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Antialopecia Activity and IR-Spectrometry Characterization of Bioactive Compounds From *Sansevieria trifasciata* P.

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Abstract

Androgenetic Alopecia (AGA) causes more than 90% of alopecia cases. Drugs for the treatment of AGA have toxic and side effects, so it is necessary to find new drugs from the natural product, such as *Sansevieria trifasciata*. This study aims to determine the hair growth activity of subfraction from *Sansevieria trifasciata* and characterize the isolate using infrared spectroscopy. The fraction of *Sansevieria trifasciata* was separated using column chromatography. The hair growth activity using the modified Tanaka method. Skin biopsies of each rabbit stained with hematoxylin and eosin. The characterization of the isolate using infrared spectroscopy (FT-IR). The separation of fractions obtained six subfractions. The hair growth activity from subfraction-C was higher with the hair growth length of 2.80 cm ± 0.00 ($p<0.05$) and similar effectiveness to 2% minoxidil. The anagen:telogen ratio of subfraction-C (78.26:21.79%, respectively). The infrared spectrum of isolate-1 and isolate-2 showed the strong signal of hydroxyl (-OH) and C-O vibration, weak speak of -CH, $-CH_2$ and $-CH_3$, and medium signal of C=O vibration. The subfraction-C of *Sansevieria trifasciata* prevents alopecia by stimulating hair growth, and the bioactive compound in the isolate is suspected to be a steroid and terpenoid group.

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Keywords: Alopecia, Infrared spectroscopy, Sansevieria trifasciata, Anagen, Telogen

1. Introduction :

Human hair growth is significant for health and well-being. Although medically hair loss is not life-threatening, hair is considered a crucial part of the body by people in various cultures worldwide. Excessive hair disorders such as hair loss in alopecia cause psychological stress [1]. Alopecia is characterized by reducing hair strands on the scalp by more than 120 strands/day. Based on the literature, Androgenetic Alopecia (AGA) causes more than 90% of alopecia cases, often used to describe alopecia in general [2]. The main etiologic factors for AGA are genetic and hormonal status [3]. The hormones that play a role are Testosterone and Dihydrotestosterone (DHT). DHT is formed by converting the hormone testosterone to 5α-DHT with the help of the enzyme 5α-reductase type 2. Excess production of 5α–DHT is a significant factor causing androgenetic Alopecia (AGA) [4–7]. The involvement of androgen receptors in this condition causes changes in the anagen and telogen phase of the hair cycle. The anagen phase of the hair growth phase becomes shorter than the telogen phase, resulting in thinner and shorter hair follicles that ultimately cannot even penetrate the epidermis [8]. This causes the anagen to telogen ratio to decline from normal conditions.

The AGA requires long-term treatment, while drugs approved by the FDA for the treatment of AGA, namely Minoxidil and Finasteride have side effects and toxic effects that cause discomfort to the user, such as allergic contact dermatitis, burning,

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ejaculation disorders, and decreased libido, have been reported [9,10]. Therefore, it is necessary to search for new drugs needed as an alternative to hair growth therapy. Currently, herbs are very popular with the public because they have been used in traditional medicine with minimal side effects than conventional drugs marketed.

The *Sansevieria trifasciata* is an empirically used herb to prevent alopecia [11]. Several phytochemical studies have reported some compounds such as terpenoids, steroids and saponin [12–14], flavonoids such as tripasciatine A and tripasciatine B, pyranoisoflavon [15-17], glycoside tannins, polyphenols, alkaloids [18,19], palmitic acid, quinolone, campesterol, pyridine, phytol, cycloeucareol, tocopherol and linoleic acid [17]. However, research on the anti-alopecia activity of this plant is still limited. The development of research from this plant shows that the extract may reduce the amount of hair loss [20 -21]. Our previous study found that the ethyl acetate fraction of *Sansevieria trifasciata* at a concentration of 20% (w/v) was effective in growing hair [22]. Therefore, in this study, the ethyl acetate fraction is separated and evaluated the antialopecia activity in vivo to obtain an active subfraction. The active subfraction isolated and characterized by FT-IR to obtain a profile of the functional group of compounds in the sample.

