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Hair-growth promoting activity of plant extracts of suruhan (Peperomia pellucida) in Rabbits

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Abstract: *Efforts in seeking effective medicine for preventing hair loss and promoting hair growth are still continues worldwide. This study investigate effects of ethanolic plant extract of suruhan, Indonesian name for Peperomia pellucida (L.) Kunth., on rabbit hairs growth. Four healty, male rabbits, aged 4-5 months, weighing 1.5 kg – 2 kg were used for the study. Six areas (2 cm x 2 cm each) on the dorsal aspects of animals were shaved and marked C-, C+, S1, S2, S3 and S4. Area C- and C+ are the shaved skin treated consecutively with CMC gel without suruhan extract (as negative control) and 2% minoxidil (as positive control). Area S1, S2, S3 and S4 are the shaved skin treated with gel containing suruhan extracts of 25% (v/v), 50% (v/v), 75% (v/v) and 100% (v/v) respectively. All treatments were given twice daily for 21 days. The length of hairs were assessed on day 7, 14 and 21, while the hair mass was measured on day 22. The results showed topical CMC gel containing ethanolic plant extracts of suruhan significantly increase the animal hairs length in a concentration-related manner. However, in comparison to both negative and positive controls, suruhan plant extracts show no significant effect on hair mass of the animals. It suggests that ethanolic plant extract of Peperomia pellucida is potent to be used as the topical formulations for hair growth.*

Keywords: *suruhan, Peperomia pellucida, hair loss, hair growth promotion, androgenetic alopecia*

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I. Introduction

In the era of hair care technology that has developed as it is today, many people still suffer from distress condition due to hair loss, thinning and baldness. This is the condition that encourages hair care practitioners around the world to keep looking for anti hair loss including traditional medicine [1,2]. In the last decade there has been a lot of pharmacological research on the hair-growth promoting activities of plant extracts using test animals and even man.

Formulation containing extract of *Hibiscus rosa sinensis*, *Calotropis gigantea* and the combination of both plants extracts reported to show better hair-growth acitivities in stress induced alopecia anmal model by using minoxidil as positive control [3]. Fresh leaf extracts of *Naringi crenulata* also showed hair growth promoting effects in rats [4]. The petroleum ether extract of *G.glabra* showed a higher proportion of anagenic hair follicles, *vis-a-vis* telogenic hair follicles, in female rats [5]. Herbal topical gel containing crude corms extract of pisang kepok (*Musa balbisiana*) significantly increase the hairs length and mass of rabbits in comparison to the normal and negative control.[6] Next, pea sprouts extract[7], *Eclipta alba* extract[8], alcoholic and aqueous extract of *Centella asiatica*, *Cyperus rotundus* and *Emblica officinalis*, also showed hair growth promoting activities in laboratory animals [9]. Pumpkin seed oil extracted from *Cucurbita pepo* L. reported to increases mean hair count up to 40% at 24 weeks in men suffer from androgenetic alopecia (AGA).[10]

In addition to the above mentioned plants, there are still numerous plant species known to be utilized in many folk medicine system, such as suruhan (*Peperomia pellucida*). This plant is reported to posse antipyretic, analgesic, anti-inflammatory, antimicrobial, refrigerant, antioxidant activities, antihyperglicemia, antihyperuricemia, burn healing, depressant effect, gastroprotective, hypotensive, cytotoxic, antisickling cell, lipase inhibitory, fibrinolytic and thrombolytic, antidiarrhoeal, antiosteoporotic.[11-13] *Peperomia pellucida* herbs have also suggested for their cosmetic potential uses such as anti-aging, antiacne, melanogenic and anti-tyrosinase activities.[14] Such cosmetic potentials allegedly related to mineral content such as sodium and potassium[15] and phytochemicals such as campesterol, stigmasterol and β-sitosterol.[16]

Considering plant extract of suruhan, the Indonesian name for *Peperomia pellucida* (L.) Kunth., has been used traditioanlly for cosmetic and contained some chemicals that should be associated with the biological aspects of beauty, this study was intended to investigate promotion effects of the plant extracts given topically on rabbit hairs growth using 2% minoxidil as reference.

