Original Article

Grape skin and seed extracts as a potential natural solution for hair loss: A bioactivity evaluation

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Abstract

Scalp yeast infections and androgenic effects can lead to hair loss respectively by disrupting hair follicle function and by causing miniaturization. Herbal extracts, including grape skin and seed extracts, show promise for hair growth improvement and hair loss prevention due to their safety and diverse biological activities. The aim of this study was to evaluate the antifungal and 5α-reductase inhibition activities of grape skin and seed extracts. The results revealed that grape skin and grape seed extracts exhibited excellent antifungal activity against *T. mentagrophytes*, *M. gypseum*, *C. albicans*, *P. ovale*, and *M. canis* in descending order. Furthermore, ethanolic grape skin and seed extracts demonstrated promising anti-hair loss activity through an increase in the proliferation of human hair follicle dermal papilla cells (HFDPC) and 5α-reductase inhibition analyzed *in vitro* on HFDPC. Therefore, the results of this study imply that grape skin and seed extracts may be utilized in the development of multifunctional hair products with anti-hair loss and antifungal actions.

Keywords: grape extract, antifungal activity, 5α-reductase, anti-hair loss

1. Introduction

Among hair loss disorders, androgenetic alopecia (AGA) is the most prevalent form characterized by progressive hair thinning, shortening, and loss of pigmentation, affecting both genders. It is suggested that AGA is primarily associated with a genetic predisposition to sensitivity to dihydrotestosterone (DHT) (Sawaya & Price, 1997). The mechanism behind AGA involves the enzyme 5α-reductase that converts testosterone into a potent androgen DHT. Androgen receptors mainly expressed in the dermal papilla cells of the hair follicle are a primary target for DHT, and this binding leads to an inhibition of hair growth (Chandrashekar, Nandhini, Vasanth, Sriram, & Navale, 2015).

Moreover, maintaining a balance among the numerous microorganisms inhabiting the skin and hair of an individual becomes vital for their health condition. While research on the microbiome of the skin and hair follicles is limited, it has been established that the invasion of fungi into the hair follicle bulge can result in permanent hair loss (Nematian, Ravaghi, Gholamrezanezhad, & Nematian, 2006; Sohnle, Collins-Lech, & Hahn, 1986).

The two drugs approved by the regulatory authorities of various countries and widely used for the treatment of alopecia are minoxidil and finasteride. The mechanism of action of finasteride, as a type II 5α-reductase inhibitor, is directly related to AGA, as it reduces the conversion of testosterone to DHT, while for minoxidil, although it has been used for the treatment of AGA for several decades, the mechanism of action is still not fully understood (Madaan, Verma, Singh, & Jaggi, 2018). One of the main assumptions revolves around the notion that, given minoxidil's vasodilatory properties, its mechanism of action

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involves increasing blood flow to the follicles. However, a recent study revealed another possible mechanism of action of minoxidil based on its antiandrogenic effect through significant downregulation of the expression of the 5α-R2 gene in HaCaT cells (Pekmezci & Türkoğlu, 2017). Despite different anti-hair loss mechanisms, both drugs cause undesirable side effects that have prompted the search for alternative treatments (Madaan *et al*., 2018).

The fact that the hair follicles provide a favorable route for the delivery of topical preparations containing compounds that penetrate these channels more efficiently than the stratum corneum has brought to the fore the potential for the topical application of herbal products. Among them are grape skin and grape seeds (*Vitis vinifera* L.), a rich resource of polyphenols with numerous documented biological benefits for skin and hair (Obreque-Slier *et al*., 2010; Yarovaya, Waranuch, Wisuitiprot, & Khunkitti, 2022). Regarding the latter, the antioxidant and anti-inflammatory properties of the grape extract are believed to help protect hair follicles from damage and stimulate hair growth (Ferri *et al*., 2017; Kwon *et al*., 2007; Takahashi, Kamiya, & Yokoo, 1998). Increases in the number of hair follicles and in hair growth length have been reported in a study on rats topically treated with grape sap (Esmaeilzadeh *et al*., 2021). The antifungal and antibacterial efficacy of rich polymeric flavan-3-ols extracts from grape seed extract has also been reported (Khawee, Aromdee, Monthakantirat, & Khunkitti, 2015; Simonetti *et al*., 2017). Furthermore, grape skin and seeds as wine-making by-products are an example of a natural resource that meets the sustainability criteria of production, which further justifies their use in cosmetics.

