

In vivo evaluation of hair growth potential of *Stachytarpheta jamaicensis* ethanolic leaves extract on sprague-dawley rats

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ABSTRACT

This study was carried out to determine the effectiveness of *S.jamaicensis* leaves extract as hair growth promoter on Sprague Dawley rats. The rats were divided into three groups; control group, positive control group and test group which received no treatment, 2% minoxidil, and *S.jamaicensis* leaves extract respectively. All of the treatments were done in 30 days. The length of the hair was measured every five days. Primary irritation test shows that there was no erythema or edema on the rats after being applied with 10% concentration of extract. Hair length with the most significant value was on rats treated with 2% minoxidil (14.58 ± 0.02 mm) towards day 30, followed by 2% extract (14.23 ± 0.05 mm), and control group (11.07 ± 0.14 mm). Histology was done every ten days to determine the anagen to telogen ratio hair phases. Anagen phase on day 30 reached 96% of rats treated with 2% minoxidil, followed by 84% with 2% extract, and control group, 83%. In conclusion, *S.jamaicensis* is an effective hair growth promoter as hair length treated with *S.jamaicensis* leaves extract showed significant difference (p value=0.05). This may be due to the presence of palmitic acid that has antiandrogenetic alopecia property. © 2014 Trade Science Inc. - INDIA

KEYWORDS

Stachytarpheta jamaicensis;
in vivo;
Hair;
Ethanolic extract

INTRODUCTION

Patterned hair loss, is an unwelcome event that may cause anxiety and concern^[1]. This problem occurs up to 50 % of people throughout their lifetime^[2]. Even though hair disorders are not life threatening, its profound impact on social interactions and on patients' psychological wellbeing is no longer undeniable^[3]. According to^[4], American Hair Loss association reported that hair loss has been emotionally disturbing and distressing besides making the affected person vulnerable in terms of physically and mentally.

Thinning of the scalp hair in middle aged as well as the older women had become a phenomenon nowadays.

Several researches have suggested that hair loss conditions are caused by hormonal imbalance or stress, and nutrition intake. In addition, other possible cause of hair loss is the long-term use of hair dyes, indirectly termed as 'colourants'. Interference with the hair function may cause diminished rate of growth and renewal, leads to a lesser number of hairs per unit area^[5]. Recent studies demonstrate that even taking medicines may cause hair loss. However, it may also occur in response to a number of triggers including fever, hemorrhage, severe illness, stress, and childbirth^[6]. The most common hair loss (alopecia) is caused by abnormal hair follicle cycling and changes in the hair follicle structure itself.

One of the products that can be found in the mar-

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ket to help preventing baldness is minoxidil. Minoxidil act as potassium channel opener and proved to be effective in 54% of all the treated subjects^[7]. However, the use of minoxidil has many side effects. A study revealed that taking either oral or topical minoxidil can lead to hypertrichosis^[8]. Another study reports that mothers using topical minoxidil had a fetus born with aplasia of the lower body part (caudal regression syndrome)^[9]. Hence, there is a need to develop plant based medicines as an alternative source for promoting hair growth. *Stachytarpheta jamaicensis* or also known as Bastard vervain and Brazilian tea, is believed to have hair growth properties. It is belong to the family of Verbenaceae. This plant is an annual weedy herbaceous plant, and reproduced from the seeds^[10]. In this study, ethanolic extract of *Stachytarpheta jamaicensis* was used to determine its potential as hair growth promoter.

EXPERIMENTAL

Extraction of *Stachytarpheta jamaicensis* leaves

An amount of 600 g shade-dried ground plant material of *S. jamaicensis* was extracted with 6 L of ethanol solvents for 48 hours at 60 °C–75 °C. The leaves extract was filtered by using Whatmann No. 1 filter paper and then separated by using a rotary evaporator at 40 °C. A sticky semi-solid material was obtained and kept at 4 °C prior to use.