II. Materials and Methods

2.1 Plant Sample and Extraction

Plant samples of suruhan (*Peperomia pellucida*) used in the study were collected from suburb of Bandar Lampung City, the province of Lampung, Indonesia. The weeds 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 extract formed.

2.2 Animals and Experimental Design

Four healty, adult, male New Zealand rabbits, aged 4-5 months, weighing 1.5 kg – 2 kg were used for the study. Both during the acclimation period and throughout the treatment, the animals were individually housed in a room with natural light cycle with the temperature range of 25 ^{o}C – 30 ^{o}C and fed with normal diet and water *ad libitum*.

The dorsal aspects of rabbits were divided into six areas, with a size of 2 cm x 2 cm each, on where the test extract were applied. The hairs on each area were shaved using a razor. These areas are then marked as depicted in **Fig.1** as follows: C-, C+, S1, S2, S3 and S4. Area C- and C+ are the shaved skin treated consecutively with CMC gel without suruhan extract (as negative control) and 2% minoxidil (as positive control). Area S1, S2, S3 and S4 are the shaved skin treated with gel containing suruhan extracts of 25% (v/v), 50% (v/v), 75% (v/v) and 100% (v/v) respectively.

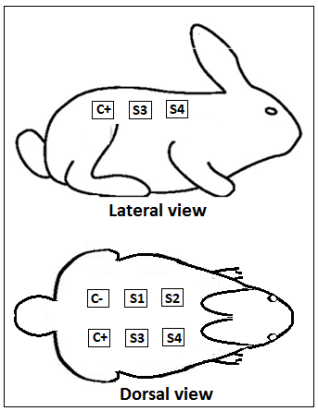

Fig.1 The dorsal aspect of rabbits where the position of treatment area are depicted. C- (negative control): shaved area treated with CMC gel without suruhan extracts; C+ (positive control): shaved skin given 2% minoxidil; S1, S2, S3 and S4 are the shaved area treated with gel containing suruhan extracts of 25% (v/v), 50% (v/v), 75% (v/v) and 100% (v/v) respectively.

2.3 Topical Gel

To make extract ready to be given topically to the test animals, the yielded ethanolic extract of suruhan were suspended in distilled water at concentrations in accordance with those 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 topical gel formed.

2.4 Gel Administrations and Observation

The CMC gel containing extract (0.1g/shaved area) was administered topically on the animal shaved skin, 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 weighed using a digital microbalance.

2.5 Statistical Analysis

The data were described as mean ± standard error (SE). One-way ANOVA and Least Significant Diference (LSD) test was used to determine the statistical significance ($p < 0.05$) of the differences between values of various experimental and control groups.

III. Results

Table 1, 2 and **3** consecutively present descriptive and analytical data of the effects of six different treatments on rabbit hairs growth on day 7, 14 and 21 after daily treatments. The results of one-way ANOVA for the data are as follows. Table 1 resulted in $F = 153{,}976176$ with $P < 0.001$; Table 2 resulted in $F = 201{,}893849$ at $P < 0.001$; and Table 3 resuted $F = 105{,}384414$ and $P < 0.001$. LSD test on the mean values

between treatments for the data in Table 1, 2 and 3 suggest that CMC gel containing ethanolic plant extracts of *Peperomia pellucida* significantly increase the animal hairs length in comparison to the negative control. However, when compared to the positive control (2% minoxidil), the effect of the suruhan extracts even at the highest concentration (100%), are statistically lower. **Table 4** presents effects of suruhan extracts on the rabbit hair mass after 21 days of treatment. The one-way Anova results in $F = 1{,}40644621$ and $P > 0{,}260$. These data suggest that CMC gel containing extract of suruhan cause less effects on the hair mass in rabbits.

