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In Vivo and *In Silico* Evaluation of *Petroselinum crispum* Leaf Fractions as Anti-Alopecia

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ABSTRACT

Androgenetic alopecia (AGA), commonly known as baldness is marked by hair loss on the scalp or other body areas, ranging from small patches to complete hair loss, potentially leading to significant psychological stress. In AGA, the activation of androgen receptors shortens the anagen phase of the hair growth cycle, resulting in follicular miniaturization and production of thinner hair follicles that fail to penetrate the epidermis. This condition is characterized by a reduced anagen-to-telogen hair ratio and elevated levels of dihydrotestosterone (DHT). Empirical use of *Petroselinum crispum* leaves for treating alopecia has prompted scientific investigation. Previous *in silico* studies identified 24 compounds in *Petroselinum crispum*, with six of them showing potential binding to the androgen receptor and compete with DHT. This study aims to examine the anti-alopecia activity of *Petroselinum crispum* leaf ethyl acetate fractions *in vivo* and *in silico*. *In vivo* evaluation employed rabbit model of alopecia, while *in silico* evaluation employed molecular docking on the androgen receptor. Ethyl acetate fractions 2 and 4 demonstrated the highest hair growth promoting activity, with significant increases in hair length (1.11±0.02 cm and 1.23±0.01 cm, respectively) and hair weight (0.125±0.02 g and 0.10±0.02 g, respectively), comparable to minoxidil. LC-MS analysis of these fractions identified several compounds, with paeonilactone C1 exhibiting the highest binding energy (-6.6 Kcal/mol), surpassing that of minoxidil. Fractions 2 and 4 of *Petroselinum crispum* leaves showed the most promising hair growth promoting activity, and could be developed into a hair tonic for the treatment and prevention of alopecia.

***Keywords*:** *Petroselinum crispum, In silico,* Anti-alopecia, Paeonilactone C1

Introduction

Alopecia or baldness is a condition characterized by hair loss on the scalp or other body parts. The severity of hair loss vary, it could be from small areas to the entire body. Hair loss may cause a psychological stress to some. According to the cause, alopecia is generally classified into androgenetic alopecia, alopecia areata, and telogen effluvium.

Androgenetic alopecia (AGA) is a disorder caused by excessive response to androgens. This condition affects up to 50 percent of males and females, marked by progressive terminal hair loss on the scalp any time after puberty. In males, hair loss is most prominent in the vertex and frontotemporal areas, while in females, the frontal hairline is typically spared with diffuse hair loss at the crown and the upper part of the head, with loss that is frequently characterized by a wider central part.[2]

Activation of the androgen receptor shortens the phase of anagen or growth in the normal hair growth cycle.

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In androgenetic alopecia, excessive androgen activation causes follicular miniaturization through shorter anagen phase, producing thinner and shorter hair follicles that are eventually incapable of penetrating the epidermis. Pathologic specimens will show a reduced 5:0 ratio of anagen to telogen hair, where the norm is 12:1. Patients with androgenetic alopecia have a higher dihydrotestosterone (DHT) production than normal.

Petroselinum crispum leaves have empirically been in use in Spain for alopecia treatment.[3] The potential anti-alopecia effect of *Petroselinum crispum* has been investigated *in silico*. Compound tracing using the KNApSAcK website has identified a total of 24 compounds from *Petroselinum crispum*, and six of the compounds were shown to bind to the androgen receptor and compete with DHT.

Further studies are needed to investigate in greater detail the potential of *Petroselinum crispum* leaves as anti-alopecia agent. The aim of this study was to examine the activity of *Petroselinum crispum* leaf fractions as anti-alopecia *in vivo* and *in silico*. The ability of *Petroselinum crispum* leaf fractions to stimulate hair growth was investigated *in vivo*, the chemical composition of the fractions were analyzed using LC-MS, and the ability of the compounds to bind to the androgen receptor was investigated *in silico*.

