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Original Article

ANTI-ALOPECIA ACTIVITY OF MORINGA (*MORINGA OLEIFERA* LAMK.) SEED OIL AGAINST DIHYDROTESTOSTERONE-INDUCED RABBITS

YULIUS BAKI KORASSA[1,2], NYI MEKAR SAPTARINI[1*], RESMI MUSTARICHIE[1], RINI HENDRIANI[3], ANTONIUS R. B. OLA[4,5], YOAN NOVICADLITHA[6], LELY A V KAPITAN[2]

[1]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Bandung-45363, Indonesia. [2]Pharmacy Study Program, Health Polytechnic of Ministry of Health in Kupang-405020, East Nusa Tenggara, Indonesia. [3]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran-45363, Bandung, Indonesia. [4]Department of Chemistry, Faculty of Science and Engineering, Nusa Cendana University-85001, Kupang, Indonesia. [5]Integrated Research Center (Bioscience) Laboratory, Nusa Cendana University-85001, Kupang, Indonesia. [6]Medical Laboratory Technologists Study Program, Health Polytechnic of Ministry of Health in Kupang-405020, East Nusa Tenggara, Indonesia

[*]Corresponding author: Nyi Mekar Saptarini; [*]Email: nyi.mekar@unpad.ac.id

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ABSTRACT

Objective: Alopecia is a condition where there is hair loss or no growth of hair, which can occur as a result of stress, heredity, hormonal factors or due to certain diseases such as diabetes mellitus. This study aimed to determine the anti-alopecia activity of moringa seed oil against rabbits induced by dihydrotestosterone (DHT).

Methods: The methods used was the alopecia rabbit model according to Matias with moringa seed oil concentrations of 7.5, 10 and 12.5%, positive control (0.1% finasteride) and negative control (1% tween 80) with parameters hair length and hair weight test.

Results: Results showed that moringa seed oil concentrations of 7.5, 10 and 12.5% had anti-alopecia activity with average hair length of 3.4±0.17, 3.9±0.20 and 4.5±0.28 cm, respectively and average hair weight of 118±23.148±30.9 and 175±47.2 mg respectively.

Conclusion: Moringa seed oil concentration of 12.5% had optimal activity for developing as anti-alopecia based on the statistical analysis value of hair length ($125x10^{-3}$>0.05) was not significantly different while hair weight ($3x10^{-3}$<0.05) was significantly different from the positive control of 0.1% finasteride.

Keywords: *Moringa oleifera* Lamk, Antialopecia, Finasteride, Growth hair

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INTRODUCTION

Alopecia is a condition where there is loss of hair or no growth of head hair, which can occur as a result of severe stress, hereditary, hormonal factors or due to certain diseases such as diabetes mellitus [1, 2]. Androgenetic alopecia (AGA) is the most common type of alopecia and is a type of hair loss that affects at least half of all men at the age of 50 y, and also nearly 70% of all men at the age of 70 y [3, 4]. Androgenic alopecia is caused by the presence of enzyme 5 α-reductase [5]. This enzyme interacts with testosterone to form 5 α-dihydrotestosterone (DHT), which then binds to a specific receptor, namely androgen receptor in the hair follicle, thereby reducing blood flow to the hair follicle, which in turn causes inhibition of hair growth, hair damage and wasting of hair follicles [6].

Alopecia therapies such as minoxidil and finasteride can have adverse side effects, especially with long-term use [7-9]. The use of finasteride can cause sexual disorders such as impotence, while minoxidil causes irritant or allergic contact dermatitis [10]. Therefore, it is necessary to develop the use of plants to be used as a safer substitute for synthetic drugs with a 5α-reductase inhibitor mechanism to stimulate hair growth and prevent alopecia [11, 12].

Moringa (*Moringa oleifera* Lamk.) seed oil is a class of edible oil that has activity as an antioxidant, antiaging, emollient, hair care and skin lightening [13]. Moringa seed oil contains phytosterol compounds, namely β-sitosterol, stigmasterol and compasterol, which can block the formation of dihydrotestosterone (DHT), which causes alopecia by inhibiting the enzyme 5 α-reductase found in hair follicles [14-16]. The content of phytosterol compounds at concentrations of 0.01% to 0.5% in plants has been shown to have anti-alopecia activity [17].