In our previous study, it was found that grape skin and seed extracts contain high quantities of polyphenols along with significant antioxidant and antibacterial properties (Khawee *et al*., 2015). However, it was of interest to extend the evaluation of their antifungal activity. Moreover, many studies on grape properties have validated a remarkable ability to promote cell growth in hair follicles and regulate physiological cycles of hair growth. However, there is still a lack of studies demonstrating the potential of grape extracts to inhibit 5α-reductase. Therefore, the hair loss prevention potential of grape extracts was also analyzed *in vitro* on human dermal papilla cells.

2. Material and Methods

2.1 Materials

Kojic acid, testosterone, minoxidil, and finasteride were obtained from Sigma-Aldrich (Taufkirchen, Germany). Tween 80 was ordered from Namsiang (Bangkok, Thailand). Sabouraud dextrose agar, sabouraud dextrose broth, and yeast extract were purchased from Difco (Detroit, MI, USA). Human hair follicle dermal papilla cells (HFDPC), ready-to-use HFDPC growth medium, HEPES Buffered Saline Solution (HBSS), trypsin ethylene diamine tetra acetic acid (Trypsin/EDTA), and trypsin inhibitor solution were ordered from Promo Cell (Heidelberg, Germany). PrestoBlue™ Cell Viability Reagent was obtained from Invitrogen (Waltham, MA, USA). Testosterone (ab174569) Human Elisa Kit was purchased from Abcam (Cambridge, UK). Dimethyl sulfoxide (DMSO) was obtained from Gibco (Gaithersburg, MD, USA).

Grape skin and grape seeds (*Vitis vinifera* L.) were provided by Village Farm & Winery (Nakhon Ratchasima, Thailand). All solvents used were of analytical grade.

2.2 Preparation of grape skin and grape seed extracts

The studied grape cultivars of *Vitis vinifera* were ripe and had a dark purple color. The waste from grapes was separated into grape skin and seeds and dried in an oven at 55 °C for 24 hr to obtain a moisture content of 8-10%. The material was then ground to pass through a 60-mesh sieve (Endecotts, London, United Kingdom). The sifted grape skin and grape seeds were stored at –20 °C until the extractions were carried out. One gram sample of either grape skin or grape seeds was extracted with 3 ml of the following alternative solvents: deionized water adjusted to pH 3 with citric acid, ethanol, or acetone. The mixtures were left in the dark at room temperature (RT) for 1 week and centrifuged at 1,200 rpm for 15 min in a centrifuge (mPW-350, MPW Med Instruments, Warsaw, Poland). The supernatants were filtered using Whatman No. 4 filter paper. The filtrates were collected and concentrated using a Buchi-3 rotary vacuum evaporator (Flawil, Switzerland) at 50 °C under reduced pressure.

2.3 Antifungal activity

2.3.1 Fungal strains and growth conditions

Candida albicans TISTR 5779, *Trichophyton mentagrophytes* DMST 19735, *Microsporum gypseum* DMST 21146, *Microsporum canis* DMST 11875 were grown in sabouraud dextrose broth (SDB), and incubated at 37 °C for 48 hr. *Pityrosporum ovale* ATCC 64061 was grown on sabouraud dextrose agar (SDA) supplemented with 1% w/v of yeast extract and 1% w/v Tween 80 at 25 °C for 72 hr.

2.3.2 Antifungal assay using agar diffusion method

All tested fungi, except *P. ovale*, were adjusted to an optical density (OD) of 0.1 at 600 nm and seeded into 20 ml of SDA. A sterilized spreader was used to spread the microorganisms evenly over the agar surface. For *P. ovale*, 100 μL of the tested yeast and 100 μL of sterile olive oil were simultaneously seeded on SDA supplemented with 1% w/v yeast extract and 1% w/v Tween 80. Using a cork borer with a diameter of 6 mm, wells were made on the SDA plate, and 45 μl of the grape skin or grape seed extract samples at a concentration of 3% w/v in 95% ethanol were added to each well. Selenium sulfide (0.25% w/v) and ketoconazole (0.2% w/v) were used as positive controls. The plates with *C. albicans* were incubated at 25 ℃ for 72 hr and all other tested fungi were incubated at 37 °C for 48 hr. The diameter (mm) of the inhibition zone was measured (Punyoyai, Sirilun, Chantawannakul, & Chaiyana, 2018).