2. Experimental

2.1 Extraction

The extraction of *Sansevieria trifasciata* leaves followed the method in the previous study [22]. Samples were obtained and collected from Kambu District, Kendari City, Southeast Sulawesi Province. The leaves were prepared in dry simplicia and ground into powder. The simplicia powder (1 kg) was macerated with ethanol as a solvent [23] and evaporated into the crude extract. Then, the extract was fractionated with n-hexane, ethyl acetate, and water. The ethyl acetate fraction was used for further separation.

2.2 Separation of ethyl acetate fraction by column chromatography

Silica gel $60H_{254}$ p.a (E. Merck®) was weighed as much as 20-40 times the weight of the sample and put into the column while compacting with a vacuum pump. The ethyl acetate fraction (10 gram) was impregnated on 200–350 mesh silica gel which was weighed as much as two times the weight of the sample. Then the sample was eluted with eluent (n-hexane: EtOAc: MeOH) in various comparisons. Sixteen subfractions were obtained, and the separation profile was visualized using TLC. The fractions with similar TLC profiles were combined. The six main subfractions (A, B, C, D, E, F) were obtained for antialopecia activity test.

2.3 Antialopecia activity

The method reported by Tanaka was followed with a slight modification[24]. This assay used five rabbits (4-5 months old, 1-2 kg BW). The animals were fed on a standard pellet diet and water. The ethical clearance for animals approved by the Committee on health research ethics of the institution of research and community service, Universitas Halu Oleo, Kendari (ethical clearance certificate number: 1201/UN29.20/PPM/2020). Briefly, the rabbit's back was shaved and made into eight compartments (2x2 cm, respectively), namely I, II, III, IV, V, VI, VII, and VIII. Each compartment was given topical application of approximately 0.2 mL of the vehicle as negative control (K(-)), minoxidil 2% as the positive control (K(+)), subfractions of ethyl acetate A, B, C, D, E, and F, respectively. The solution was applied to the rabbit back once daily for 18 days. The hair growth was observed by taking five strands of rabbit fur in each compartment every three days, on days 3, 6, 9, 12, 15, and 18 of treatment. The hair length was measured using a caliper. Data were collected and analyzed statistically. After 18 days of treatment, the rabbits were randomly selected and sacrificed. Skin biopsies were performed from each rabbit compartment site, and skin was stored in phosphate-buffered formalin for paraffin cutting. The skin was cut horizontal sections (1-2 mm) and stained with hematoxylin and eosin. The phase of the hair follicle cycle (anagen and telogen), the proportion of hair growth, and the anagen/telogen ratio were calculated with the help of an ocular micrometer.

2.4 Isolation Bioactive Compounds by TLC-Preparative

The isolation was carried out using the subfraction with the most effective hair growth effect, Subfraction-C with the TLC-Preparative method. Silica Gel $60F_{254}$ p.a (E. Merck®) was used as a stationary phase size of 20x20 cm. The TLC-Preparative plate was activated first at 100ºC for 1 hour. The sample (100 mg) was dissolved using 10 mL of chloroform: methanol (1:1), then applied with a capillary tube to the surface of the TLC plate. After that, the plate was eluted with n-hexane: ethyl acetate as a mobile phase (8:2). The band formed was observed under ultraviolet light at wavelengths 254 and 366. The isolate obtained from the TLC-preparative was taken by scraping the silica where the compound band was visible. The silica powder was dissolved with a mixture of chloroform: methanol (1:1) (10 ml), then centrifuged for 15 minutes at 5000 rpm to precipitate the stationary phase. The

supernatant was evaporated into powder and collected as an isolate.

2.5 Characterization of isolate

The functional groups in the isolate were characterized by using infrared spectroscopy with the KBr pellet method. Isolate-1 and isolate-2 (1 mg respectively) were mixed with KBr powder (10 mg), then ground until smooth. Futhermore, the mixture was compressed in a mould to obtain thin pieces (pellets). The characterization of sample pellets was carried out with an FTIR (Perkin Elmer FTIR 100) spectrometer at a wavelength of 4000-400 cm^{-1} [25].