Research related to moringa seed oil as anti-alopecia is still rarely done, so it is necessary to carry out studies through ongoing research to support data that can confirm information regarding the development of moringa seed oil as anti-alopecia. The novelty of this research was the anti-alopecia activity test of Moringa seed oil from East Nusa Tenggara using cold pressing in rabbits induced by dihydrotestosterone. Moringa seed oil from East Nusa Tenggara is abundant in nature, namely 35-40%, with phytosterol components which have potential as anti-alopecia based on *in silico* test, so it was necessary to carried out *in vivo* tests to determine the activity and effectiveness of the right dose of Moringa seed oil as anti-alopecia.

MATERIALS AND METHODS

Materials

Dihydrotestosterone hormone (merck), moringa seeds (East Baumata Village, Kupang Regency), absolute ethanol (braun), ethanol (medika), Lieberman burchard, distilled water (berno farma), concentrated sulfuric acid (merck), chloroform (merck), methanol (merck), tween 80 (merck), finasteride (combiphar), anhydrous acetic acid (merck), *n*-hexane (merck), ethyl acetate (merck), kiesel gel GF 254, New Zealand male white rabbit.

Moringa seed oil test material preparation

Moringa seeds were weighed as much as 1.5 kg, peeled from the skin and the seeds were taken and then weighed. Moringa seeds were put in oil press (cold press). The oil obtained was then filtered from the dregs using a flannel cloth and stored in a closed container and then the yield value was calculated using the formula [18, 19].

$$\text{Yield} = \frac{\text{Weight of obtained oil}}{\text{Weight of moringa seed}} \times 100\%$$

Phytochemistry test

Lieberman burchard reaction

Take 1 ml of moringa seed oil, put it in a test tube, then dissolve it in 0.5 ml of chloroform and add 0.5 ml of anhydrous acetic acid. The

mixture was then added with 1-2 ml of concentrated H_2SO_4 through the tube wall; positive results containing steroids were indicated by the formation of a bluish-green color [20].

Salkowski test

Take 1 ml of moringa seed oil and put it in a test tube, then add 1-2 ml of concentrated H_2SO_4 through the tube wall. The presence of unsaturated steroids was indicated by the appearance of a red ring [21].

Steroid identification by TLC method

Moringa seed oil was spotted on the stationary phase of Kiesel Gel GF 254 and the mobile phase: *n*-hexane: ethyl acetate (4:1). The results were indicated by the presence of sulfuric acid anisaldehyde stains, the presence of steroids was indicated by the appearance of a purple color on the plate spots and the calculated Rf value of the steroid [22].

Acrolein test

Take 1 ml of moringa seed oil, add 3 drops of H_2SO_4 and then heated slowly over a fire. The presence of fatty acids was indicated by a strong smell of acrolein, which was distinguished from the smell of SO_4 [23].

Translucent spot test

The translucent test was carried out by dropping 2-3 drops of moringa seed oil on filter paper, the presence of stains on the filter paper indicated the presence of fat [24].

Anti-alopecia test of moringa seed oil

Ethical clearance application

Application for research Ethical Clearance was made at the Health Research Ethics Committee, Faculty of Medicine, Padjadjaran University, Bandung, with letter number 4106/UN6. 0.1/PT/2021.

Test animal amount preparation

The anti-alopecia test was carried out *In vivo* on rabbits using the modified Matias method [25-27]. The test animal used was a New Zealand male white rabbit aged 3-4 mo with a body weight of 2-3 kg. The number of rabbits used based on the Federer formula was 4 with each rabbit being given 6 treatments in the back area, namely the normal area (without treatment), sample area (treat with moringa seed oil concentrations of 7.5, 10 and 12.5%), positive control area (treat with 0.1% finasteride) and negative control area (treat with 1% tween 80) [28-39].

Alopecia induced in rabbits

After 1 (one) week of adaptation, the rabbit's back was then induced subcutaneously using the hormone dihydrotestosterone (DHT) at a dose of 0.01 grams/0.1 ml every day for 20 d while observing the index of alopecia by measuring the induced area using vernier caliper [31]. The index of alopecia occurrence could be seen in the table 1.

Table 1: Alopecia index [25]

Alopecia index	Description
0	No hair loss
1	Diffuse thinning throughout the interscapular area
2	There was alopecia with an area of 1 cm^2
3	Alopecia developed in the posterior area of approximately 2 cm^2
4	Alopecia greater than or equal to 4 cm^2

Anti-alopecia test of moringa seed oil

After the rabbit was stated to have alopecia with wide posterior area of 2 cm^2 (alopecia index 3), the rabbit's back was then divided into 6 smearing areas, each of which was rectangular in shape with an area of 2 x 2 cm and a distance between the areas of 1 cm. Area I was not applied as a blank, area II was applied with moringa seed oil with a concentration of 7.5%, area III was applied with moringa seed oil with a concentration of 10%, area IV was applied with moringa seed oil with a concentration of 12.5%, area V was applied with 0.1% finasteride (positive control) and area VI was applied with 1% tween 80 (negative control) [31, 32].