Materials and Methods

Chemicals

Organic solvents (ethyl acetate >99%, n-hexane >99%, and methanol >99%) were of Sigma-Aldrich Chemicals, Germany. Silica gel 60 GF254, silica gel 60 G, TLC plate, $Ce(SO_4)_2$, 5% minoxidil, and sodium carboxymethyl cellulose (Na-CMC) were of Merck Chemicals,

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Indonesia. All chemicals were of analytical grade and were purchased from local suppliers.

Animals

Rabbits aged 5–6 months weighing between 2–3 kg were used for the study. The rabbits were kept in cages under hygienic condition at the Pharmacology Laboratory. The cages were kept at ambient temperature (25 ± 5°C), and the rabbits were provided with standard rodent feed (Hobi, Makassar, Indonesia) and allowed free access to drinking water. Ethical approval for the use of the experimental animals was given by the local ethics committee with an approval number: 1406/UN.29.20/PPM/2020.

Plant collection and identification

The dried leaves of *Petroselinum crispum* were collected from UPT Materia Medica Batu (-7.8675151795688025, 112.51927839544109) in November, 2020. The plant material was identified with voucher number: 253/ll/2020.

Plant extraction and fractionation

The dried powdered leaves of *Petroselinum crispum (*1 kg) were extracted by maceration with 96% ethanol. The ethanol extract was partitioned by solvent-solvent extraction using n-hexane and ethyl acetate. The ethyl acetate fraction was further fractionated by Vacuum Liquid Chromatography (VLC) using the nonpolar-to-polar mobile phase gradient system with a combination of n-hexane, ethyl acetate, and methanol. All fractions were analyzed by Thin Layer Chromatography (TLC). Fractions with similar TLC pattern were merged to obtain seven major fractions (F1 – F7).

Phytochemical analysis

The chemical constituents of the fractions were identified by LC-MS using an HPLC connected to a Q-TOF spectrometer (Waters, Milford,MA, USA) equipped with a positive ion mode ESI source. A full scan was performed from m/z 100 to m/z 1200 at a source temperature of 120°C. The mobile phase used was a mixture of water and acetonitrile modified with formic acid, at a flow rate of 0.3 mL/min. The ionization mode was ESI+ (Electrospray Ionization positive) with an m/z range of 50-1200. Low collision energy was employed to detect relevant ions. Data processing was conducted using UNIFI software (version 1.8, Waters Corporation, Milford, MA, USA) to identify significant peaks and calculate mass errors in ppm. The analysis results included the identification of compounds, retention times, observed m/z, and signal intensities. The result was compared with database that collected more than 1200 compounds based on chemical structure, molecular formula, and molecular mass from various web-based resources.

Evaluation of in vivo hair growth promoting activity

Hair growth stimulatory activity test was carried out using Tanaka's method.[4] Rabbits' backs were shaved in ten squares, five on the right side and five on the left side, guided with a marker (Figure 1). Each square was sized 2 x 2 cm^2 and spaced 1 cm from another square. The hair within the squares was shaved clean and smeared with 96% ethanol as antiseptic.

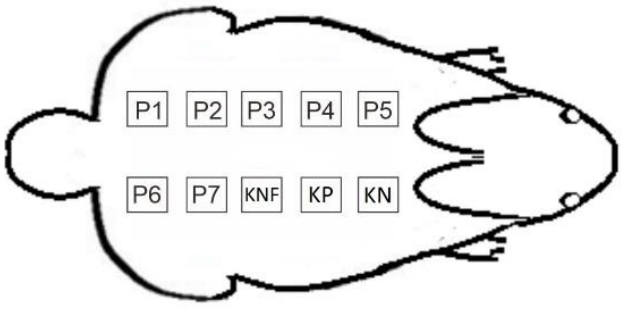

Figure 1: Illustration of the treatment for the rabbits

The rabbits were divided into ten (10) groups, and treated as follows; P1 was smeared with fraction 1 (15%), P2 with fraction 2 (15%), P3 with fraction 3 (15%), P4 with Fraction 4 (15%), P5 with Fraction 5 (15%), P6 with Fraction 6 (15%), P7 with Fraction 7 (15%), KN with Na-CMC (negative control), KP with minoxidil (positive control), and KNF served as a normal control to which no treatment was administered.