Animals

Healthy male Sprague Dawley rats, weighing 120–150 g, were used for the study. The animals were kept at the experimental cabin under standard environmental conditions and were allowed for free access normal diet and water ad libitum. The animals were housed in cages at room temperature of 25 ± 3 °C on a normal day-night cycle^[11].

Primary skin irritation test and in vivo hair growth activity

One rat was selected at random and 4 cm² of the hair from the dorsal area of the Sprague-Dawley rat was shaved until complete removal of hair was obtained. The area was cleaned using alcohol swab. The dorsal area was applied with 1 ml of 2 % of the extract and signs of toxicity were determined based on standards

for skin irritation by^[12]. The dose was increased to 10 % to find signs of toxicity. Another rat tested with 2 % minoxidil served as the positive control.

In vivo hair growth activity included 18 rats. 4 cm² of hair from the dorsal area of the rat was shaved and was cleaned by using alcohol swab. The rats were divided into three groups; control group, positive control group and test group which received no treatment, 2% minoxidil, and *S. jamaicensis* leaves extract respectively. All of the groups had been given the same concentration of treatment. The concentration was determined based on primary skin irritation test. The treatment was continued for 30 days based on^[7].

Hair length determination

The hairs had been plucked from the shaved area of each rat from each group. This was done on the 10th, 15th, 20th, 25th, and 30th day of treatment by using sterile forceps. The average length in millimeter of the hair had been calculated.

Histological studies and determination of percentage of anagen and telogen phases

On 10th, 20th, and 30th day of the treatment, one rat from each group undergone euthanication. The skin biopsies were taken from the shaved area. The histological study was carried out. Anagen and telogen were calculated per 4cm² area. Data was then been tabulated as in Figure 2.

Identification of *S. jamaicensis* leaves extract

GC-MS analysis was performed using an Agilent-5973 network system. A mass spectrometer with an ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic column used for the analysis was an HP-5 capillary column (30 m × 0.32 mm i.d., film thickness of 0.25 μm). Helium was used as a carrier gas at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. With the column held initially at 60 °C for 2 min, 1 μL of essential oil solution in hexane (HPLC grade) was injected and analyzed, and then the temperature was increased to 240 °C with a 3 °C/min heating ramp

Statistical analysis

Data were expressed as mean ± S.E.M. The vari-

ance of the data was statistically evaluated by ANOVA via SPSS software 18th edition. Value of $p \leq 0.05$ was considered statistically significant.

RESULTS & DISCUSSION

Skin irritation study analysis

Rat dorsal portion was applied with the *S. jamaicensis* leaves extract to observe for any appearance of toxicity. The skin was analyzed after 24 and 48 hours interval. The extract concentration was increased from 2 % to 10 %. Observation on both group of rats treated with extract and minoxidil showed no indication for appearance of erythema and edema. Even when the doses of *S. jamaicensis* leaves extract were increased up to 10 % concentration, the rat's skin was still tolerable to the concentration given.

Analysis of hair growth rate

For post-natal adults, the hair growths are theoretically occurring at two processes: follicular neogenesis and telogen to anagen transition of the pre-existing hair follicle units^[13]. The anagen phase's duration will determine the length of the hair and also will depend on the continuous proliferation and differentiation of matrix cells at the follicle base as reported by^[14]. Based on Figure 1, the hair length on day 5 and 10 gave a significant difference on rats applied topically with 2 % *S. jamaicensis* extract. There was also significant increment shown in day 15, 20, 25, and 30 by 2 % minoxidil. *S. jamaicensis* with 2 % concentration extract showed significant difference, $p=0.05$ when compared to control group.

