



HAIR GROWTH ACTIVITY AND ANTIOXIDANT ACTIVITY OF *ANGIOPTERIS EVECCTA* (A EVECCTA)

Khin Phyu Phyu, Myo Nanda Aung*, Maung Maung Mya, Aye Win Oo, Mu Mu Sein Myint, Khine Khine Lwin, Phyo Wai Zin

Department of Medical Research.

*Corresponding Author: Myo Nanda Aung

Department of Medical Research.

Article Received on 25/04/2019

Article Revised on 15/05/2019

Article Accepted on 05/06/2019

ABSTRACT

One of the problems often experienced by humans is baldness or hair growth that is not normal. *Angiopteris evecta* is one of the traditional indigenous medicinal plants which have many pharmacological properties. Hair growth activity of *A evecta* was determined in adult rabbit. Skin irritation test of rabbit was carried out by using ethanol and watery extracts of *A evecta*. Irritation degree was negligible. Mean length of hair growth for negative control was 0.74 cm and that of standard minoxidil was 1.5 cm. For watery extract, mean length of hair growth of 10 %, 20 % and 40 % were 0.82 cm, 1.28 cm and 1.4 cm, respectively. For ethanolic extract, mean length of hair growth of 10 %, 20 % and 40 % were 0.81 cm, 1.29 cm and 1.39 cm, respectively. The rabbit treated with ethanolic and watery extracts of *A evecta* showed increased in hair length when compared with those of control group ($p < 0.01$). Therefore, it can be concluded that ethanolic and watery extracts of *A evecta* had increased in hair growth activity. In antioxidant activity, IC_{50} values of watery extract of *A evecta* was 1.5595mg/ml and IC_{50} values of ascorbic acid was 1.1040 mg/ml. *A evecta* rhizome contained high amount of Zn (51.32 ± 0.01 ppm) and Fe (25.28 ± 0.02 ppm) which were above the permissible limits set by WHO, 2008 in edible plants (Fe=20 ppm and Zn=27.4 ppm) and the absence of toxic contaminants (Cd, Cr, As and Pb) in their rhizomes of *A evecta*.

KEYWORDS: *Angiopteris evecta*, hair growth activity, antioxidant activity.

INTRODUCTION

One of the problems often experienced by humans is baldness or hair growth that is not normal. Baldness is usually caused by hormonal disorders, side effects of medications, food intake, dermatological diseases, infections and stress.^[1] Baldness (alopecia) can be on all the hair on the body (alopecia universalis) or the whole head of hair (alopecia totalis). The cause of this disease is still unclear. Androgenetic alopecia is one of alopecia which is prevalent in the community.^[2] Various cosmetic products have been developed to overcome the problem of hair loss and baldness, both derived from synthetic materials or from natural materials. It has been proven that synthetic materials are used and they can have side effects such as local irritation and erythema.^[3] According to the Food and Drug Administration (FDA) minoxidil is a safe and effective drug that is given for androgenetic alopecia patients.^[4]

There has been a global trend toward the use of natural substance present in medicinal plants and dietary plants as therapeutic antioxidants. Many antioxidant compounds, naturally occurring in plant sources have

been identified as free radical or active oxygen scavengers. This species is also known to be of importance for its ethno-medicinal uses. People use to take the rhizome of *Angiopteris evecta* with honey for longevity. Myanmar is rich in plants and many of which have medical values. Among them, *A evecta*, Family - Marattiaceae is one of the traditional indigenous medicinal plants which have many pharmacological properties such as antimicrobial, antihyperglycemic, analgesic activities. *A evecta* can also reduce hair loss, improve and nourish hair.^[5] This study was to evaluate hair growth activity and antioxidant activity of rhizomes of *Angiopteris evecta* (*A evecta*).

Objectives

1. To assess the skin irritancy potential and hair growth activity of ethanol and watery extracts of *A evecta*.
2. To compare the hair growth activity of different concentrations of *A evecta* extracts with 2% minoxidil.
3. To determine the antioxidant activity and heavy metals (Cd, Pb, As, Cr, Cu, Fe, Zn and Mn) of rhizome of the *A evecta*.

MATERIAL AND METHODS

Sample collection and identification

The plant material used in this study was *A evecta* (*A evecta*) which was obtained from Western Bago Yoma. Plant was collected and identified by a competent taxonomist from the Department of Botany, West Yangon University, Yangon.

Laboratory Animal

Experimental animals used were 6 male albino rabbit, 4-5 months old, obtained from Laboratory Animal Services Division. Male rabbits were used as test animals because male rabbits are more stable than female rabbits in hormonal changes.