The test preparation was administered by applying it 2 times a day, namely in the morning and evening with an administration volume of 1 ml for each part for 28 d. The first day of application was considered day 0. Observations were made by taking 6 strands of rabbit hair every 7 d, calculated on days 7, 14, 21, and 28. The hair was taken by cutting, straightening, and attaching it to a tape, then measuring the length of the hair using a caliper. On the 28th day, each area was shaved and then the hair was weighed [32].

Parameters for assessing hair growth included hair length, hair weight and thickness of each treatment group compared to the positive control group (0.1% finasteride). For hair length measurements were taken every week for 28 d by observing the growth of each treatment group. Hair weight was related to the size of the hair strands [32].

Data analysis

Data on hair length and hair weight were processed statistically to see whether there were significant differences between the test and control areas. To see the normality and homogeneity of the data, a normality test (Shapiro-Wilk) and a homogeneity test (Levene) were done. Normal and homogeneous data distributions were processed for analysis of variance (ANOVA) tests. Data distribution that was not normal or homogeneous was processed using non-parametric statistics, namely the Kruskal-Wallis test. Then proceed with the Mann-Whitney test to saw that there were significant differences in each test group with a confidence level of 95% [28].

RESULTS

Moringa seed oil preparation

The nett yield of moringa seeds from 1.5 kg of whole moringa seeds was 850 g and after pressing, 65.24 ml of oil was obtained with yield of 7.67%. The yield obtained was in accordance with the range of moringa seed oil obtained by cold pressing, namely 4-10% with the color of moringa seed oil ranging from light to dark clear yellow with a distinctive aroma and slightly bitter taste [33, 34].

Phytochemistry test

Table 2 showed that moringa seed oil contained steroid compounds and fatty acids, where both components belong to the lipid group [35]. Moringa seed oil contained a high content of oleic acid (68-76%), linoleic acid (58-62%), behenic acid (7%), arachidic acid (3%) [36]. Moringa seeds had a phytosterol content of 27.47% [37]. The phytosterol components in moringa seed oil include brassicasterol, ergostadienol, methylene cholesterol, campasterol, campestanol, stigmasterol, ergostadienol, clerosterol, β-sitosterol, stigmastanol, avenasterol, stigmastadienol, isoavenasterol and stigmastenol [15, 16].

Table 2: Results of steroid and fatty acid tests of moringa seed oil

Compound	Method/Reagent	Result	Reference	Description
Steroids	Liebermann-Burchard	Bluish-green colour formed	[20]	detected
	Salkowski test	Red coloured ring formed	[21]	detected
	TLC mobile phase *n*-hexane: ethyl acetate (4:1)	The appearance of purple sulfuric acid anisaldehyde spots	[21]	detected
Fatty acid	Acrolein test	Strong scent of acrolein was smell	[23]	detected
	Translucent spot test	Transparant spot	[24]	detected