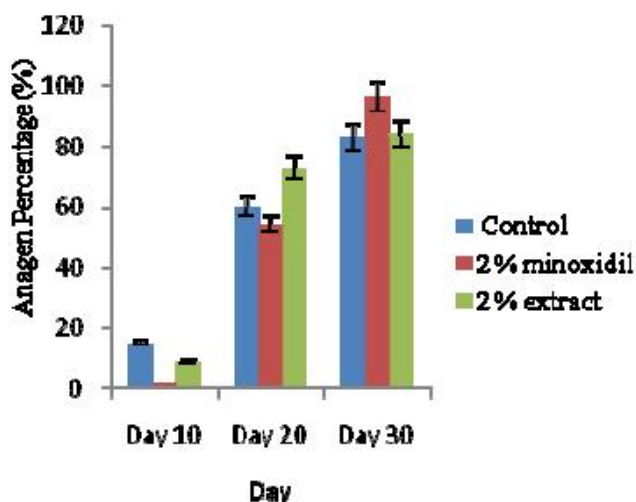


Figure 1 : Graph of hair length (mm/day) against day

Histological evaluation and the percentage of anagen and telogen phases

In order to produce new hair, the existing hair follicles need to undergo cycles of growth (anagen), regression (catagen) and rest (telogen). Histologically, anagen phases are characterized as long and straight follicles, with the follicles angled for the hair coat to lie flat along the body surface^[14]. Besides that, the hair bulb is rigid and present deep in the subcutis layer^[15].

Figure 2 explains on anagen percentage obtained from the histological studies as depicted in Figure 3. The histological study was done every 10 days based on^[4]. Since catagen phase lasts only 3-4 days in mice^[14] therefore the catagen phases is excluded from being counted in the histology slide as also being done by^[16]. Figure 3 shows anagen phase percentage is increasing from day 10 to 30 while the telogen phase is decreasing which was *vice versa* with anagen phase^[16]. Even though the hair length (Figure 1) showed to have significant increment, but the percentage of anagen phase of hair follicle (Figure 2) for group treated with 2% extract of *S. jamaicensis* marked slight difference to the control group. This might be due to the time limitation of the matrix cells of the follicle base to have continuous proliferation and differentiation hence reducing the number of anagen in the cells.

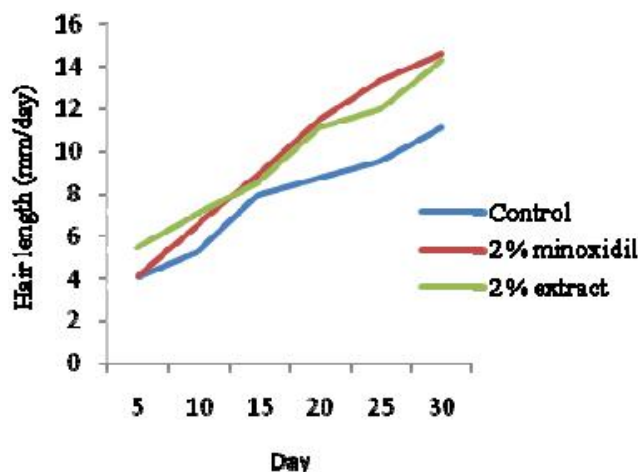


Figure 2 : Graph of anagen percentage (%) after 10, 20, and 30 days of treatment

Figure 3 shows the histological phases of anagen and telogen in control group, positive control group and treated group. For these figure, anagen phase was identified by the presence of inner and outer root sheath. Anagen hair consists of surrounding connective tissue

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and three groups of cell layers which are the hair shaft, inner root sheath (IRS), and outer root sheath (ORS). The hair shaft has the multicellular cortex encased in a cuticular layer of flattened cells, with the medulla layer being placed centrally in the cortex^[17]. Telogen phase was characterized by wrinkling of inner and outer root sheath as well as dermal papilla been released out as shown in Figure 3(c), 3(e) and 3(f). The hair bulb was shrunken and located in the dermis above the subcutis layer^[15]. In day 30 as depicted in Figure 3(g), 3(h) and

3(i), most of the hair phases were in anagen phase as being predicted in the study as further supported by^[18].