Place of study

Department of Medical Research, Ministry of Health and Sports, Myanmar.

Study design

Laboratory based control parallel study design.

Study period

August 2016 to August 2017.

Extraction method

Hundred grams of dried rhizome powder of *A evecta* was extracted with 96 % ethanol by soxhlet apparatus for 8 hours and filtered. Filtrate was evaporated by rotary evaporator at 70 °C and ethanolic extract was placed in desiccator.

Hundred grams of dried rhizome powder of *A evecta* was extracted with 1000 mL of distilled water by different temperature for two activities. Control water bath for 6 hours at 60°C for antioxidant activity and 90°C for 8 hours for hair growth activity. Filtrates were separately evaporated by freeze dryer (Operon, -70 °C) and dried extracts were placed in desiccator.

Preparation of test solutions fir hair growth activity

Different concentrations of 10%, 20% and 40% *A evecta* watery and ethanolic extract solution were prepared. 500 mg of Carboxymethyl cellulose and sprinkled over hot water stirring vigorously until completely mixed and 10, 20, 40 g of *A evecta* extract was added and then 100 ml of distilled water was added. Then mix until homogeneous solution by using vortex mixer.

Testing skin irritancy potential

Safety was evaluated based on the Primary Irritation Index (PII) by Draize test⁽⁸⁾. Three male rabbits were kept acclimation period for 7 days. The area on the back of rabbit was shaved prior to the experiment. The shaved areas of skin were divided into three marked areas, (4 x4) cm for each. The two marked areas were used for application of ethanol and watery extracts of *A evecta*, respectively and the remaining area was used as blank sample. Each 0.5 g of the watery and ethanolic extracts was applied to specific shaved areas and observed at 24

hours, 48 hours and 72 hours respectively. Erythema and edema were evaluated according to the scoring system for skin reactions.^[6]

Table 1: Classification system for skin reaction.

Reaction	Score
Erythema	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to eschar formation	4

The Score of Primary Irritation (SPI) was calculated for the rabbit as the following. Scores for erythema and edema at 24, 48 and 72 hours were summed and divided by the number of the observations for the treated sites.

$SPI \text{ for rabbit} = \frac{\sum \text{Erythema and edema grade at 24, 48 and 72 hrs}}{\text{Number of observation}}$

The difference between the SPI scores from the treated site and control site was calculated and used for Primary Irritation Index determination. The irritation degree was categorized as negligible, or slight, moderate or severe irritation based on the PII (Table 2).

Table 2: Response categories of irritation in rabbit.

Category	Primary Irritation Index (PII)
Negligible	0-0.4
Slight irritation	0.5-1.9
Moderate irritation	2-4.9
Severe irritation	5-8

Testing on hair growth activity with different extracts of *A evecta*

Rabbits were used 3 male, aged 4-5 months. Prior to use, rabbit were acclimatized for 7 days, to get used to live in the neighborhood and the new treatment. Then backs were cleaned of feathers and rested for 24 hours. The three rabbits were sheared back and divided into four plots with a size of 2 x 2 cm and given treatment as follows. For the first rabbit, plot I was given 2% minoxidil solution as a positive control, in plot II; treatment was not given as a negative control, in plot III; was given *A evecta* ethanolic extract 40% and in plot IV: *A evecta* watery extract 40% was given respectively.

For the second rabbit, plot I: was given 2% minoxidil solution as a positive control, plot II: treatment was not given as a negative control, plot III: *A evecta* ethanolic extract 20%, plot IV: *A evecta* watery extract 20% was given respectively.

For the third rabbit, plot I: 2% minoxidil solution as a positive control, plot II: treatment was not given as a negative control, plot III: *A evecta* ethanolic extract 10%, plot IV: *A evecta* watery extract 10% was given

respectively. Both one mL of each *A. evecta* extract was given 2 times a day. Hair length was measured on every 3 days for 18 days. Five strands of hair were used to measure the length of hair in each plot and average length of the five strands was calculated. The way the measurement was to measure the length of the hair using a caliper with cm units.

Data Analysis

Having obtained the data from the research, the data processing was done by using statistical analysis of variance (ANOVA) ⁽⁷⁾ for hair growth activity and elements concentration data were analyzed by using, Microsoft Excel version 2007. Results were presented as mean± SD. 50% Inhibitory Concentration (IC₅₀) was

calculated by regression analysis and STATA Version.11.

