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Research Article

The Potential of Moringa (*Moringa oleifera Lamk*) Seed Oil as Anti-Alopecia

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ABSTRACT

Objective: This review article aimed to examine the potentiality of moringa seed oil as anti-alopecia. **Method**: The data was collected by studying national and international journal articles using several search engines, namely Google and Google Scholar websites, Research Gate, Sciencedirect and Scimagojr. The keywords for this article include moringa seed oil, fatty acids, phytosterol, and anti-alopecia. **Results**: The result was tabulated in a table and described according to the mechanism of action of the active compounds found in moringa seed oil, fatty acids, phytosterol, and anti-alopecia. Moringa seed oil contain the phytosterol compounds (β-sitosterol, ergosterol and campesterol) show the activities that obstruct the formation of the dihydrotestosterone (DHT) compound known to be the cause of alopecia. The fatty acid compounds found in moringa seed oil (lauric acid, linoleic acid, palmitoleic acid, palmitic acid, and oleic acid) reinforce its potential to be an anti-alopecia. **Conclusion**: The phytosterol and fatty acid compounds supported the growth of hair to be fertile and healthy.

Key words: Moringa Seed Oil, Fatty Acids, Phytosterol, Anti-alopecia.

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INTRODUCTION

Alopecia is the condition of partial or complete absence of hair from an area of the body where it usually grows, usually the scalp, which often occurs due to heavy stress, heredity, or illness such as diabetes mellitus.[1] One can also experience the condition because of having 5 α-reductase enzymes, also known as the cause of adrogenetic alopecia. The enzyme interacts with testosterone and forms dihydrotestosterone (DHT) that later connects to a specific receptor called the androgen receptor on the hair follicle. The receptor reduces blood flow to hair follicles and, as a result, inhibits hair growth, causes hair damage, and shrinks hair follicles.[2] Figure 1 shows the mechanism of action of DHT blockers and 5 α-reductase blockers.[3]

5 α-reductase is an enzyme in the body that can change the testosterone hormone into dihydrotestosterone (DHT). The enzyme consists of two types, which are type 1 and type 2. Type 1 commonly grows in a newborn's scalp, skin, and heart organ. Whereas the latter forms in the genital skin, heart, and prostate.[2] Dihydrotestosterone is the most common cause of hair loss as it foundationally connects with a specific receptor on the hair follicle called the androgen receptor. This receptor shrinks the hair follicle and eventually makes the hair thinner, more vulnerable, and more easily falls out due to the damage caused in the hair follicle and root.[3]

Androgenic alopecia affects almost every man in their 50s. About 70% of them are above 70 years old. Not only that, but the condition also affects women.[4] Treating alopecia can be done using oral finasteride or 2% to 5% of topical minoxidil, which helps to prevent 5 α-reductase both in women and men.[5]

The treatment of alopecia with herbal plants can be a safe and effective alternative due to its minimum side, long-term effect.[1] Moringa (*Moringa oleifera* Lamk) seed oil falls into the classification of edible oil with acts as an antioxidant, antiaging, emollient, haircare, and skin lightening.[6] Two components of fatty acids that potentially become anti-alopecia are lauric acid and linoleic acid.[7] Moringa seeds contain the compound β-sitosterol.[8] β-sitosterol compound functions as a blocker of dihydrotestosterone on androgen receptors on hair follicles.[9]

There have been numerous researches on isolation and identification of moringa seed oil's compound, yet the information regarding the potential activity of moringa seed oil is still lacking. Therefore, this review article aimed to analyze the potential of active compounds in moringa seed oils and develop it as an anti-alopecia herb.

METHODS

The data collected in this review was done through a direct searched on reference journals, both national and international scaled, using several search engines as follows: Google and Google Scholar website, Research Gate, Sciencedirect and Scimagojr. To got desired results, researcher used the following keywords to found the appropriate scientific journal keywords: moringa seed oil, fatty acids, phytosterol, and anti-alopecia.

The conduct of this research considers two criteria the inclusion and exclusion criteria. The inclusion criterion is a criterion describing the subject of research that can represent the research samples. To decide the representation of this study, the researcher used the following criteria it must be a reputable journal both at national and international scales, and the publication year must be within the last 23 years. Otherwise, the latter criterion describes the condition where a scientific journal cannot represent samples due to its incapability to meet the sample requirement, in that the journal article is not entirely accessible.[10]