2.3.3 Determination of the minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs)

A broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) and

minimum fungicidal concentrations (MFCs) of grape seed and grape skin extracts. Briefly, 50 µl of two‑fold serial dilutions of grape seed and grape skin extracts in SDB were prepared in a 96‑well plate. Then, 50 µl of tested fungal culture was added into each well to make a final concentration of approximately 10^6 CFU/ml. In each test, all tested fungi in SDB and *P. ovale* in SDB supplemented with 1% w/v of yeast extract and 1% w/v Tween 80 were used as the positive controls, and broth alone was the negative growth control. The plates were incubated at 37 °C for 72 hr. The MICs and MFCs were then determined. The MFC value was determined by removing 10 µl of the broth from each well and spotting onto the corresponding agar, then incubating for 72 hr at 25°C for *C. albicans* and at 37 °C for the other fungi (Taweechaisupapong, Ngaonee, Patsuk, Pitiphat, & Khunkitti, 2012).

2.4 *In vitro* anti-hair loss activity

2.4.1 Human hair follicle dermal papilla cell (HFDPC) culture

The HFDPCs were purchased from Promo Cell (Heidelberg, Germany) and cultured in Follicle Dermal Papilla Cell Growth Medium Kit (Promo Cell) supplemented with 1% (v/v) penicillin/streptomycin (PS, Gibco BRL). The growth medium contained growth factors of fetal calf serum 0.04 ml/ml, bovine pituitary extract 0.004 ml/ml, basic fibroblast growth factor (recombinant human) 1 ng/ml, and insulin (recombinant human) 5 µg/ml. Dermal papilla cells were incubated in the appropriate cell culture conditions of 95% relative humidity (RH), 5% CO_2, and 37°C. The cells were sub‑cultured until they grew to about 80% confluence using HEPES buffered saline solution (HBSS), trypsin/EDTA solution, and trypsin inhibitor solution (Reiter, Pfaffi, Schönfelder & Meyer, 2009).

2.4.2 Human hair follicle dermal papilla cell (HFDPC) viability assay

The cytotoxicity of grape skin and grape seed extracts against human dermal papilla cells was examined using the PrestoBlue™ cell viability protocol. Briefly, dermal papilla cells (10^4 cells/well) were seeded into each well of a 96‑well microliter plate and incubated for 24 hr. For this assay, the stock solutions of 3% w/w grape extracts were dissolved in HFDPC medium, and 1% minoxidil and 1% finasteride were dissolved in 30% ethanol. The cells were treated with 50 µl of samples of grape skin and grape seed extracts (0.012–0.2 mg/ml) in HFDPC medium, minoxidil (0.05-0.2 mg/ml), and finasteride (0.05-0.2 mg/ml) in HFDPC medium (positive control), and with a blank control containing HFDPC medium only. After 24‑hr incubation, 10 µl of PrestoBlue™ reagent in HFDPC medium was added to each well and incubated at 37°C for 2 hr. The absorbance was determined at 570 nm using a Multiskan MS microplate reader (Thermo Electron Corp., Waltham, MA, USA) at 0, 30, 60, 90, and 120 min. The % cell viability was calculated as follows:

$$\% \text{ cell viability} = \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \times 100,$$

where $\text{Abs}_{\text{blank}}$ is the absorbance of cells in the medium, and $\text{Abs}_{\text{sample}}$ is the absorbance of cells treated with the sample in the medium.