Measurement of DPPH Radical Scavenging Activity and heavy metals

Stock solution of 100 mg of watery extract of *A. evecta* was dissolved in 10 mL of 50% ethanol. Different concentrations of test solution; 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10 µg/mL were prepared with 50% ethanol by serial dilution. Two mL of different concentrations of test solution was mixed with 2 mL of 60 µM DPPH solution and vortex and kept for 30 minutes in dark container. Radical Scavenging Activity was measured by Spectrophotometer. Heavy metals *A. evecta* was measured by ICPOES.

RESULTS AND DISCUSSION



Figure 1: The whole plant of *Angiopteris evecta* and its rhizome.

The yield percentage of 96 % ethanol and watery extracts was 4.96% and 4.13 % respectively.

Phytochemical analysis of *A. evecta* revealed that flavonoids, polyphenols, tannins, saponins, amino acid, glycosides, carbohydrate and reducing sugar were present, and alkaloids, steroids, triterpene, cyanogenic glycoside compounds were not present.

In antioxidant activity, IC₅₀ values of watery extract of *A. evecta* was 1.5595mg/ml and IC₅₀ values of ascorbic

acid was 1.1040 mg/ml. *A. evecta* rhizome contained high amount of Zn (51.32±0.01 ppm) and Fe (25.28±0.02 ppm) which were above the permissible limits set by WHO, 2008 in edible plants (Fe=20 ppm and Zn=27.4 ppm) and the absence of toxic contaminants (Cd, Cr, As and Pb) in their rhizomes of *A. evecta*.

Skin irritation testing on rabbits

The primary irritation score for the watery and ethanolic extract were found 0.1 and 0.2 respectively and which were falls under the category of negligible irritant.

Table 3: Score of irritation and edema after application of watery and ethanolic extract of *A. evecta*.

Rabbit no.	Reaction	Score for skin irritation								
		Watery extract			Ethanolic extract			Negative control		
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
1.	Erythema	0	0	0	1	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0
2.	Erythema	1	0	0	0	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0
3.	Erythema	0	0	0	1	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0	0

Hair Length measurement results can be seen in Table 4 and 5. The process of measuring the five strands of hair did to get great results because of long rabbit hair was not all the same. With the calculated average of the five strands of hair was able to yield very good results.

Based on table 4 and 5, the test extract and positive control showed an increase in rabbit hair length compared with negative control. On the third day there had been a difference between the extract test 40%, 20% and 10% with a negative control.

Table 4: Hair length of rabbit treated with different percentage of ethanolic extracts and 2% minoxidil.

Group	Mean Hair Length (cm)					
	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
Positive Control (2% Minoxidil)	0.27	0.47	0.68	0.92	1.21	1.5
Negative Control	0.07	0.17	0.33	0.46	0.69	0.74
Ethanolic Extract 10%	0.08	0.2	0.36	0.5	0.74	0.81
Ethanolic Extract 20%	0.18	0.37	0.55	0.82	1.1	1.29
Ethanolic Extract 40%	0.26	0.45	0.63	0.88	1.16	1.39

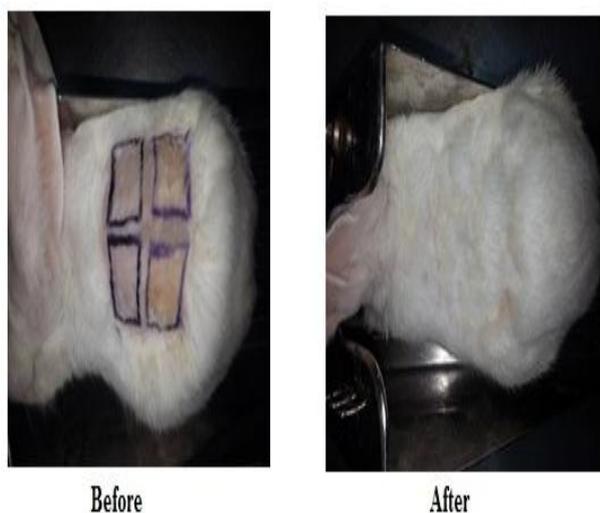
On day 18, mean hair lengths of positive group was significantly increased to compare with negative control ($p < 0.05$). In 20% and 40 % ethanolic extract groups showed statistically not significant compare with positive control ($p > 0.05$). That means hair growth activities of

20% and 40 % ethanolic extract groups were comparable with 2 % minoxidil group. Whereas hair growth activity of 10% ethanolic extract group was not potent as 20% and 40% ethanolic extract groups.

Table 5: Hair length of rabbit treated with different percentage of watery extracts and 2% minoxidil.