The test sample preparations were smeared on the rabbits' backs twice using a brush. Smearing on the rabbits' backs was performed in the morning and late afternoon for three weeks. Measurement of hair length was done on days 3, 6, 9, 12, 15, 18, and 21, and hair weight was determined on day 22.

Preparation of protein structure

The protein coordinates were downloaded from https://www.rcsb.org/pdb/ (PDB ID: 4K7A). The receptor was in the form of a protein macromolecule, the organism was *Homo sapiens*, and the resolution value was 2.21 Å. Preparation was performed using BIOVIA Discovery Studio 2017 to separate residual solvent (water), native ligand, and other non-standard residuals to obtain native ligand and receptor file free of solvent and other non-standard residuals in PDB file extension. Then, every file in the PDB file extension was optimized using AutoDock Tools and stored in PDBQT file extension.[5]

Preparation of Petroselinum crispum Mill. compounds

The compounds from the ethyl acetate fraction identified by LC-MS were downloaded from KNApSAcK's website (http://www.knapsackfamily.com/) to obtain the ID codes. The compounds' ID codes were then downloaded from KNApSAcK-3D's website (http://knapsak3d.sakura.ne.jp/), and the mol* format was converted into PDB using the software Open Babel 2.4.1.[6] The PDB format was converted into PDBQT using MGLTools 1.5.6.[7]

Redocking of Ligand Molecule

Molecular docking was conducted using AutoDock Vina with grid coordinates at x = -2.5616, y = 0.4597, and z = -6.6180, set into 14 × 14 × 24. The docking result was analyzed, and the ligand-receptor interaction model was visualized using BIOVIA Discovery Studio 2017. An RMSD (root mean square deviation) value was obtained using the PyMOL software. If the native ligand docking result had an RMSD value < 3, then the docking process was accepted and declared valid.[8]

Docking of Petroselinum crispum compounds and receptor

The *Petroselinum crispum* compound docking used AutoDock Vina following all earlier redocking procedures, where all compounds were made using Notepad. Virtual screening was carried out with automated docking via script.

Interaction analysis

Data analysis was conducted based on the binding energy generated from the molecular docking. A binding energy value indicated the binding power of a test compound to the receptor. The lower the binding energy value, the stronger the bond between the compound and the receptor. The interaction that occurred between the test compound and the androgen protein could be seen from the type of interaction formed between the test compound and the target protein.[9] The docking result was analysed and visualized for the ligand-receptor interaction model using AutoDock Tools and BIOVIA Discovery Studio 2017.

Statistical analysis

Values were expressed as mean ± standard error of mean from three replicates. Data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. P values < 0.05 were regarded as significant.

Results and Discussion

Extract and fraction yeild

Extraction of 1 kg of *Petroselinum crispum* leaves resulted in 100 g of extract (10% yield). Liquid-liquid extraction produced 214 g of ethyl acetate extract (21.5% yield). The fractionation of the fraction resulted

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in 20 fractions, from which 7 final fractions (F1, F2, F3, F4, F5, F6, and F7) were obtained after TLC analysis.

Phytochemical constituents of Petroselinum crispum leaf fraction

The phytochemical constituents of *Petroselinum crispum* leaf ethyl acetate fraction are presented in Table 1 and Figures 2-8. It is estimated that only 20 to 30% of the 350,000 known plant species have been phytochemically examined in detail, therefore, the actual number of secondary metabolites in the plant kingdom is likely to exceed 200,000.[10] The TLC analysis of the ethyl acetate fraction of *Petroselinum crispum* showed similar results as that obtained previous research.[11] LC-MS analysis of fractions 1 – 7 identified some compounds in *Petroselinum crispum* which have been previously found in other plants. Some of these compounds are Sappanone B which has been found in the Heartwood of *Caesalpinia sappan* L,[12] digiprolactone found in *Cosmos caudatus* leaves,[13] and Paeonilactone C1 found in *Paeonia officinalis*.[14]

Table 1: Compounds identified from LC-MS analysis of *Petroselinum crispum* leaf ethyl acetate fractions