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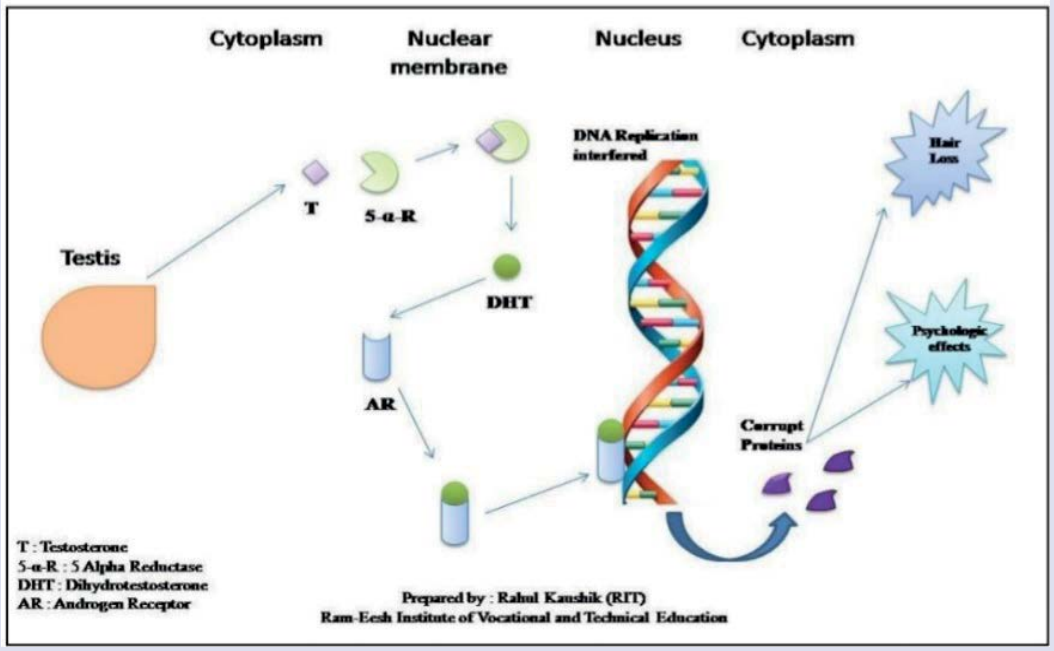

Figure 1: Proposed mechanism of action of DHT blockers and 5 α-reductase blockers.[3]

Study that become focus of this review article was to discuss about secondary metabolite components of moringa seed oil and the method used to obtain moringa seed oil. The results obtained then tabulated in tabular form and described based on its mechanism of action as anti-alopecia.

RESULTS

Data and review article related to the potential of moringa seed oil as anti-alopecia exclusion to few and limited. Therefore, to expand the search for article, researcher used article published in the last 23 years used search engine such as, Google, Google Scholar website, Research Gate, Sciencedirect and Scimagojr. Result of the search for article that were done then identified and screened. Amount of article were 55 articles. The articles obtained then processed by critical appraisal skills program (CASP) and 10 inclusion articles were obtained that contained information related to the secondary metabolite components of moringa seed oil including sterol compounds and fatty acids that has anti-alopecia activity and methods for obtaining oil by cold press and extraction using *n*-hexane as solvent.

The number of exclusion articles was 45 because the articles were still in incomplete article form or in abstract form. The review result of inclusion articles obtained gives information regarding the active compounds of moringa seed oil as an anti-alopecia and methods of extracting moringa seed oil. The review article require journal and sources from the supporting article to explain the background, methods and discussion. The articles that obtained based on searching result were 32 articles distributed in the article and documented in the bibliography. The search results chart is shown in Figure 2.

The discussion of this review article leads to the finding of the component study of moringa seed oil to potentially become an anti-alopecia. The result shows the potential of moringa seed oil to be an anti-alopecia as it contains two groups of compounds, which are sterol and fatty acid groups and supporting compounds such as tocopherols and isoflavones.[3,11,12] Table 1 shows the results on the compounds of moringa seed oil.

DISCUSSION

Moringa seed oil extraction process

There are two methods to the extraction of moringa seed oil. There are cold presses and soxhletation using *n*-hexane solvent.[15,18] The cold press method is commonly used to extract components in biological matters such as oil and fats in seeds from within the cell seed structure compressed using a pressing tool. The advantage of this method is that it will not break the active compounds within the matter. Yet, the process requires specific tools and techniques to extract pure and quality oil. Materials that can undergo the cold press method contain 30-70% oil.[23] On the other hand, the soxhletation method advantages in its ability to optimally extract a compound using small amount of sample matter and solvent.[24] Consequently, this method requires a heating technique that can damage the structure of fatty acids and sterol.[25] Moringa seed oil extraction using *n*-hexane as a solvent due to the oil that will be extracted from moringa seeds has nonpolar properties, so a nonpolar *n*-hexane solvent is needed.[17]

Moringa seed oil extracted by soxhletation method used *n*-hexane as solvent has higher oil value yield than used cold press method, but the result of chemical content analysis of free fatty acid produced by cold press method has higher levels than used extraction method with *n*-hexane as solvent.[22] The acidity value of moringa seed oil used cold press method was quite low when compared to extraction method used *n*-hexane as solvent. This could be associated with water added during the grinding of seeds used cold press method which can increased the action of lipolytic enzymes, but the acidity value would be higher if the process was done in direct contact with high-temperature air.[26] The cold press method did not extract campestanol, brassicasterol and ergostadienol but the oil produced had the highest tocopherol content.[22]

Characterization of fatty acids in moringa seed oil

The characterization of fatty acid as the result of hydrolyzing moringa seed oil was conducted using Gas Chromatography-Mass Spectrometry