Anti-alopecia test of moringa seed oil

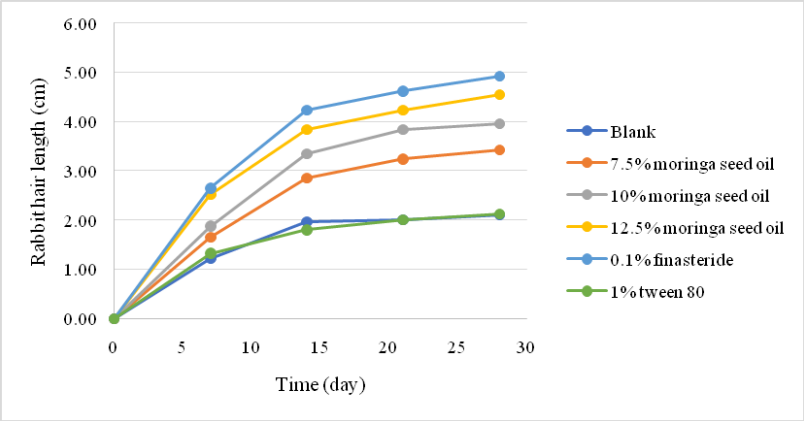

Fig. 1: Graph of rabbit hair length

Based on fig. 1, it could be seen that moringa seed oil concentrations of 7.5, 10 and 12.5% had anti-alopecia activity based on hair length data with average values of 3.4±0.17, 3.9±0.20 and 4.5±0.28 cm. Based on the graph of average hair length for 28 d, it could be seen that there was an increase in rabbit hair length growth with each treatment. The blank graph and negative control (1% Tween 80) were lower than the treatment graph for moringa seed oil concentrations of 7.5, 10 and 12.5% and the positive control (0.1% finasteride). Fig.1 also showed that there was an increase in the growth of rabbit hair length, which was different from each treatment.

Table 3: Results of the average length of rabbit hair

Sample test	Rabbit hair length (cm)	Statistic analysis	
	Average±SD	Significant difference to negative control (p)	Significant difference to positive control (p)
Blank	2.1±0.32	0.00<0.05*	0.00<0.05*
7.5% moringa seed oil	3.4±0.17	0.00<0.05*	0.00<0.05*
10% moringa seed oil	3.9±0.20	0.00<0.05*	0.00<0.05*
12.5% moringa seed oil	4.5±0.28	0.03<0.05*	0.125>0.05
Positive control (0.1% finasteride)	4.9±0.15	0.00<0.05*	-
Negative control (1% tween 80)	2.1±0.32	-	0.00<0.05*

Description: *significant difference between moringa seed oil concentrations and control

Statistical analysis based table 3 showed that moringa seed oil treatment with concentrations of 7.5, 10 and 12.5% and time (days) affected rabbit hair length ($1x10^{-2}$<0.05). Treatment and day had an interaction in influencing rabbit hair length ($3x10^{-3}$<0.05) where the 7.5% and 10% treatments were significantly different from the positive and negative controls ($1x10^{-2}$<0.05), while the 12.5% treatment was not significantly different with the positive control ($125x10^{-3}$>0.05) but significantly different from the negative control ($3x10^{-3}$<0.05).

Weight of rabbit hair measurement

Moringa seed oil concentrations of 7.5, 10 and 12.5% had hair growth activity based on hair weight with average values of 118±23, 148±30.9 and 175±47.2 mg respectively, positive control was 280±92.7 mg and negative control was 60±18.2 mg.

Based on the graph of the average hair weight for 28 d (fig. 2), it showed that there was an increase in hair weight in the treatment of moringa seed oil at concentrations of 7.5, 10 and 12.5% where the higher the concentration of moringa seed oil, the greater the hair weight of rabbits, which means hair growth activity occurs, while In the positive control treatment of 0.1% finasteride, it was seen that there was a greater increase in hair weight when compared to the moringa seed oil treatment, negative control and blank. In the blank treatment and negative control showed an average hair weight with a lower value when compared to the positive control and moringa seed oil concentrations of 7.5, 10 and 12.5% with values of 62.5 and 60 mg; this means that the blank and negative control 1% tween 80 did not have a big effect on hair growth.

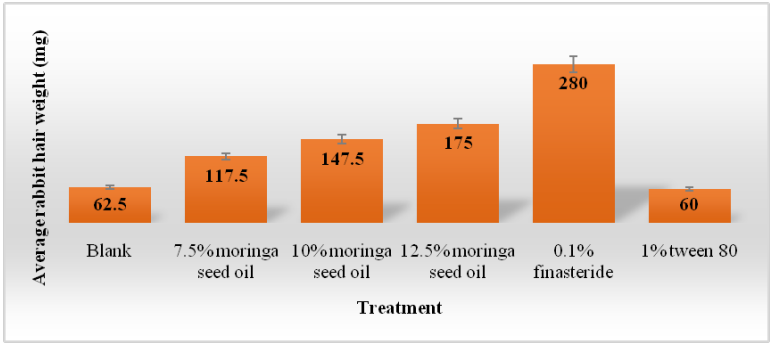

Fig. 2: Graph of average rabbit hair weight

Table 4: Results of measuring the weight of rabbit hair

Sample test	Rabbit hair weight (mg)	Statistic analysis	
	Average±SD	Significant difference to negative control	Significant difference to positive control
Blank	62.5±9.5	1.000>0.05	0.000<0.05*
7.5% moringa seed oil	117.5±23.62	0.512>0.05	0.001<0.05*
10% moringa seed oil	147.5±30.9	0.128>0.05	0.008<0.05*
12.5% moringa seed oil	175±47.2	0.025<0.05*	0.046<0.05*
Positive control (0.1% Finasteride)	280±92.7	0.000<0.05*	-
Negative control (1% tween 80)	60±18.2	-	0.000<0.05*

Description: *(Significant difference between moringa seed oil concentrations and control).