Fraction No	Compound name	Observed m/z	Observed RT (Minutes)
	Fraction 1		
1.	3-(3′,4′-Dihydroxybenzyl)-7-hydroxychroman-4-one	287.0907	9.37
2.	Bergaptol	203.0329	9.71
3.	cis-Caffeic acid	203.0327	7.77
4.	Sappanone B	303.0858	9.46
5.	Xanthotoxin	217.0485	9.13
	Fraction 2		
1.	2,4,4′,6′-Tetrahydroxy-benzophenone	247.0591	9.19
2.	3-(3′,4′-Dihydroxybenzyl)-7-hydroxychroman-4-one	287.0904	9.37
3.	Digiprolactone	197.1159	6.75
4.	Paeonilactone C1	319.1165	9.28
5.	Xanthotoxin	217.0483	8.72
	Fraction 3		
1.	Aviprin	305.1014	7.73
2.	d-Catechin	291.0856	8.44
3.	Digiprolactone	197.1159	6.76
4.	Digitopurpone	271.0594	8.24
5.	Paeonilactone C1	319.1167	9.30
	Fraction 4		
1.	Aviprin	305.1013	7.73
2.	d-Catechin	291.0854	8.46
3.	Digiprolactone	197.1160	6.76
4.	Digitopurpone	271.0591	8.26
5.	Candidate Mass $C_{34}H_{40}O_9$	593.2762	9.95
	Fraction 5		
1.	1,3-Dihydroxy-2-hydroxymethylanthraquinone-3-O-*β*-D-xylopyranose(1-6)-*β*-D-glucopyranoside	565.1560	6.10
2.	Aviprin	305.1010	7.75
3.	Candidate Mass $C_34H_{40}O_9$	593.2769	9.96
4.	Candidate Mass $C_{45}H_{36}O_3$	625.2740	9.90
5.	Candidate Mass $C_{18}H_{33}NO$	280.2626	9.78
	Fraction 6		
1.	1,3,6-Trihydroxy-2-methyl-anthraquinone-3-*O*-*β*-*D*-xylopyranose(1-6)-*β*-D-(6-*O*-acetyl) Glucopyranoside	607.1667	7.29
2.	1,3-Dihydroxy-2-hydroxymethylanthraquinone-3-*O*-*β*-*D*-xylopyranose(1-6)-*β*-*D*- glucopyranoside	565.1553	6.10
3.	Aloenin	433.1114	6.32

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4.	Galloylpaeoniflorin	633.1831	8.01
5.	Kaempferol-3-*O*-rutinoside	595.1655	6.20
	Fraction 7		
1.	1,3,6-Trihydroxy-2-methyl-anthraquinone-3-*O*-*β*-*D*-xylopyranose(1-6)-*β*-*D*-(6-*O*-acetyl) glucopyranoside	607.1674	7.30
2.	1,3-Dihydroxy-2-hydroxymethylanthraquinone-3-*O*-*β*-*D*-xylopyranose(1 6)-*β*-*D*-glucopyranoside	565.1561	6.11
3.	3′,4′-Dimethoxy-isoflavan-7,2′-di-*O*-*β*-*D*-glucoside	649.2140	8.22
4.	Galloylpaeoniflorin	633.1827	8.02
5.	Kaempferol-3-*O*-rutinoside	595.1676	6.21

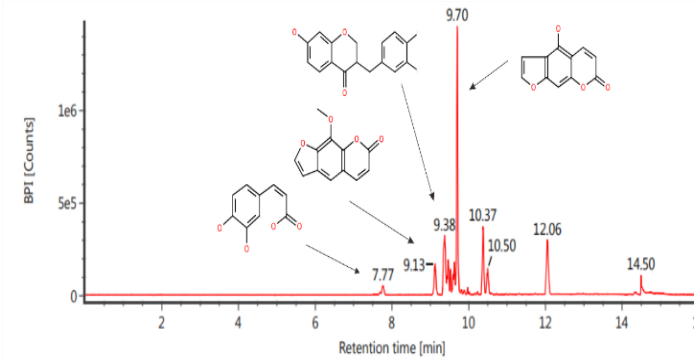

Figure 2: Chromatogram and compounds identified from LC-MS analysis of fraction 1 of *Petroselinum crispum* leaf ethyl acetate fraction