2.4.3 Determination of remaining testosterone in human hair follicle dermal papilla cell (HFDPC)

The 5α‑reductase activity was determined by means of remaining testosterone using the method modified from McCoy and Ziering (2012). Dermal papilla cells (10^4 cells/well) were placed into 96‑well plates and incubated for 24 hr at 95% RH, 5% CO_2, and 37°C. In accordance with the cell viability assay, the cell treatments (50 µl) were divided into the following groups: grape skin and grape seed extracts (0.012–0.2 mg/ml) in HFDPC medium, minoxidil (0.05-0.2 mg/ml) and finasteride (0.05-0.2 mg/ml) in HFDPC medium (positive control), and blank control containing HFDPC medium only. Testosterone was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 40 ng/ml and a 100 µl aliquot was added to each well except for the blank control. After 24 hr of incubation, the supernatant was collected. The Testosterone Human Elisa Kit Assay was carried out following the Abcam protocol. The absorbance was measured at 450 nm using a microplate reader.

3. Results and Discussion

The antimicrobial assay of grape extracts conducted by agar diffusion method against five fungal strains is shown in Table 1. Overall, the agar diffusion assay revealed comparable zones of inhibition for 3% w/w ethanolic grape extracts and 0.2% ketoconazole and 0.25% selenium sulfide against all fungal strains. The ethanolic grape seed extract was able to inhibit all fungal strains with an inhibition zone in the range of 22.17 ± 0.94 mm to 23.76 ± 0.01 mm and appeared closely similar to 0.2% ketoconazole (22.13 ± 0.47 − 23.67 ± 1.01 mm) and 0.25% selenium sulfide (23.40 ± 0.13 − 24.76 ± 1.03 mm) except against *P. ovale* that showed a significantly smaller inhibition zone (20.60 ± 0.22 mm) compared to both reference standards (22.73 ± 0.01 mm and 23.12 ± 0.85 mm, respectively). Moreover, a significantly higher antifungal activity of ethanolic grape seed extract compared to ethanolic grape skin extract was found against *C. albicans* (22.17 ± 0.94 mm and 18.18 ± 0.34 mm, respectively), and *M. canis* (23.08 ± 0.32 mm and 19.84 ± 0.03 mm, respectively) (p<0.05). Other grape extracts demonstrated significantly smaller inhibition zones (p<0.05).

The antifungal activities (MICs and MFCs) of 3% w/w grape skin and 3% w/w grape seed extracts compared to 0.2% ketoconazole and 0.25% selenium sulfide are shown in Table 2. In accordance with the results of the agar diffusion assay, the ethanolic extracts of grape skin and seed showed greater antifungal activity compared to aqueous and acetone extracts against all fungal strains, with MIC/MFCs in the range of 0.25 − 1 µl/ml and mg/ml, repectively. Ethanolic extract of grape skin exhibited the lowest MIC of 0.25 µl/ml and MFCs in the range of 0.5 − 0.75 mg/ml against *T. mentagrophytes,* and *M. gypseum*, while ethanolic extract of grape seed performed similarly against *C. albicans*, *T. mentagrophytes*, *M. gypseum*, and *P. ovale*. The MICs against

Table 1.   Antifungal efficacy of 3% w/w grape skin extract and 3% w/w grape seed extract by agar diffusion method

Grape part	Extraction solvent	Inhibition zone (mm)				
		C. albicans	*T. mentagrophytes*	*M. gypseum*	*M. canis*	*P. ovale*
Skin	Water pH 3	16.48 ± 0.12^a	16.50 ± 0.63^a	16.44 ± 0.27^a	17.88 ± 0.61^a	18.04 ± 1.00^{ab}
	Ethanol	18.18 ± 0.34^b	22.22 ± 0.54^{bc}	22.16 ± 0.65^{bc}	19.84 ± 0.03^b	19.72 ± 0.24^{ac}
	Acetone	16.40 ± 0.55^a	16.10 ± 0.42^a	17.56 ± 1.00^a	16.30 ± 0.55^a	16.28 ± 0.01^b
Seed	Water pH 3	19.73 ± 1.00^b	20.12 ± 1.20^b	20.04 ± 1.21^b	19.80 ± 0.66^b	18.18 ± 1.11^{ab}
	Ethanol	22.17 ± 0.94^c	23.72 ± 1.00^c	23.76 ± 0.01^c	23.08 ± 0.32^c	20.60 ± 0.22^c
	Acetone	16.05 ± 0.01^a	16.12 ± 0.10^a	18.40 ± 0.54^a	18.54 ± 1.00^b	18.48 ± 1.01^a
Ketoconazole 0.2%		22.13 ± 0.47^c	22.41 ± 0.22^c	23.67 ± 1.01^c	22.82 ± 0.21^c	22.73 ± 0.01^d
Selenium sulfide 0.25%		23.40 ± 0.13^c	24.72 ± 0.54^c	24.76 ± 1.03^c	24.08 ± 1.12^c	23.12 ± 0.85^d

