

Table-3: Hair length of rabbits on day 21 after daily topical administration of leaf extracts of cocor bebek (*Kalanchoe pinnata*)

Treatment	Hair length of rabbits (mm)				Mean ± SD
	1	2	3	4	
K-	7.55	7.02	8.57	13.94	9.268±3.178 ^a
E1	9.23	9.01	9.81	14.35	10.600±2.523 ^b
E2	13.01	9.31	11.75	14.72	12.198±2.278 ^c
E3	13.48	12.91	12.97	15.31	13.666±1.126 ^d
E4	15.22	14.31	13.43	16.39	14.834±1.266 ^f
K+	13.87	13.76	13.01	16.05	14.169±1.308 ^e

K- (negative control) is shaved skin treated with CMC gel without plant extracts; K+ (positive control) is shaved area given 2% minoxidil; whereas E1, E2, E3 and E4 are the shaved skin treated with CMC gel containing suruhan extracts 25%, 50%, 75% and 100% respectively. Mean±SD values followed by different superscripts are significantly different at $\alpha=0.05$.

Although leaf extracts of cocor bebek significantly affects hair length of rabbits, but the topical administration of the gel does not effective enough to increase hair mass of the test animals.

Statistical analysis results, as shown in Table 4, clearly show an F-value of 1.664 with P =0.30019. These data suggest that CMC gel containing leaves extract of cocor bebek cause less effect on the hair mass in rabbits.

Table-4: Hair mass of rabbits on day 22 after daily topical treatment with leaf extracts of cocor bebek (*Kalanchoe pinnata*)

Treatment	Hair mass of rabbits (mg)				Mean ± SD
	1	2	3	4	
K-	0.24	0.14	0.68	1.16	0.553±0.468
E1	0.25	0.13	0.75	1.22	0.587±0.500
E2	0.46	0.12	0.76	1.26	0.652±0.484
E3	0.43	0.32	0.79	1.31	0.712±0.450
E4	0.54	0.32	0.83	1.41	0.776±0.473
K+	0.37	0.31	0.86	1.38	0.730±0.496

K- (negative control) is shaved skin treated with CMC gel without plant extracts; K+ (positive control) is shaved area given 2% minoxidil; whereas E1, E2, E3 and E4 are the shaved skin treated with CMC gel containing suruhan extracts 25%, 50%, 75% and 100% respectively. Mean±SD values followed by different superscripts are significantly different at $\alpha=0.05$.

DISCUSSION

The findings of this study confirm the greatness of cocor bebek, *Kalanchoe pinnata*, as a plant with many medical uses. The medical benefits of this plant are undoubtedly because these plants are rich in bioactive compounds including alkaloids, triterpenes, glycosides, flavonoids, steroids, bufadienolides, lipids and organic acids, glut-5(6)- and 3-one, taraxerone, 3 β -friedelanol, β -amyrin-3-acetate, and β -sitosterol [10 - 11]. Flavonoid fraction of *K. Pinnata* contains quercetrin and quercetin 3-O- α -L-arabinopyranosyl-(1 \rightarrow 2)- α -L-rhamnopyranoside, these two compounds showed a high gastroprotective effect [12]. Based on an *in vivo* and *in vitro* studies that managed to reveal the antidiabetic action of *K. pinnata* extract, Patil *et al.*, [13] suspected that this plant contains substance similar to the currently used drug glibenclamide [13]. Other compounds extracted from this plant, that is expected to be a key role in the healthcare are campesterol and 5,6,7,8,4' pentahydroxy flavanone due to their antioxidant, antimicrobial and cytotoxic activities [14].

Among the above chemicals, compounds belong to flavonoid group were found to have broad application in cosmetic industry [15]. One characteristic of the flavonoid that supports its usefulness in the cosmetic industry is easy to penetrate the skin tissue [16]. Plant extracts of *Chrysanthemum zawadskii* var. *latilobum* (Asteraceae) and *Polygonum multiflorum* Thunb. (Polygonaceae) have also reported to show anti hair loss activity [17]. Phytochemical analysis of these two plant also found flavonoids as a significant bioactive component [18, 19].

The most common type of hair loss both in men and women is androgenetic alopecia (AGA). AGA is believed associated with the abnormality of androgens such as testosterone (T) and its derivative dihydrotestosterone (DHT). However, recently, authors have argued against the use of the term AGA in women, as the role of androgens in female pattern hair loss is debatable [20]. Beyond the debate on the cause of the androgenetic-related hair loss between male and female, researches on seeking the effective medicine for

promoting hair growth either *in vitro* or *in vivo* using animal models still continues. Among the result of the studies suggested that hair loss can be prevented by eating foods rich in biotin and cystein or using certain topical plant extracts or herbal oils [21-23]. Overall, from a variety of literature review, Semwal *et al.* (2011) stated there are dozens of substances suspected effect on hair growth, including saponin, alkaloids, ecliptine, wedelic acid, luteolin, triterpine, glycosides, β -sitosterol, hentriacontanol, vitamin A, vitamin C, iron calcium oxalic, malic acid, α pinene, β pinene, fatty acid, sterol compounds, polyphenols, steroids, volatile oil and essential oil [24].

CONCLUSION

Although it does not give a clear effect on hair mass, but the leaves extract of cocor bebek, *Kalanchoe pinnata* (Lam.) Pers., significantly improves the hair length of rabbits. Therefore it is inferred that plant extract of cocor bebek is potential to be use as a hair growth promoting agent.

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