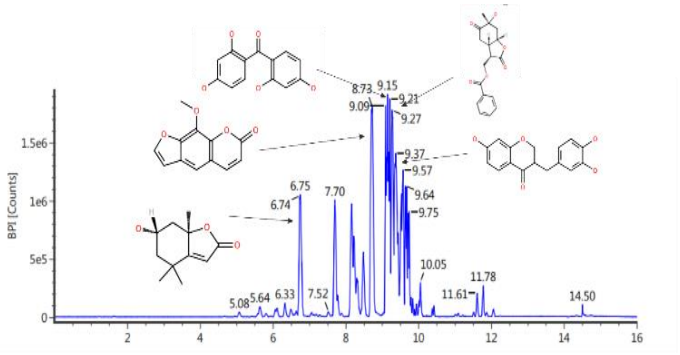

Figure 3: Chromatogram and compounds identified from LC-MS analysis of fraction 2 of *Petroselinum crispum* leaf ethyl acetate fraction

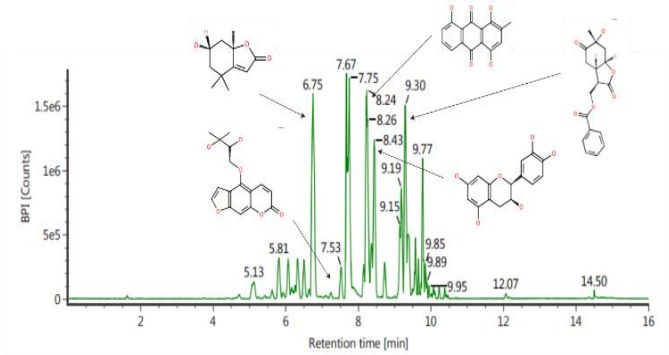

Figure 4: Chromatogram and compounds identified from LC-MS analysis of fraction 3 of *Petroselinum crispum* leaf ethyl acetate fraction

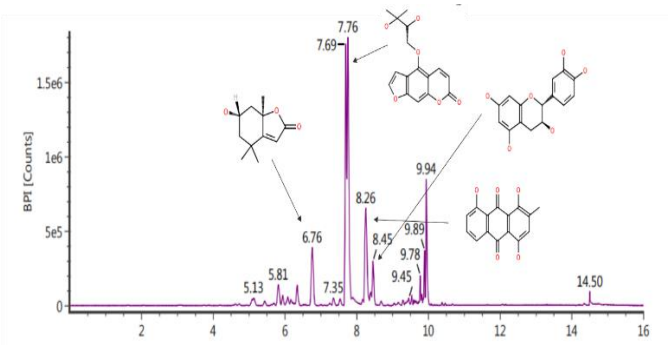

Figure 5: Chromatogram and compounds identified from LC-MS analysis of fraction 4 of *Petroselinum crispum* leaf ethyl acetate fraction

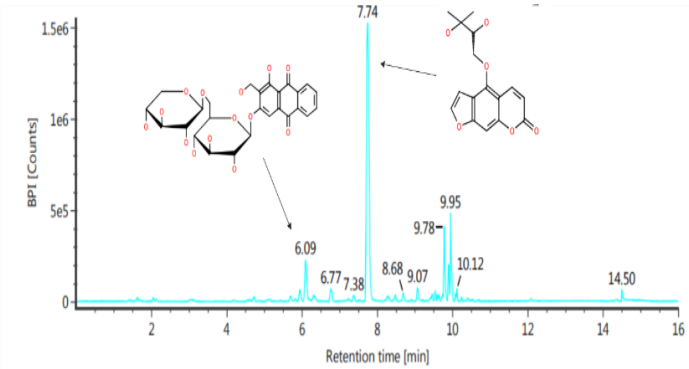

Figure 6: Chromatogram and compounds identified from LC-MS analysis of fraction 5 of *Petroselinum crispum* leaf ethyl acetate fraction