Table 1 Rabbit hairs growth on day 7 after daily topical treatment with plant extracts of suruhan (*Peperomia pellucida*)

Treatment	Hairs lenght of rabbits (mm)				Mean + SD
	1	2	3	4	
C-	2,18	1,99	2,21	2,16	2,135±0,099[a]
S1	2,96	3,69	3,15	3,57	3,343±0,344[b]
S2	3,64	3,93	3,48	4,03	3,770±0,254[c]
S3	4,52	4,37	4,31	4,94	4,535±0,284[d]
S4	5,62	5,78	5,63	5,81	5,709±0,101[e]
C+	6,09	5,715	5,689	5,79	5,821±0,184[e]

Mean±SE values followed by the same superscript are not differ at $\alpha = 0.05$. C- (negative control) is shaved skin treated with CMC gel without plant extracts; C+ (positive control) is skin given 2% minoxidil; whereas S1, S2, S3 and S4 are the shaved skin treated with CMC gel containing suruhan extracts 25%, 50%, 75% and 100% respectively.

Table 2 Rabbit hairs growth on day 14 after daily topical treatment with plant extracts of suruhan (*Peperomia pellucida*)

Treatment	Hairs lenght of rabbits (mm)				Mean + SD
	1	2	3	4	
C-	4,72	4,27	4,22	4,48	4,421±0,229[a]
S1	6,33	6,61	7,08	6,85	6,716±0,324[b]
S2	6,86	6,91	7,22	6,99	6,995±0,159[c]
S3	7,98	7,90	7,94	8,01	7,956±0,050[d]
S4	8,52	8,29	8,45	8,21	8,368±0,142[e]
K+	8,87	8,43	8,93	8,39	8,655±0,284[f]

Mean±SE values followed by the same superscript are not differ at $\alpha = 0.05$. C- (negative control) is shaved skin treated with CMC gel without plant extracts; C+ (positive control) is skin given 2% minoxidil; whereas S1, S2, S3 and S4 are the shaved skin treated with CMC gel containing suruhan extracts 25%, 50%, 75% and 100% respectively.

Table 3 Rabbit hairs growth on day 14 after daily topical treatment with plant extracts of suruhan (*Peperomia pellucida*)

Treatment	Hairs lenght of rabbits (mm)				Mean + SD
	1	2	3	4	
C-	6,13	6,21	6,26	6,22	6,205±0,054[a]
S1	8,48	8,74	8,82	8,66	8,675±0,145[b]
S2	9,74	9,53	9,25	9,17	9,423±0,262[c]
S3	10,67	10,38	10,66	10,36	10,518±0,171[d]
S4	11,58	10,77	11,32	10,43	11,025±0,521[e]
C+	11,63	10,96	12,07	10,60	11,315±0,660[e]

Mean±SE values followed by the same superscript are not differ at α =0.05. C- (negative control) is shaved skin treated with CMC gel without plant extracts; C+ (positive control) is skin given 2% minoxidil; whereas S1, S2, S3 and S4 are the shaved skin treated with CMC gel containing suruhan extracts 25%, 50%, 75% and 100% respectively.

Table 4 Rabbit hairs mass on day 22 after daily topical treatment with plant extracts of suruhan (*Peperomia pellucida*)

Treatment	Hairs mass of rabbits (mg)				Mean + SD
	1	2	3	4	
C-	0,024	0,038	0,037	0,038	0,0343±0,007
S1	0,023	0,043	0,040	0,042	0,0372±0,009
S2	0,035	0,061	0,039	0,046	0,0452±0,011
S3	0,032	0,044	0,059	0,048	0,0459±0,011
S4	0,038	0,052	0,043	0,049	0,0457±0,006
C+	0,038	0,053	0,043	0,050	0,0460±0,006

Mean±SE values followed by the same superscript are not differ at α =0.05. C- (negative control) is shaved skin treated with CMC gel without plant extracts; C+ (positive control) is skin given 2% minoxidil; whereas S1, S2, S3 and S4 are the shaved skin treated with CMC gel containing suruhan extracts 25%, 50%, 75% and 100% respectively.