Identification of *S. jamaicensis* leaves compound

The compositions of *S. jamaicensis* leaves compound was determined by GC-MS and summarized as in TABLE 1 below. Palmitic acid or chemically known as n-hexadecanoic acid contains antiandrogenetic alopecia property^[19] which is needed to prevent baldness.

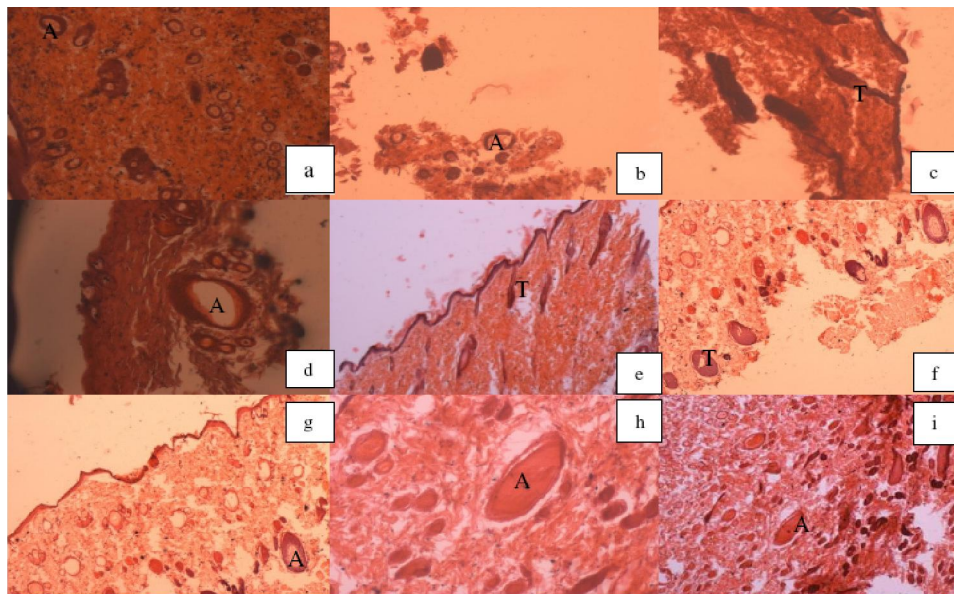


Figure 3: Day 10 (a) Anagen phase of control group (b) Anagen phase of rat applied with 2 % minoxidil (c) Telogen phase of rat applied with 2 % extract; Day 20 (d) Anagen phase of control group (e) Telogen phase of rat applied with 2 % minoxidil (f) Telogen phase of rat applied with 2 % extract; Day 30 (g) Anagen phase of control group (h) Anagen phase of rat applied with 2 % minoxidil (i) Anagen phase of rat applied with 2 % extract; A= anagen, T= telogen.

TABLE 1 : Compounds present in *S. jamaicensis* leaves ethanolic extract from GC-MS analysis

No	Retention time	Area	Library/ ID	Quality	Common name
1	11.985	5.93	Cyclopenta[c] pyran-4-carboxylic acid, 7 methyl-, methyl ester	94	Genipin
2	17.386	11.25	n-Hexadecanoic	96	Palmitic acid
			Tridecanoic acid	91	Tridecanoic acid
3	18.800	13.18	Phytol	93	Phytol
4	19.097	15.68	9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)-	91	Alpha-linolenic acid
			Cis,cis,cis-7,10,13-Hexadecatriena	91	Alpha-linolenic acid
			9,12,15-Octadecatrien-1-ol,(z,z,z)-		

CONCLUSION

There was significant difference in the hair length between 2% extract of *S. jamaicensis* and control group. As a conclusion, *S. jamaicensis* ethanolic leaves extract gave promising result to become an alternative plant based hair growth promoter most probably due

to the presence of palmitic acid acted as antiandrogenetic alopecia property.

ACKNOWLEDGEMENTS

This project was financially supported by Faculty of Applied Sciences, Universiti Teknologi MARA and RIF Grant (600-RMI/DANA 5/3/RIF (65/2012)).

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