Different superscripts in the same column indicate significant differences ($p < 0.05$) between grape skin and grape seed extracts and between each reference shampoo product using one-way ANOVA with Tukey's multiple comparisons. Reported values are mean ± SD of triplicate assay for each sample.

Table 2.   MIC and MFC of 3% w/w grape skin and 3% w/w grape seed extracts

Grape part	Extraction solvent	*C. albicans*		*T. mentagrophytes*		*M. gypseum*		*M. canis*		*P. ovale*	
		MIC* (μl/ml)	MFC** (mg/ml)	MIC (μl/ml)	MFC (mg/ml)	MIC (μl/ml)	MFC (mg/ml)	MIC (μl/ml)	MFC (mg/ml)	MIC (μl/ml)	MFC (mg/ml)
Skin	Water pH 3	0.5	1.0	0.75	1.0	0.75	1.0	1.0	1.0	0.75	1.0
	Ethanol	0.5	1.0	0.25	0.5	0.25	0.75	0.75	0.75	0.5	0.75
	Acetone	0.5	1.0	0.5	0.5	0.5	0.75	0.75	1.0	0.5	0.75
Seed	Water pH 3	0.75	0.75	0.5	1.0	0.5	1.0	0.5	1.0	0.75	1.0
	Ethanol	0.25	0.75	0.25	0.5	0.25	0.75	0.5	0.5	0.25	0.5
	Acetone	0.25	0.75	0.5	0.75	0.25	0.75	0.5	0.75	0.25	0.75
Ketoconazole 0.2%		0.125	0.25	0.25	0.25	0.125	0.25	0.25	0.25	0.25	0.25
Selenium sulfide 0.25%		0.125	0.25	0.25	0.5	0.125	0.25	0.125	0.25	0.125	0.25

* MIC = Minimum Inhibitory Concentration
** MFC = Minimum Fungicidal Concentration

T. mentagrophytes (0.25 μl//ml) of both ethanolic grape extracts were the same as those of 0.2% ketoconazole and 0.25% selenium sulfide, while the MFCs was 0.25% for selenium sulfide only (0.5 mg/ml). Overall, both grape extracts showed antifungal activities comparable to those of the reference standards with ethanolic grape seed extract being slightly superior to grape skin extract. The effectiveness of antifungal activity for both grape extracts ranked in descending order was as follows: *T. mentagrophytes*, *M. gypseum*, *C. albicans*, *P. ovale*, and *M. canis*.

This is in accordance with other studies on the antifungal activity of *Vitis vinifera*. A study evaluating the antifungal activity of ethanol/water grape seed extract (7:3 v/v) showed significant inhibition of *C. albicans* in an experimental murine model of vaginal candidiasis, which was correlated with the high content of polyphenols, particularly flavan-3-ols (Simonetti *et al.*, 2014). In other studies, the antifungal activities of grape seed extracts against dermatophytes of *T. mentagrophytes*, *M. gypseum*, and *Malassezia furfur* were reported and found to be greater with ethanol/water grape seed extract compared to ethanol or methanol alone (Simonetti *et al.*, 2017). In a recent study, *Vitis vinifera* juice extract loaded on chitosan nanoparticles showed an antifungal effect against *C. albicans* and *Aspergillus niger* and demonstrated complete wound-healing after 7 days on experimental rats with wounded skin fungal

infection (Elshaer, Elwakil, Eskandrani, Elshewemi, & Olama, 2022). In addition, in our previous study, it was found that both grape skin and seed extracts showed antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, and *Escherichia coli* (Khawee *et al.*, 2015). These results indicate that grape extracts, as a high polyphenol resource, are effective antimicrobial agents that can find applications in cosmetic and pharmaceutical hair products.