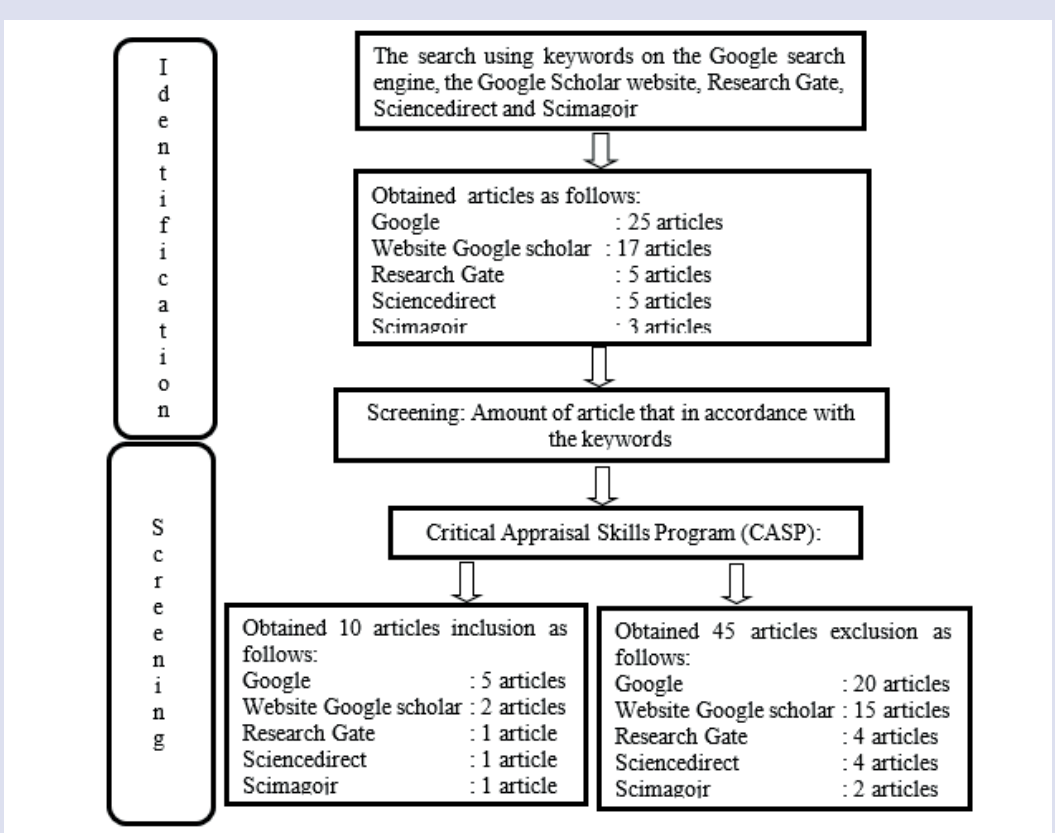

Figure 2: Articles search chart.

Table 1. Study results on the active compound of moringa seed oil

No	Compounds found in moringa seed oil	Oil extraction method	Reference
1	Palmitoleic, margaric, stearic, oleic, linoleic, linolenic, arachidic, gadoleic, behenic, erucic, lignoceric, cerotic acids	Extraction using n-hexane solvent	13
2	Oleic, palmitoleic, stearic, behenic, and arachidic acids	Extraction using n-hexane	14
3	Myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic, gadoleic, behenic acids	Cold press	15
4	Methyl-oleate	Methanolysis of moringa seed oil	16
5	Lauric, palmitoleic, palmitic, oleic, stearic, arachidic, eicocenic, behenic, lignoceric acids, and methyl-oleate	Soxhletation using n-hexane solvent	17
6	24-methylenecholesterol, campesterol, campestanol, stigmasterol, ergostadienol, clerosterol, stigmastanol, *β*-sitosterol, avenasterol, 28-isoavenasterol, and tocopherol	Soxhletation using n-hexane solvent	18
7	24-methylenecholesterol, campesterol, campestanol, stigmasterol, ergostadienol, clerosterol, stigmastanol, *β*-sitosterol, avenasterol, 28-isoavenasterol, and tocopherol	Soxhletation using n-hexane solvent	19
8	α–tocopherol, ɣ–tocopherol, ɛ–tocopherol, brassicasterol, ergostadienol, methylene cholesterol, campasterol, campestanol, stigmasterol, ergostadienol, clerosterol, β-sitosterol, stigmastanol, avenasterol, stigmastadienol, isoavenasterol, and stigmastenol	Soxhletation using n-hexane, petroleum ether, and chloroform: methanol solvents	20
9	Brassicasterol, ergostadienol, methylene cholesterol, campasterol, campestanol, stigmasterol, ergostadienol, clerosterol, β- sitosterol, stigmastanol, avenasterol, stigmastadienol, isoavenasterol, stigmastenol, and tocopherol	Cold press	21
10	Brassicasterol, ergostadienol, methylene cholesterol, campasterol, campestanol, stigmasterol, ergostadienol, clerosterol, β-sitosterol, stigmastanol, avenasterol, stigmastadienol, isoavenasterol, stigmastenol, and tocopherol	Cold press	22