The results of statistical analysis using one-way ANOVA based on hair weight data tabel 4 showed that the moringa seed oil with concentrations of 7.5, 10 and 12.5% treatments were not significantly different between the three. There was a significant difference between 2.5% moringa seed oil treatment and negative control (tween 80), whereas the 2.5% moringa seed oil treatment with the positive control (finasteride 0.1%), there was no significant difference, which means they had the same activity. Statistical data showed that moringa seed oil concentrations of 7.5, 10, and 12.5% have activity on rabbit hair growth, but the 12.5% concentration provided more optimal activity.

DISCUSSION

The anti-alopecia activity of moringa seed oil was supported by the presence of phytosterol compounds, which based on *in silico* test, the compounds had the potential to became anti-alopecia drug candidates based on test simulations using autodock with PDB code: 7BW1. The ergostadienol compound in moringa seed oil was predicted to had anti-alopecia potential through inhibiting the enzyme 5 α-reductase, which involves the amino acids Glu57 and Tyr91 [38].

Moringa seed oil has the ability as an anti-alopecia because it contains fatty acid and phytosterol compounds [39-41]. Mechanism of linoleic acid and beta-sitosterol in moringa seed oil as anti-alopecia could be seen in fig. 3.

Fig. 3 showed the mechanism of β-sitosterol compound as anti-alopecia by inhibiting the enzyme 5 α-reductase, which can convert the hormone testosterone into the form of dihydrotestosterone (DHT) [42]. DHT is the most common cause of hair loss, the fundamental reason for hair loss occurs because DHT binds to specific receptors on the hair follicles, namely the androgen receptors, which cause constriction of the hair follicles so that the hair gradually becomes thinner, easily brittle and falls out due to damage to hair follicles and roots or miniaturization [43]. Fatty acids in moringa seed oil such as linoleic acid and lauric acid have properties for supporting nutrition for hair, slowing hair loss, accelerating hair growth, protecting hair, caring for hair and stimulating hair growth. Stimulating hair growth through the mechanism of inhibiting the enzyme 5 α-reductase [6, 44].

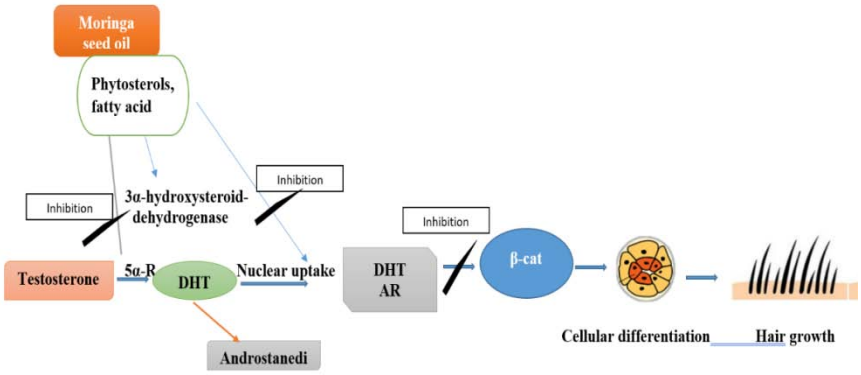

Fig. 3: Mechanism of linoleic acid and β-sitosterol in moringa seed oil as anti-alopecia [42]

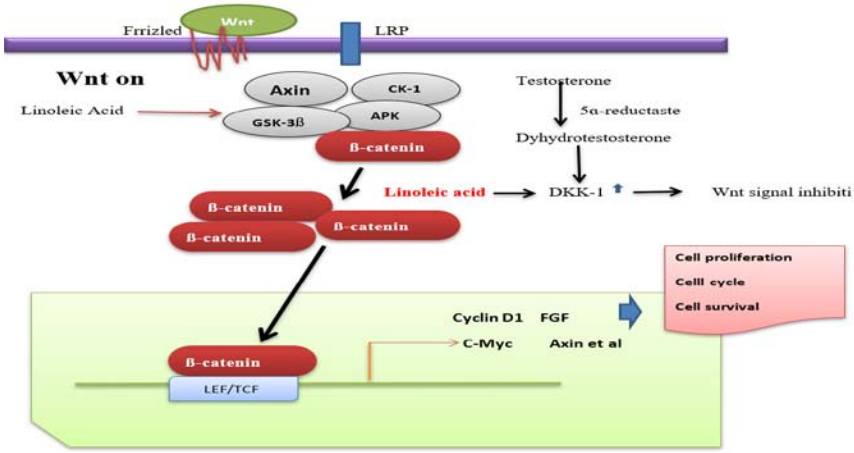

Fig. 4: Mechanism of linoleic acid as anti-alopecia [45]