2.6 Data analysis

Hair growth data are presented in Mean ± SD. Data were compared with the control group using IBM SPSS Statistic 24 one-way ANOVA.

3. Result and Discussion

The *Sansevieria trifasciata* has the potential to prevent hair loss through its ability to stimulate hair growth. Previous studies showed that extract and the ethyl acetate fraction of *Sansevieria trifasciata* could stimulate hair growth. Furthermore, in this study, the ethyl acetate fraction was separated using column chromatography. The fractionation obtained six combined subfractions (A, B, C, D, E, F) and was used to antialopecia test using the modified Tanaka method. The experimental parameters were hair growth length, hair follicle cycle phase (anagen and telogen), hair growth proportion, and anagen/telogen ratio calculated using an ocular micrometer. Hair growth measurements were carried out every three days until day 18 using a caliper. Six subfractions, namely subfractions A, B, C, D, E, and F, and two control groups, namely negative control (K(-)) and positive control (K(+)), were applied to the rabbit's back.

The ability of *Sansevieria trifasciata* subfraction as antialopecia could be observed on potency and effectiveness indicators. The parameter used in this study was the hair growth of rabbits given subfraction treatment. The results in Table-1 showed that each subfraction of *Sansevieria trifasciata* had significant hair growth activity from day 6 to day 18 ($p<0.05$) compared to the negative control group. However, there was no significant difference in hair length in the subfraction B group on the 15th until the 18th day of treatment with the negative control. The subfraction c showed the highest increase significantly ($p<0.05$) in hair growth compared to other subfractions with a hair growth length of 2.80 cm ± 0.00 and had similar effectiveness to 2% minoxidil (Table-1). The hair growth pattern of the subfraction-c group was similar to the minoxidil 2% group. Minoxidil was the first and only topical drug approved by the FDA to treat AGA. The exact mechanism of action of minoxidil on hair growth remains unclear but may be mediated through the opening of potassium channels, leading to increased skin blood flow and increased blood vessel levels, vascular Endothelial Growth Factor (VEGF) hair growth promoter in the dermal papilla [26].

Table 1. Measurement of hair growth length of rabbits treated with subfraction A, B, C, D, E and F of Sansevieria trifasciata P. for 18 days

Treatment Group	Mean of hair growth length of rabbit (cm) ± SD					
	Days					
	3	6	9	12	15	18
K(-)	0.23 ±0.06	0.40 ± 0.10	0.53 ± 0.15	0.80 ± 0.26#	1.07 ± 0.15#	1.57 ± 0.36#
K(+)	0.27 ±0.00	0.57 ± 0.06	0.77 ± 0.06	1.23 ± 0.15*	1.87 ± 0.06*	2.43 ± 0.06*
A	0.30 ±0.00	0.63 ±0.00*	0.90 ± 0.10*	1.30 ± 0.10*	2.00 ± 0.46*	2.27 ± 0.40*
B	0.23 ±0.06	0.53 ± 0.12	0.83 ± 0.10*	1.30 ± 0.20*	1.63 ± 0.40	1.93 ± 0.32
C	0.27 ±0.06	0.60 ± 0.10*	0.87 ± 0.15*	1.40 ± 0.36*	2.17 ± 0.58*	2.80 ± 0.00*
D	0.7 ±0.06	0.57 ±0.06	0.77 ± 0.06*	1.23 ± 0.15*	1.93 ± 0.32*	2.17 ± 0.29*
E	0.27 ±0.06	0.55 ± 0.07	0.80 ± 0.17*	1.30 ± 0.30*	1.97 ± 0.40*	2.20 ± 0.35*
F	0.23 +0.06	0.50 ± 0.10	0.77 ± 0.12*	1.20 ± 0.10*	1.97 ± 0.40*	2.17 ± 0.40*