(GC-MS). The initial procedure of deciding the characterization of moringa seed oil's fatty acids started by the preparation of the sample, the soxhletation of moringa seed oil using *n*-hexane solvent or through the pressing method to get pure oil, determination of free fatty acid levels, saponified number determination, hydrolyzation of moringa seed oil, esterification of free fatty acid resulted from the hydrolysis, and the identification of fatty acids using GC-MS.[17]

The acid number is milligrams of potassium hydroxide (KOH) needed to neutralize free fatty acids in one gram of oil, while the free fatty acid (FFA) level is the content of free fatty acids contained in oil wich a molecular weight of the fatty acids considered to be equal to predominant fatty acid and expressed in percent.[17]

Determination of the saponification number of moringa seed oil using titrimetric method by titrating the sample solution and the blank solution until the saponification number is known. When doing a test of saponification number, moringa seed oil is reacted with excess KOH. In determining the amount of saponification, fatty acids and free fatty acids from moringa seed oil are reacted with KOH in alcohol to form soap. The amount of KOH required in the saponification process depends on the number of triglyceride molecules contained from moringa seed oil which is composed of short carbon chain fatty acids that have a relatively small molecular weight so that it has a large saponification number and oils with large molecular weights have a small saponification number.[27]

Moringa seed oil hydrolysis occurs in under alkaline conditions because water cannot completely hydrolyze moringa seed oil, so a strong alkaline solution such as KOH is used. The reaction that occurs between moringa seed oil and base is a saponification reaction. The saponification reaction of the oil yield a product in the form of soap or fatty acid salts. If the fatty acid salt product is added with water and acid catalyst in the form of sulfuric acid (H_2SO_4) it will form free fatty acids.[17]

The formation of free fatty acids into ester compounds is called esterification which is done by reacting fatty acids with alcohols. Free fatty acids from moringa seed oil obtained from the hydrolysis process are added with methanol and sulfuric acid as a catalyst to form methyl ester compounds. The esterification process is done to analyze fatty acid levels using GC-MS because the requirements for the compounds needed for analysis purposes must be volatile while the fatty acids obtained from hydrolysis are non-volatile. One of the esterification products from the volatile fatty acids of moringa seed oil is methyl ester.[17] Moringa seed oil analysis using the GC-SM method from hydrolyzed fatty acids (methyl ester compounds) showed the presence of 10 peaks on the chromatography which can be seen in the Figure 3.

Based on Figure 3, there are 6 peaks identified as fatty acid compounds, starting from the fourth peak to the tenth peak. The fourth peak with a retention time of 27,285 has an area of 1.07% which is suspected to be lauric acid because it has a mass spectra that corresponds to the mass spectra of acid which corresponds to the mass spectra of dodecanoic acid (methyl laurate) from WILEY229.LIB with entry number 79499 and entry number from the fifth to the tenth peak in a row are: 123056; 124633; 142893;144208;134626;161700. Fatty acid analysis using KG-SM can be seen in table 2.

Table 2 showed that fatty acid content of moringa seed oil includes lauric acid, palmitoleic acid, palmitic acid, oleic acid, stearic acid and arachidic acid. The highest fatty acid content of moringa seed oil was oleic acid or 9-octadecanoic acid with a percent area value of 38.08%.[17]

Fatty acids contained within the moringa seed oil are the components of triglycerides. To examine fatty acids and their compositions, triacylglycerol or triglycerides fats in oil can be changed into methyl ester fatty acid using the derivatization method.[28] This method is a chemical process that changes a compound into another compound if it inherits appropriate characteristics to do analysis using gas chromatography.[29] Fatty acid structure is shown in Figure 4.

Identification and determination of sterols in moringa seed oil

The steroid is an organic compound with three rings of carbon-6 atoms as well as one ring of carbon-5 atom which structures can be defined based on alkyl groups on carbon atoms numbered 10, 13, and 17 that is estran, androstane, pregnant, nor pregnant, colestan, stigmastan, ergostan, colane, and spirostan groups. Commonly, steroid dissolves in an organic, non-polar solvent like petroleum ether.[31]

Sterol compounds are lipid that cannot be saponified and found in higher plant species known as phytosterol, and grouped in the form of triterpenes with the cyclopentane perhydrophenanthrene ring form. Phytosterol compounds are bound to simple glucosides or in free form.[32] Phytosterol groups structure is shown in Figure 5.

Identification and determination of sterol compounds in moringa seed oil could be done used Gas Liquid Chromatography (GLC) based on the method described in Official Journal of the European Community (L248). Sterol analysis was performed on a Hewlett-Packard 5890 gas chromatograph equipped with DB-5 FSOT capillary column (30 m x 0.25 mm x 0.25 µm). The carrier gas pressure (H_2) was 75 kPa at FID 300

Table 2: Fatty acid analysis using GC-MS.