Since ethanol was the most efficient solvent for achieving the highest yield of polyphenols, as well as antioxidant and antimicrobial activities in our previous and current studies, the ethanolic extracts were selected for further studies on human follicle dermal papilla cells (Khawee *et al.*, 2015). The cytotoxicity of ethanolic grape skin and grape seed extracts on HFDPC at various concentrations (0.012 – 0.6 mg/ml) was assessed in comparison to minoxidil and finasteride reference standards (Figure 1). Cell proliferation of HFDPC treated with both grape extracts increased in a dose-depended manner and exceeded 100% at all concentrations compared to untreated control cells. Cell proliferation increased by up to 128% and 138% after treatment with concentrations as low as 0.012 mg/ml of grape seed and grape skin extracts, respectively. Treatment of HFDPC with 0.3 mg/ml of grape skin extract and 0.06 mg/ml and 0.3 mg/ml of grape seed extract significantly increased cell proliferation
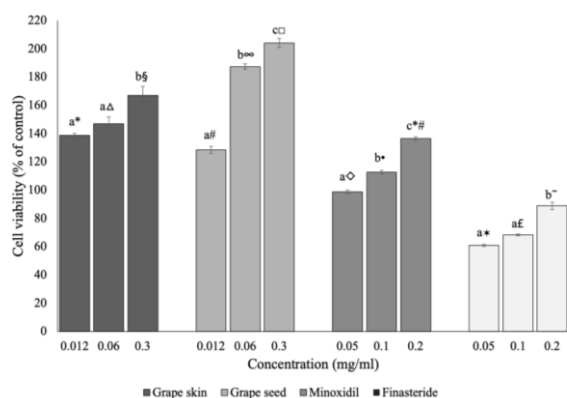
Figure 1. Cell proliferation assessment of grape skin and grape seed extracts compared to minoxidil and finasteride as reference standards. The reported values are expressed as a percentage of control and presented as mean ± SD of the triplicate assay for each sample. Data with different letters have a significant difference ($p<0.05$) between different concentrations within the sample. Data with different symbols have a significant difference ($p<0.05$) between samples and reference standards.

(167%, 187%, and 204%, respectively) compared to other concentrations within the type of extract ($p<0.05$). This result indicates that both grape extracts not only preserved the viability of the cells but also elevated dermal papilla cell proliferation.

In an earlier study, grape seeds' proanthocyanidins increased the proliferation of mouse hair follicle cells and converted the hair cycle in mice from the telogen phase to the anagen phase, potentially through effects on signal transduction pathways (Takahashi *et al*., 1998). Moreover, epigallocatechin-gallate (EGCG), commonly found in flavan-3-ol, in green tea and grape seeds improved hair growth in hair follicles *ex vivo* and increased the proliferation of HDPCs *in vitro* (Kwon *et al*., 2007).

In accordance with other studies, minoxidil increased cell proliferation of HFDPC in a dose-dependent manner at all concentrations (Chaksupa, Sookvanichsilp, Soonthornchareonnon, Moongkarndi, & Gerdprasert, 2022).

However, for finasteride, the results were inconsistent with the recent study, where HFDPC cell viability was exceeding 80% after treatment with finasteride in the concentration range of 0.01-100 μM (Rattanachitthawat, Pinkhien, Opanasopit, Ngawhirunpat, & Chanvorachote, 2019). In contrast, in this study, treatment with concentrations of 0.05 – 0.1% finasteride reduced HFDPC cell viability to 61% and 68%, respectively, while 0.2% finasteride maintained cell viability in a non-cytotoxic range above 80% compared to untreated cells. Although there was no explanation for this phenomenon in other studies, we do not rule out that the discrepancy in results could be due to the different methodologies, reagents, and cell resources used in studies.