*$p<0.05$ significance to control
#$p<0.05$ significance to Minoksidil 2%

Another parameter used in this study to assess the antialopecia activity of the subfraction was the appearance of anagen (A) and telogen (T) on microscopic histopathological of rabbit skin (Fig-1). The histopathology result found that anagen clearly showed the inner and outer root hair sheaths and the absence of signs of apoptosis in the outer root sheath. In contrast, telogen histopathology showed follicles with central wrinkling of the hair canal tricholemal keratinization [27]. Anagen and telogen are two of the hair growth phases. Under normal conditions, the duration of the anagen (growth phase) will be longer than the duration of the telogen (resting phase). However, in alopecia, the duration of the anagen phase

is reduced even though the telogen phase remains constant. This condition leads to a reduced AT ratio [28-29]. Based on this, the number of anagen and telogen obtained from the histopathological is made in ratio to make it easier to assess the antialopecia activity (Fig-2).

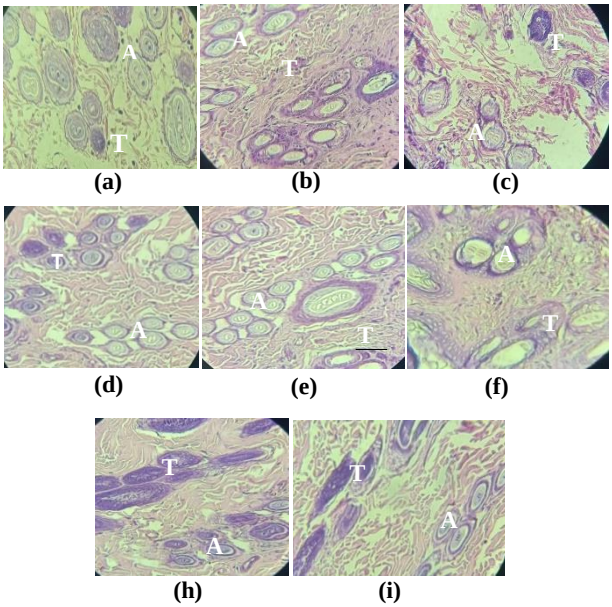

Fig 1. Comparison of hair loss pattern in rabbit skin section in each group 40x enlargement by hematoxylin-eosin staining. (a) The skin of animals is treated with vehicles. (b) The skin of animals treated with minoxidil solution. (c) The skin of the animal is treated with a subfraction-A solution. (d) The skin of the animal is treated with a subfraction-B solution. (e) The skin of animals is treated with the subfraction-C solution. (f) The skin of animals is treated with the subfraction-D solution. (g) The skin of the animal is treated with the subfraction-E solution. (h) The skin of the animal is treated with the subfraction-F solution. A = Anagen and T = Telogen

The anagen and telogen ratio result showed that the anagen ratio could be increased by the administration of subfraction, which indicated a longer duration of hair growth than the telogen phase. The highest anagen ratio occurred in the group given subfractions-C compared to other subfractions. The pattern of increasing AT ratio of subfractions-C was similar to the minoxidil group. The anagen:telogen ratio of subfractions A, B, C, D, E, and F, KN, and K(+) were respectively 66:34%; 74.07:25.93%; 78.26:21.79%; 58.73:30.18%; 66.67:33.33%; 75.61:24.39%; 74.14:25.86%; 79.59:20.41%. This result indicated that the subfraction of *Sansevieria trifasciata* could prevent alopecia by stimulating hair growth and prolonging the duration and ratio of the anagen phase.

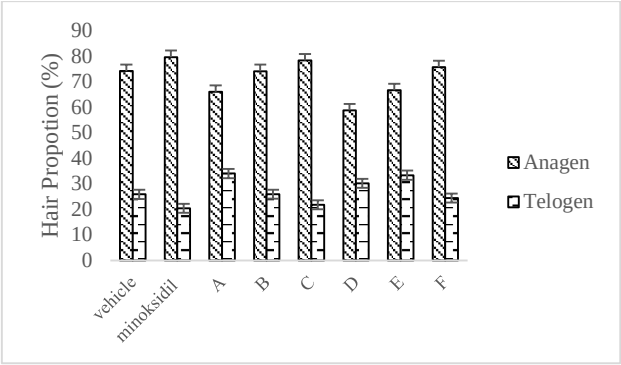

Fig 2. Hair growth proportion (anagen versus telogen) of rabbit skin following treatment. Subfraction-A (A), Subfraction-B (B), subfraction-C (C), subfraction-D (D), subfraction-E (E), subfraction-F(F).