Fig. 4 showed the activity of linoleic acid in moringa seed oil as anti-alopecia through the mechanism of activating β-catenin signaling and inducing the growth of Human hair dermal papilla cells (HFDPC) by increasing the expression of cell cycle proteins such as cyclin D1 and cyclin-dependent kinase 2 [45, 46]. Linoleic acid can also increase several growth factors, such as vascular endothelial growth factor, hepatocyte, and keratinocyte growth factor. Linoleic acid can also significantly inhibit the expression of Dickkopf-related protein-1 (DKK-1) and primary alopecia signaling by DHT, so that can relieve testosterone-induced signaling molecules and induced HFDPC growth by activating β-catenin signaling [45, 47].

CONCLUSION

Moringa seed oil concentrations of 7.5, 10 and 12.5% had anti-alopecia activity with average hair length of 3.4±0.17, 3.9±0.20, and 4.5±0.28 cm, respectively and average hair weight of 118±23, 148±30.9, and 175±47.2 mg respectively. Moringa seed oil with a concentration of 12.5% had optimal activity for developing as anti-alopecia based on a statistical analysis of hair length ($125x10^{-3}$>0.05), which was not significantly different with positive control, while based on a statistical analysis of hair weight ($3x10^{-3}$<0.05) was significantly different from the positive control of 0.1% finasteride.