Peak	tR (Minute)	Area (%)	MW/ Molecular Formula	Compound Name
1	23.15	15.85	204/$C_{15}H_{24}$	Beta-patchoulen
2	25.369	5.27	204/$C_{15}H_{24}$	Naphthalene
3	26.097	9.93	204/$C_{15}H_{24}$	1.2.4-Methenoazulen
4	27.285	1.07	214/$C_{13}H_{26}O_2$	Dodecanoic acid/methyl lauric
5	36.078	1.79	268/$C_{17}H_{32}O_2$	9-hexadecanoic acid/methyl palmitoleic
6	36.506	8.52	270/$C_{17}H_{34}O_2$	Methyl hexadecanoate/methyl palmitate
7	40.026	38.08	296/$C_{19}H_{36}O_2$	9-octadecanoic acid / methyl oleic
8	40.495	1.84	298/$C_{19}H_{38}O_2$	Octadecanoic acid/methyl stearate
9	42.799	16.13	284/$C_{18}H_{36}O_2$	Octadecanoic acid/stearic acid
10	44.147	1.51	326/$C_{21}H_{42}O_2$	Eicosanoic acid/methyl arachidic

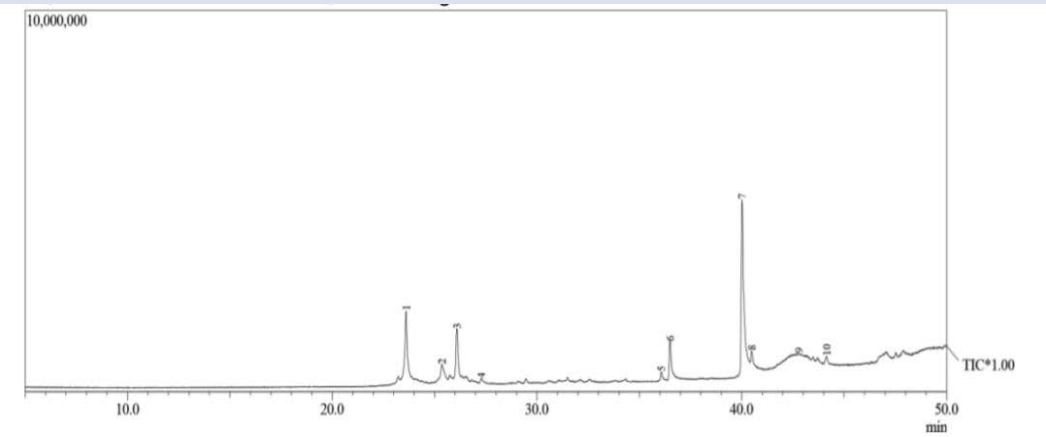

Figure 3: Chromatogram (CG) sample methyl ester from moringa seed oil.[17]

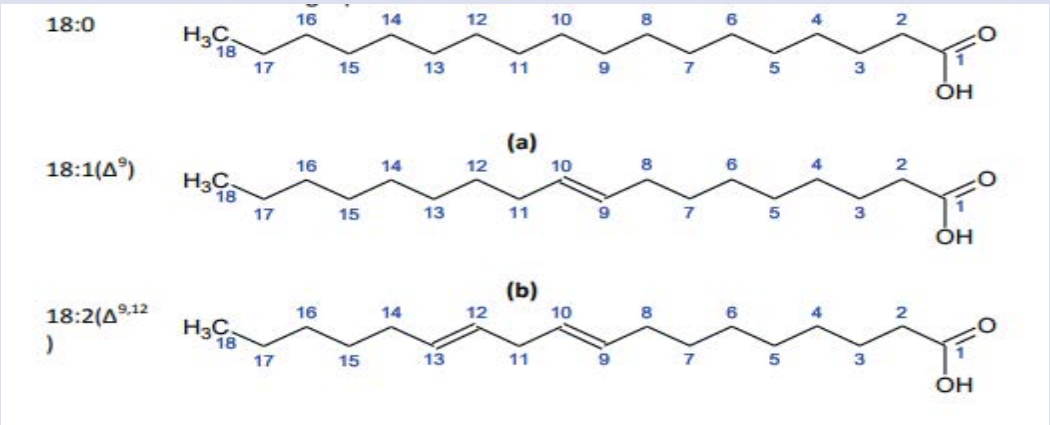

Figure 4: Fatty acid structure (a) stearic acid (b) oleic acid (c) linolenic acid.[30]