In androgenetic alopecia, miniaturization of the dermal papilla is associated either with the direct action of testosterone or with conversion by the enzyme 5α-reductase to the more potent 5α-DHT. The action of these androgens on human hair follicles causes inhibition of dermal papilla cell proliferation and induction of programmed cell death by androgens (Sawaya & Price, 1997). Therefore, the experiment using the Testosterone ELISA kit was used as an indirect method to identify the conversion of testosterone to DHT by 5α-reductase produced by dermal papilla cells (Figure 2). Testosterone was added to the tested grape extracts and reference standards at an initial concentration of 40 ng/ml, and the remaining testosterone content was measured after its conversion to DHT as a factor responsible for hair loss. The effectiveness of the sample in inhibiting the activity of 5α-reductase can be assessed by the amount of testosterone remaining. It was found that both grape skin and seed extracts at all concentrations inhibited 5α-reductase to a similar extent and were comparable to finasteride. Moreover, both grape extracts at concentrations of 0.06 mg/ml and 0.3 mg/ml demonstrated significantly higher effectiveness in inhibition of 5α-reductase compared to minoxidil ($p<0.05$). These results can be explained by the difference in the mechanisms of action of the two drugs. While finasteride selectively inhibits type II 5α-reductase, minoxidil dilates the blood vessels of the scalp, promoting blood flow to the hair follicles without affecting hair follicle enzymes, which makes the effectiveness in this context limited (Chandrashekar *et al*., 2015; Mata *et al*., 2020).
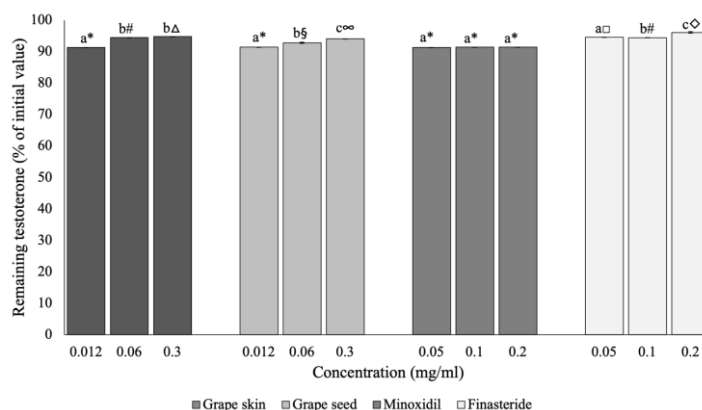

Figure 2. Testosterone remaining in hair follicle dermal papilla cells after treatment with ethanolic grape seed and skin extracts in comparison to minoxidil and finasteride. The results indirectly represent inhibition of 5α-reductase activity and conversion of testosterone to dehydrotestostetone (DHT). Data with different letters have a significant difference ($p<0.05$) between different concentrations within the sample. Data with different symbols have a significant difference ($p<0.05$) between samples and reference standards.

Although minoxidil and finasteride are still at the forefront of conventional therapeutic agents used to treat alopecia, their side effects have prompted the search for effective alternative therapies with limited side effects among plant-based resources. To the best of our knowledge, this is the first study to show the inhibitory activity of grape extracts on 5α-reductase tested in HFDPC. However, there were several studies reporting the potent inhibiting capacity of EGCG and other flavonoids containing catechol moieties against 5α-reductase (Hiipakka, Zhang, Dai, Dai, & Liao, 2002; Kwon *et al*., 2007). The proposed mechanism of competitive 5α-reductase inhibition might be explained by the interaction of catechol groups in flavonoids with amino acid residues in the carboxyl-terminal portion of the protein important for binding of nicotinamide adenine dinucleotide phosphate (NADPH) by 5α-reductase. Other mechanisms of inhibition associated with their ability to form complexes with certain metal ions and proteins, as well as their antioxidant and pro-oxidant activities are unlikely (Hiipakka *et al*., 2002). These findings suggest that grape extracts have the potential to be applied to cosmetic products for hair loss.

4. Conclusions

In this study, ethanol was found to be superior to water and acetone solvents in terms of the bioactivities of grape skin and seed extracts. Moreover, both ethanolic grape extracts exhibited excellent antifungal activity against *T. mentagrophytes*, *M. gypseum*, *C. albicans*, *P. ovale*, and *M. canis*, ranked in descending order. In addition, a promising anti-hair loss activity through an increase in the proliferation of HFDPC and indirectly assessed 5α-reductase inhibition were discovered for grape skin and seed extracts showing their potential as alternatives to conventional hair loss medications. The results of this study imply that grape skin and seed extracts may be useful in the development of hair products with multifaceted benefits including anti-hair loss and antifungal properties. However, human studies are required to validate their effectiveness.

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