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Nil

AUTHORS CONTRIBUTIONS

All the authors contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. McElwee KJ, Sinclair R. Hair physiology and its disorders. Drug Discov Today Dis Mech. 2008;5(2):e163-71. doi: 10.1016/j.ddmec.2008.04.001.
2. Abbas A, Naqvi SAR, Rasool MH, Noureen A, Mubarik MS, Tareen RB. Phytochemical analysis, antioxidant and antimicrobial screening of seriphidium oliverianum plant extracts. International Hormesis Society; 2021.
3. Kumar N, Rungseevijitprapa W, Narkkhong NA, Suttajit M, Chaiyasut C. 5α-reductase inhibition and hair growth promotion of some Thai plants traditionally used for hair treatment. J Ethnopharmacol. 2012;139(3):765-71. doi: 10.1016/j.jep.2011.12.010, PMID 22178180.
4. Hariani E, Jusuf NK. Evidence-based treatment of alopecia areata. Period Dermatol Venereol. 2017;29(2).
5. Yason Y. Optimizing the composition of propylene glycol and sorbital as humectants in the anti-hair loss cream formula of saw palmetto [Serenoa repens] extract: application of factorial design. Yogyakarta: Faculty of Pharmacy, Sanata Dharma University; 2007.
6. Kaushik R, Gupta D, Yadaf R. Review artikel: alopecia: remedies. Int J Pharm Sci Res. 2011;2(7):1631-7.
7. Roy RK, Thakur M, Dixit VK. Hair growth promoting activity of Eclipta alba in male albino rats. Arch Dermatol Res. 2008;300(7):357-64. doi: 10.1007/s00403-008-0860-3, PMID 18478241.
8. Gregoriou S, Papafragkaki D, Kontochristopoulos G, Rallis E, Kalogeromitros D, Rigopoulos D. Cytokines and other mediators in alopecia areata. Mediators Inflamm. 2010;2010:928030. doi: 10.1155/2010/928030, PMID 20300578.
9. Choi HI, Kim DY, Choi SJ, Shin CY, Hwang ST, Kim KH. The effect of cilostazol, a phosphodiesterase 3 (PDE3) inhibitor, on human hair growth with the dual promoting mechanisms. J Dermatol Sci. 2018;91(1):60-8. doi: 10.1016/j.jdermsci.2018.04.005, PMID 29678305.
10. Legiawati L, Suseno LS, Sitohang IBS, Yusharyahya SN, Pawitan JA, Liem IK. Combination of adipose-derived stem cell conditioned media and minoxidil for hair regrowth in male androgenetic alopecia: a randomized, double-blind clinical trial. Stem Cell Res Ther. 2023;14(1):210. doi: 10.1186/s13287-023-03440-2, PMID 37605227.
11. Jain PK, Das D, Das C. Prospect of herbs as hair growth potential. Innovare J Med Sci. 2017;5(1):6-14.
12. Kaur A, Singh TG, Dhiman S, Arora S, Babbar R. Novel herbs use din cosmetics for skin an D hair care: a review. Plant Arch. 2020;20:3784-93.
13. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam.) leaves. J Agric Food Chem. 2003;51(8):2144-55. doi: 10.1021/jf020444+, PMID 12670148.
14. Cabeza M, Bratoeff E, Heuze I, Ramirez E, Sanchez M, Flores E. Effect of β-sitosterol as inhibitor of 5α-reductase in hamster prostate. Proc West Pharmacol Soc. 2003;46:153-5. PMID 14699915.
15. Anwar F, Bhanger MI. Analytical characterization of moringa oleifera seed oil grown in temperate regions of Pakistan. J Agric Food Chem. 2003;51(22):6558-63. doi: 10.1021/jf0209894, PMID 14558778.
16. Rahman IMM, Barua S, Nazimuddin M, Begum ZA, Rahman MA, Hasegawa H. Physicochemical properties of moringa oleifera lam. Seed oil of the indigenous-cultivar of Bangladesh. J Food Lipids. 2009;16(4):540-53. doi: 10.1111/j.1745-4522.2009.01165.x.
17. Goodman DS. Topical preparation for the treatment of hair loss. United States Patent US. 2022;6(358):541 B1.
18. Dewatisari WF, Rumiyanti L, Rakhmawati I. Phytochemical yield and screening of sanseviera sp leaf extracts. Appl Agric Res. 2017;17(3):197-202.
19. Arta Y, Suryani D, Syafitri N, Hanafiah A, Elvira D. Use of the palm oil yield guess application in the palm oil farmer group in segati village, Langgam District. J Community Serv Appl Sci. 2022;3(01):1-15.
20. Hayati EK, Halimah N. Phytochemical test and brine shrimp lethally test against artemia salina leach anting-anting (Achalypha indica Linn.) plant ekstract. Alchemy. 2010;2:53-103.
21. Ningsih IY, Muslichah S, Puspitasari E, Dianasari D. Phytochemistry practical manual. rev 8th ed. Sec. Pharmaceutical biology. University of Jember; 2014. p. 8.
22. Bhawani SA, Sulaiman O, Hashim R, Ibrahim MN. Thin-layer chromatographic analysis of steroids: a review. Trop J Pharm Res. 2010;9(3):301-13. doi: 10.4314/tjpr.v9i3.56293.
23. Fitriana YAN, Fitri AS. Uji lipid pada minyak kelapa, margarin, dan gliserol. Sainteks. 2019;16(1):19-23. doi: 10.30595/sainteks.v16i1.7013.
24. Amrita. Qualitative analysis of oils and fats. Ministry of Electronics and Information Technologi. amrita. olabs.edu. In; 2015.
25. Matias JR, Malloy V, Orentreich N. Animal models of androgen-dependent disorders of the pilosebaceous apparatus. 1. The androchronogenetic alopecia (AGA) mouse as a model for male-pattern baldness. Arch Dermatol Res. 1989;281(4):247-53. doi: 10.1007/BF00431058, PMID 2774656.
26. Pandit S, Chauhan NS, Dixit VK. Effect of cuscuta reflexa roxb on androgen-induced alopecia. J Cosmet Dermatol. 2008;7(3):199-204. doi: 10.1111/j.1473-2165.2008.00389.x, PMID 18789055.
27. Patel S, Sharma V, S Chauhan NS, Thakur M, Dixit VK. Evaluation of hair growth promoting activity of phyllanthus niruri. Avicenna J Phytomed. 2015;5(6):512-9. PMID 26693408.
28. Amin J, Purba SEL, Anwar E, Djajadisastra J. Green tea (Camellia sinensis L.) ethanolic extract as hair tonic in nutraceutical; physical stability, hair growth activity on rats, and safety test. Int J Pharm Pharm Sicences. 2014;6(5):94-9.
29. Priskila V. Physical stability test and hair growth activity test of male white rats from hair tonic preparation containing water extract of kepok banana weevil (Musa balbisiana) [thesis] Depok Fakultas Farmasi Universitas Indonesia; 2012. p. 28-30.
30. Purwantini I, Munawaroh R, Darwati NBS. Combination of the and Mangkokan leaves extract to promote hair growth. Traditional Medicine. 2008;13:43.
31. Kasmawati H, Mustarichie R, Halimah E, Ruslin R, A Sida NA. Antialopecia activity and IR-spectrometry characterization of bioactive compounds from Sansevieria trifasciata P. Egypt J Chem. 2022;65(13):19-24. doi: 10.21608/ejchem.2022.104463.4825.
32. Sulastri L, Indrawati T, Taurhesia S. Hair growth activity test of a combination of green tea and gotu kola herb water extract. Pharmaciana. 2016;6(1):39-46.