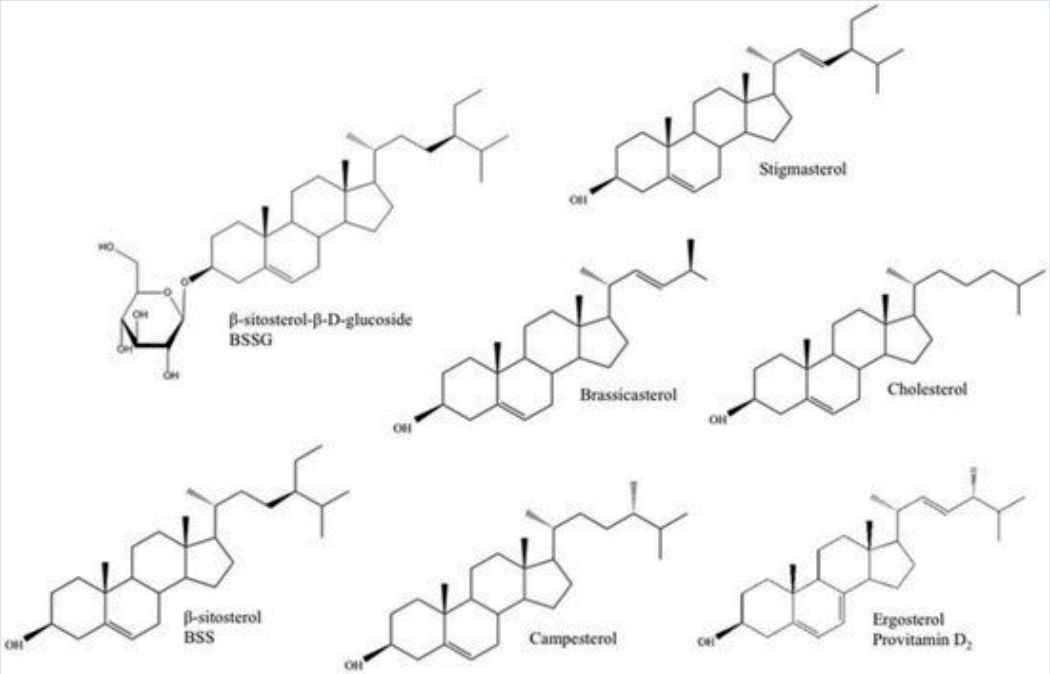

Figure 5: Phytosterol groups structure.[33]

Table 3: Compared the result of the identification of sterol compounds in moringa seed oil used the cold press method and extraction with *n*-hexane as solvent.

Determination sterols by GLC	GLC%	
	Cold Press	Extraction with n-hexane
cholesterol	0.13 (0.020)	0.13 (0.019)
brassicasterol	Not detected	0.06 (0.010)
24-methylenecholesterol	0.85 (0.160)	0.88 (0.116)
campesterol	14.03 (0.931)	15.13 (0.996)
campestanol	Not detected	0.35 (0.069)
stigmasterol	17.27 (1.225)	16.87 (1.01)
ergostadienol	Not detected	0.39 ((0.071)
clerosterol	0.95 (0.230)	2.52 (0.916)
β-sitosterol	49.19 ((3.896)	50.07 (3.998)
stigmastanol	1.05 (0.411)	0.86 (0.196)
Δ 5-avenasterol	12.79 (1.614)	8.84 (1.198)
28-isoavenasterol	1.01 (0.421)	1.40 (0.312)
Δ 7,14-stigmastanol	0.83 (0.223)	0.44 (0.102)
Δ 7-avenasterol	0.94 (0.095)	1.11 (0.089)

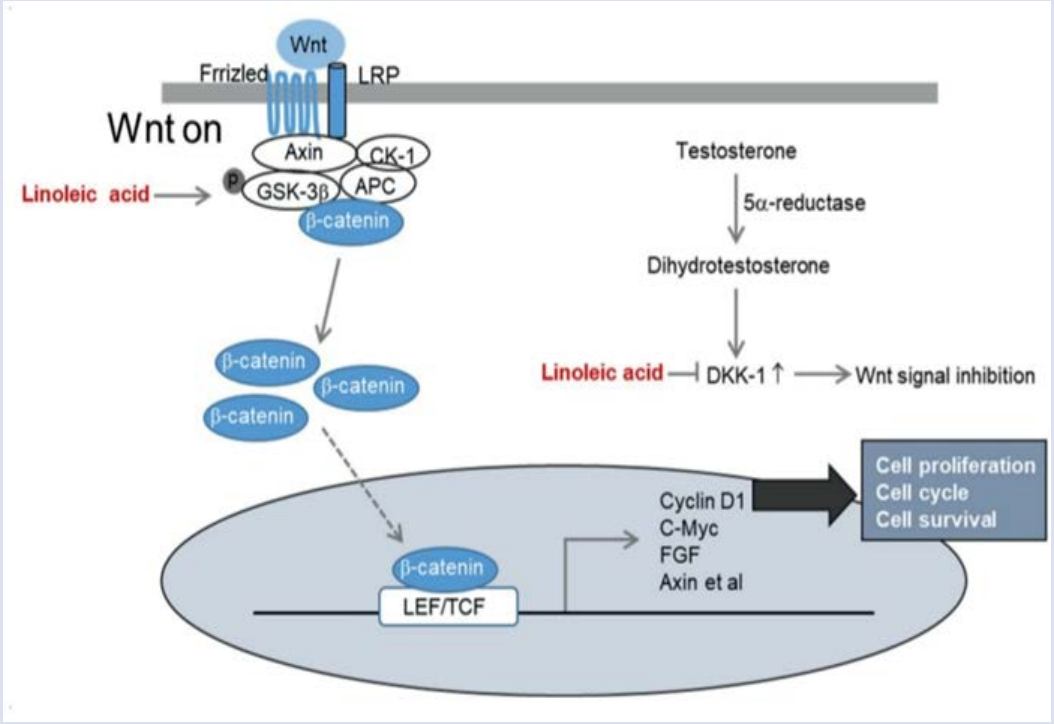

Figure 6: The mechanism of linoleic acid in hair loss improvement.[43]

°C and injector 280 °C. The column temperature was maintained at 260 °C for 40 minutes. The internal standard used in the test was R-cholestanol.[22]

Moringa seed oil sterol compounds were identified and measured by comparing the retention time at the peak area of known and unknown components with standard sterol compounds. The sample used was moringa seed oil from cold press and extraction with *n*-hexane solvent using soxhletation method.[22] The table compared of the identification of sterol compounds in moringa seed oil used the cold press method and extraction with *n*-hexane as solvent can be seen in table 3.