Group	Mean Hair Length (cm)					
	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
Positive Control (2% minoxidil)	0.26	0.49	0.68	0.91	1.22	1.47
Negative Control	0.06	0.15	0.33	0.43	0.65	0.74
Watery Extract 10%	0.08	0.18	0.38	0.54	0.75	0.82
Watery Extract 20%	0.18	0.37	0.55	0.82	1.1	1.28
Watery Extract 40%	0.24	0.44	0.59	0.89	1.17	1.4

On day 18, compared with negative control group, mean hair lengths of positive group was significantly increased ($p < 0.05$). Both 20% and 40 % watery extract groups showed statistically not significant compare with positive control ($p > 0.05$). Hair growth activity of 2 % minoxidil group was nearly the same as that of 20% and 40 % watery extract groups. Among the three different concentrations, 40% of watery and ethanolic extracts gave the best hair growth activity.

**Figure 2: Hair length of rabbit treated with different percentage of ethanolic extracts and 2% minoxidil.****Figure 3: Hair length of rabbit treated with different percentage of watery extracts and 2% minoxidil.**

Ability as a hair grower compounds likely caused secondary metabolites contained in *A. eveccta* roots namely quinone, flavonoid, monoterpenes, sesquiterpenes, Polyphenol, tannins and triterpenoids. Polyphenol compounds work to help fight the formation of free radicals. Polyphenols as well as flavonoid have the ability to change or reduce free radicals and also as an anti-free radical.^[9] One of the causes of hair loss is a free radical, so that the polyphenol compounds present in *A. eveccta* roots can prevent free radicals and accelerate the growth of hair. In addition tannin compounds have properties that can bind to and protect protein. Protein is one of the molecules necessary for the hair to grow. According to Kurniawan,^[10] saponins on the human body works to increase blood flow to the hair follicles, when blood flow to the follicles Hair is reduced then it will affect the hair follicles and causes hair loss. Sa'diahet

al.^[11] had performed a negative control using ethanol 70% and showed growth similar to normal hair growth. It is assumed that the ethanol used did not have the effect of hair growth.

CONCLUSION

The rhizomes of *A. evecta* had high antioxidant activity like ascorbic acid and it contained high amount of zinc which enhance hair growth activity. In this study, 40% ethanol and watery extracts showed good hair growth activity comparable as minoxidil.

ACKNOWLEDEMENT

I would like to thank Director General (Department of Medical Research) for allowing me to perform this research work. I am also grateful to the staff of Pharmacology Research Division and Laboratory Animal Services Research Division for their help in this study.

REFERENCES

1. Derm Net NZ. Hair loss, balding, hair shedding. Archived from the original on 14 November 2007; Retrieved, 2007-12-07.
2. StoughD, Stenn K, Haber R, Parsley W.M, Vogel J.E, Whiting D.A, Washenik K ,Mayo ClinProc, 2005; 80(10): 1316-22.
3. Resmi M, Wiwiek I, Abdul M and Danni R. Activity of *A. evecta* for baldness treatment. *Journal of Chemical and Pharmaceutical Research*, 2016; 8(5): 821-830.
4. Varothai, S, Bergfeld, W.F. "Androgenetic alopecia: an evidence-based treatment update." *American journal of clinical dermatology*, 2014; 15(3): 217–30.
5. Ashin Naga Thain. Illustrated Medical Dictionary, (2).
6. More B.H, Sakharwade S.N, Tembhone S.V, Sakarkar D.M, Evaluation for skin irritancytesting of formulations containing extract of *Buteamonosperma* for its topical application. *International Journal of Toxicology and Applied Pharmacology*, 2013; 3(1): 10-13.
7. Hossain A.I, Faisal M, RahmanS, Jahan R, Rahmatullah M, *BMC Complement Alternat Med*, 2014; 14: 169-73.
8. Draize J.H, Woodward G, Calvery H.O. Methods for the study of irritation and toxicity ofsubstances applied topically to the skin and mucous membrane. *The Journal of Pharmacology and Experimental Therapeutics*, 1944; 82: 377-390.
9. Robinson T. Kandungan O. T. T, Penerbit I.T.B, Bandung, 1995; 85-95.
10. Kurniawan P. Daun W.M.R, Dan M.H. Available at. [http://www.tabloidcempaka.com/index.php/read/kesihatan/detail/101/Daun-Waru Menumbuh](http://www.tabloidcempaka.com/index.php/read/kesihatan/detail/101/Daun-Waru%20Menumbuh).