33. Amelia P, Fithriyah N. Chairul. Analysis of α-tocopherol (vitamin E) in moringa oleifera lamk. seed oil using high-performance liquid chromatography. J Valence Chem. 2014;4(2):142-7.
34. Widyanastuti NA, Susilo B. Study of hydraulic press extraction of moringa seed oil (Moringa oleifera) with a variety of heat treatments. Bioproses Komoditas Tropis. 2013;1(2):1-10.
35. Mamuaja CF. Lipid A. Uns rat Press. Kampus Unsrat Bahu Manado; 2017.
36. Manzoor M, Anwar F, Iqbal T, Bhanger MI. Physico-chemical characterization of Moringa concanensis seeds and seed oil. J Americ Oil Chem Soc. 2007;84(5):413-9. doi: 10.1007/s11746-007-1055-3.
37. Salman AN, Prangdimurti E, Dase H. Reducing the bitter taste of moringa seeds (Moringa oleifera) and its potential as a food to prevent hypercholesterolemia. Online public access catalog IPB university E-library; 2022. p. 1-50.
38. Korassa YB, Saptarini NM, Mustarichie R, Hendriani R, Tjitda PJP. *In silico* study of 12 phytosterol compounds in moringa (Moringa oleifera Lamk.) Seed Oil On 5α-reductase Enzyme Inhibition Activity as Anti-Alopecia. Int J App Pharm. 2022;14(4):49-57. doi: 10.22159/ijap.2022.v14s4.OP11.
39. Aney JS, Tambel R, Kulkarni M, Bhise K. Pharmacological and pharmaceutical potential of Moringa oleifera: a review. J Pharm Res. 2009;2(9):1424-6.
40. Hardman R. Herbal principles in cosmetics: properties and mechanisms of action. Vol. 1. Taylor and Francis Group; 2010. p. 264-7.
41. Suhery WN, Febrina M, Permatasari I. Microemulsion formulation of a combination of virgin coconut oil and rice bran oil for hair growth. Trad Med J. 2018;23(1):40-6. doi: 10.22146/mot.29213.
42. Gupta AK, Talukder M, Bamimore MA. Natural products for male androgenetic alopecia. Dermatol Ther. 2022;35(4):e15323. doi: 10.1111/dth.15323. PMID 35044013.
43. Ho BSY, Vaz C, Ramasamy S, Chew EGY, Mohamed JS, Jaffar H. Progressive expression of PPARGC1α is associated with hair miniaturization in androgenetic alopecia. Sci Rep. 2019;9(1):8771. doi: 10.1038/s41598-019-43998-7, PMID 31217429.
44. Muhammud A, Amin ARM, Bakar RA, Jaafar R. The effectiveness of coconut oil mixed with herbs to promote hair growth. Int J Ethics Eng Manag Educ. 2014;1(3):27-30.
45. Ryu HS, Jeong JY, Lee CM, Lee KS, Lee JN, Park SM. Activation of hair cell growth factors by linoleic acid in Malva verticillata seed. Molecules. 2021;26(8):2117. doi: 10.3390/molecules26082117, PMID 33917070.
46. Mohammed MK, Shao C, Wang J, Wei Q, Wang X, Collier Z. Wnt/β-catenin signaling plays an ever-expanding role in stem cell self-renewal, tumorigenesis and cancer chemoresistance. Genes Dis. 2016;3(1):11-40. doi: 10.1016/j.gendis.2015.12.004, PMID 27077077.
47. Kwack MH, Sung YK, Chung EJ, Im SU, Ahn JS, Kim MK. Dihydrotestosterone-inducible dickkopf 1 from balding dermal papilla cells causes apoptosis in follicular keratinocytes. J Invest Dermatol. 2008;128(2):262-9. doi: 10.1038/sj.jid.5700999, PMID 17657240.