The sterol fraction of moringa seed oil resulted mainly from campesterol, stigmasterol, β-sitosterol and Δ5-avenasterol compounds. The β-sitosterol compound from the determination result showed the most dominant amount using the cold press method or extraction with *n*-hexane solvent, besides that there were also other steroid compounds such as cholesterol, brassicasterol, Δ7-avenasterol, 24-methylenecholesterol, campestanol, ergostadienol, clerosterol, stigmastanol, 28-isoavenasterol and Δ7,14-stigmastanol. Table 3 also showed that the cold press method did not extract campestanol, brassicasterol and ergostadienol compounds while *n*-hexane solvent extraction method could extract all components of the sterol compounds mentioned in the table.[22]

Mechanism of action of moringa seed oil compounds as an anti-alopecia

The potential of moringa seed oil to be an anti-alopecia exists due to the phytosterol compound contained, that is β-sitosterol. The mechanism in which this compound plays a role as an anti-alopecia happens through the blocking of 5 α-reductase enzymes, which then prevents the formation of dihydrotestosterone (DHT) the cause of hair loss. This compound blocks the binding of dihydrotestosterone (DHT) in the androgen receptor located in the hair follicle and leads to the stimulation of hair growth on the scalp.[9] In addition, β-sitosterol has been shown to act as an inhibitor of 5α-reductase activity by showing significant hair growth capacity, however, so far there have been no reports and studies that support the activity of β-sitosterol compounds from Moringa seed oil and its specific components as antialopecia. Therefore, research that focuses on investigating the regenerative effects of hair and the main components of moringa seed oil as an antialopecia is needed.[9] The compound of phytosterol with 0.01% to 0.25% concentration has proven to have the effects of anti-alopecia.[34] Not only that, but moringa seed oil also contains peptide oil compounds that function as micro-circulation on the scalp as well as tocopherol or tonic for hair.[3,35,36]

Beside phytosterols, moringa seed oil also contains isoflavones, where these compounds belong to the class of phytoestrogen known as flavonoid. Phytoestrogens are basically a group of compounds from plants that have the ability to act like the hormone estrogen. In all existing isoflavone groups, genistein and daidzein types carry the most estrogenic function although its fewer in amount and further studies are needed regarding the components of these compounds in moringa seed oil. After converted, daidzein then will be metabolized in the intestine to form equol. Equol is compound that act as anti-androgens, but the mechanism is very specific and still needs to be investigated further regarding to the specific mechanism because this compound is known to not actually bind to the androgen receptor (AR) but binds to dihydrotestosterone (DHT) instead with high affinity. This shows that the biological activity and physiological processes of dihydrotestosterone (DHT) can be modified by changing its shape from a DHT receptor complex to a nuclear receptor according to adaptation or transformation treatment.[37]

Another study showed that the steroid content in moringa seed oil has estrogen-like activity and can reduce testosterone levels.[38] Estrogen can modify the occurrence of androgen metabolism in different hair follicle subunits, so that 5-α dihydrotestosterone levels can be reduced, besides that it can also affect growth factors and cytokine transcription which is an important hormone in the process of normal hair growth.[39]

The potential of moringa seed oil to be an anti-alopecia is also supported by the discovery of an oleate acid compound that can deescalate hair loss rate and accelerate hair growth. Moringa seed oil contains

a high oleate acid compound (68-76%).[14] Oleate acid falls into the monounsaturated fatty acids group, which is a solid antioxidant.[40] Fatty acids have the efficacies to stimulate hair growth, provide nutrition for hair, slow down hair loss, accelerate hair growth, protect hair, also act as hair care and tonic.[3,41]

Moringa seed oil has a linoleic acid component which can significantly increase the proliferation of dermal papilla cells which contribute to the regeneration of hair growth. In addition, linoleic acid can induce β-catenin signal activation, where in the β-catenin signaling pathway, there is no external signal that causes phosphorylation of β-catenin by glycogen synthase kinase 3 (GSK-3) which is a serine/threonine protein kinase to mediate the addition of phosphate molecules to the serine/threonine amino acid residue, so that β-catenin becomes dispersed and degraded. When the β-catenin ligand binds to its receptor, the activity of GSK-3 is suppressed so that it will inhibit the degradation of β-catenin. Furthermore, β-catenin moves into the nucleus which will eventually induce hair cell proliferation.[42,43] The mechanism of action of linoleic acid as an anti-alopecia can be seen in Figure 6.