The chemical compounds in the herb could affect the pharmacological effects. Therefore, in this study, characterization of compounds in the active subfraction was carried out using FT-IR. First, subfraction-C was used for further separation of compounds using preparative thin-layer chromatography. Based on the separation results, two types of isolates were obtained, namely isolates-1 and isolate-2. Each isolate was further characterized using Fourier transformation infrared (FTIR) to overview the functional groups contained in the isolate.

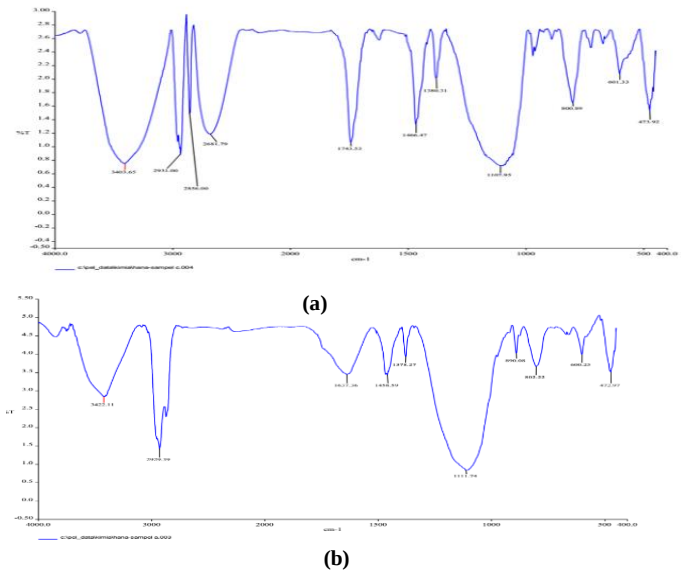

Fig 3. Caracterization of isolate-1 and isolate-2 using FT-IR. (a) isolate-1; (b) isolate-2

The peak which appears in the spectrum indicates certain functional groups. The results in Fig-3 showed a strong signal in wavenumbers of samples 1 and 2, respectively 3403.65 cm-1 and 3422.11 cm-1, indicating the presence of hydrogen bonds (-OH) group. Weak signals appear in the spectrum of isolate-

1 and isolate-2 respectively at wavenumbers 2931.00 cm^{-1}, 2856.00 cm^{-1}, and 2929.39 cm^{-1} indicated the vibration of -CH[30]. The medium signal that appeared at 1743.53 cm^{-1} and 1637.36 cm^{-1} was a C=O stretching carbonyl group on both isolates. Strong signals at wavenumbers 1107.95 cm^{-1} and 1111.74 cm^{-1} indicated the presence of C-O stretching groups [30], [31]. Weak signal of $-CH_2$ and $-CH_3$ stretching showed in spectrum isolate-1 and isolate-2 respectively at wavenumber 1466.47 cm^{-1} and 1380.31 cm^{-1}; 1458.59 cm^{-1} and 1378.27 cm^{-1} [31].

The presence of hydroxyl without signal of the aromatic group, aliphatic methyl, and methylene in the infrared spectrum result indicated the presence of steroids or terpenoids [32] compounds in both isolates. These compounds caused the ability of subfraction-C to stimulate hair growth. Other studies reported that the steroid could prevent alopecia [33] by suppressing the T-cell-mediated immune attack on the hair follicle mechanism [34]. Besides that, the terpenoids in other studies could stimulate hair growth by activating the Wnt/β-catenin pathway. Monoterpenoids have increased the amount of human follicle dermal papillae and vascular endothelial growth factor (VEGF) required for hair growth [35].

4. Conclusion

The subfraction-C of *Sansevieria trifasciata* prevent alopecia by stimulating hair growth, and the bioactive compound in the isolate suspected to be a steroid and terpenoid group

5. Conflicts of interest

There are no conflicts to declare

6. Formating of funding sources

This research received no external funding

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