IV. Discussion

Though whole plant extract of suruhan show insignificat effects on hair mass of treated rabbits (**Table 4**), but as can be seen in **Table 1, 2,** and **3,** the extracts significantly affect hair growth of the animals in a concentration-related manner. What biochemical aspects of suruhan are that allow this plant extracts to affect hair growth? Some phytochemical screening studies reported that *Peperomia pellucida* plant extracts contain a high-value mineral composition comprising potassium, calcium and iron as the main elements that make this weed would serve as a good source of protein and energy as well as micronutrients.[17]

In addition to mineral elements such as sodium, potassium, calcium, zinc, iron, manganese, lead and phosphorus [18], *Peperomia pellucida* extracts also contain stigmasterol, analogue of pheophytin and b-sitosterol-D-glucopyranoside [19], alkaloid, glycoside, reducing sugar, flavonoid, tannin, steroid, terpenoid, α-amino acid, neutral compound, phenolic compound and starch.[20] By extracting other species of *Peperomia* from Peru, Lira and colleagues found β-caryophyllene, α-humulene, epi-α-bisabolol, sabinene, cryptone and caryophyllene oxide.[21]

Some of above mentioned chemicals and derivatives are known to be contained in other plant species that have been suggested as hair-growth promoter herbs including *Ocimum gratissum*, *Cucurbita pepo, Wedelia sp.* The phytochemicals are including phenolics compounds, tocopherols, sterols [22]; β-myrcene, α-terpinene, γ-terpinene, ortho-lymene, α-bergamotene, trans caryophyllene, α-humulene, aromadendrene, 3-decynex, thymol and carvocrol [23]; eugenol, carvacrol, nerol & eugenol methyl ether.[24] From *Cucurbita maxima* researchers found both unsturated and sturated fatty acid such as linoleic, oleic, palmitic and stearic acids; several sterols including α-tocopherol, stigmasterol, brassicasterol, campesterol, sitostanol, avenasterol, erythrodiol, uvaol and β-sisosterol; several phenolic acids such as protocatechuic, caffeic, syringic, vanillic, p-coumaric and ferulic.[25, 26]

By treating rats *in vivo* using mixture of local myrtus, galls and oak extracts, Khidhir and Mahmood [27], suggested that the effect of the extracts on hair growth may be mediated through the regulation of growth factors in dermal papilla cells. Such plants, Myrtus for example, containing citric acid, malic acid, resin, tannin, sugar, flavonoids, anthocyanin arabinosides, anthocyanin glucosides, kaempferol, quercetin, myricetin 3-o-glucoside, myricetin 3, 3-di-o-galactoside, myricetin 3 rutinoside, aesculin, scopoletin, caffeic acid, myricetin 3-o-rhamnoside or myricitrin, esculetin-6-oglucoside or esculin, hesperetin 7-o-rhamnoglucoside or hesperidin, hesperetin-2-o-methylchalcone-4-orhamnoglucoside. The essential oil content including 1, 8-cineole, α-pinene, methyl eugenol, terpineole, trans-carveole,cis-carveole, geraniol, methyl geranate, α-terpinyl acetate, neryl acetate, β-caryophyllene, myrcene, sabinene, myrcene, p-cymene, c-terpinene, linalyl acetate, car-3-ene, phellandrene, methyl eugenol, methyl butyrate, methyl benzoate, benzyl alcohol, isobutyl butyrate, myrtenylacetate, limonene, α-terpineol, linalool, eucalyptol, p-cymol, β-pinene, geraniol, camphene, butyl butyrate and myrtenol. [28]

Overall, from a variety of literature review, Semwal et al. [29] 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.

V. Conclusion

Topical gel containing ethanolic plant extracts of suruhan of all concentration levels applied in this study showed a higher mean of hair length in rabbits, so it inferred that crude extract of Peperomia pellucida is potent to be used as alternative herbal for the hair growth promotion.

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