Another mechanism of hair loss is due to hormonal imbalance. When dihydrotestosterone, which is converted from testosterone by the enzyme 5α-reductase and produced in excess. When dihydrotestosterone binds to derma papilla cells, the expression of dickkopf-related protein (DKK-1), will induce apoptosis, which causes hair matrix cell death which gives a specific picture of hair loss. An important aspect of dihydrotestosterone-induced hair loss is reduced cell proliferation through inhibition of β-catenin signaling. Linoleic acid will activate β-catenin signaling effectively by inhibiting the expression of DKK-1 by dihydrotestosterone.[43,44]

Linoleic acid has also been reported to have anti-alopecia activity starting from the anagen phase by showing the potential for hair growth through inducing growth factors such as vascular endothelial growth factor (VEGF), insulin like growth factor-1 (IGF-1), and keratinocyte growth factor (KGF), as well as through suppression of transforming growth factor -β (TGF-β).[45]

Other components of moringa seed oil as anti-alopecia are tocopherols and lauric acid. The content of tocopherol compounds in moringa seed oil acts as anti-oxidant that helps effective circulation in the scalp due to increased oxygen uptake in the blood, thus playing an important role in promoting hair growth and preventing hair loss. Moringa seed oil contains lauric acid with high affinity and low molecular weight so that it can penetrate the hair cavity and stimulate hair growth. Lauric acid is a good nutrient and can penetrate the hair cavity easily and give the effect of hair growth.[3,41]

CONCLUSION

The content of phytosterol compounds and fatty acids in moringa seed oil has potential as an anti-alopecia. The mechanism of action of the compound as an anti-alopecia occurs through inhibition of the 5 α-reductase enzyme by preventing the formation of dihydrotestosterone (DHT) to bind to androgen receptors in hair follicles, so that can prevent hair loss. Other components such as tocopherols can act as antioxidants to protect the scalp and help effective circulation in the scalp to provide nutrients for the hair.

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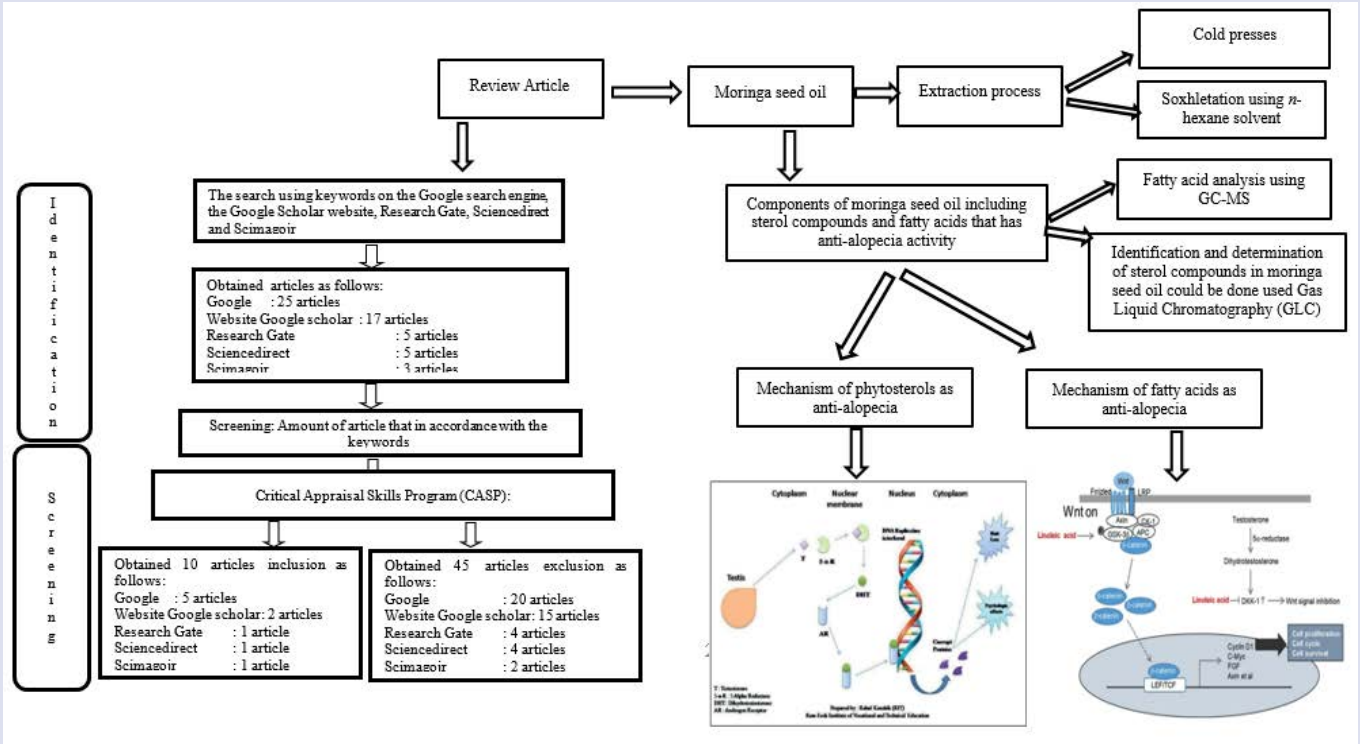
GRAPHICAL ABSTRACT



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