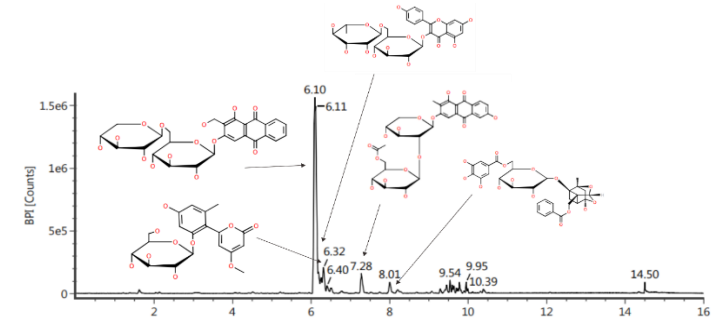

Figure 7: Chromatogram and compounds identified from LC-MS analysis of fraction 6 of *Petroselinum crispum* leaf ethyl acetate fraction

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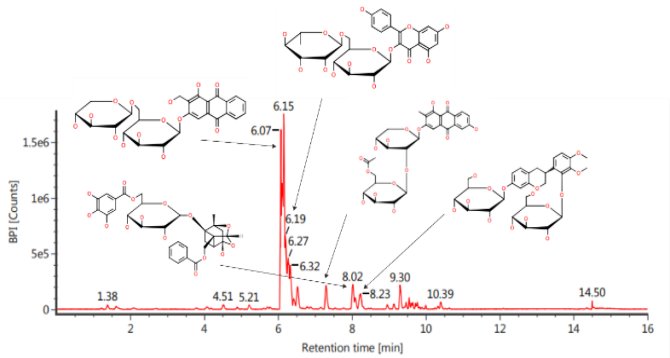

Figure 8: Chromatogram and compounds identified from LC-MS analysis of fraction 7 of *Petroselinum crispum* leaf ethyl acetate fraction

In vivo hair growth promoting activity of Petroselinum crispum

Effect on hair length

The effect of *Petroselinum crispum* ethyl acetate fractions on hair length after 21 days of treatment is shown in Table 2 and Figure 9. On day 21, there was a significant increase in hair length of rabbits in the P2, P3, P4, P5, P7 and KP (positive control) groups compared to those of the KNF (negative control) and KN (normal control) groups. This indicated that these fractions have hair growth promoting effect. Fractions F2 and F4 showed the highest hair growth promoting effect, with the hair length measuring 1.10 ± 0.02 mm and 1.23 ± 0.01 mm, respectively in these groups. These values were comparable to that obtained for the KP (positive control) group, which resulted in hair length measuring 1.16 ± 0.02 mm.

Effect on hair weight

The hair weight of rabbits after treatment with ethyl acetate fractions of *Petroselinum crispum* are presented in Table 3 and Figure 10. After 21 days of treatment, the hair weight in the P1, P2, P4, P5, P7 and KP (positive control) groups increased significantly compared to the KNF (negative control) group. This shows that fractions F1, F2, F4, F5, and F7 have hair growth promoting activity. Again, fractions F2 (P2), and F4 (P4) showed the highest hair weights, measuring 0.125 ± 0.02 g and 0.10 ± 0.02 g, respectively, which were not different from the hair weight of rabbits in the KP (positive control) group with values of 0.091 ± 0.01 g.

In silico hair growth promoting activity of Petroselinum crispum

Fractions 2 and 4 exhibited the highest hair growth promoting activity. These fractions were further investigated *in silico*. The compounds identified by LC-MS analysis were subjected to molecular docking with the androgen receptor. The *in silico* study was intended to predict the class of compounds and compounds with the best hair growth promoting activity, and a correlation between the *in vivo* and *in silico* activity can be established. Root mean square deviation (RMSD) was used as a means of ensuring the validity of the procedure. The validity of the docking procedure can be determined using the root mean square deviation (RMSD) criteria of the molecular weight of minoxidil between the redocked pose and the experimental pose (crystallography). It docking procedure is considered valid if RMSD is ≤ 3 Å.[8] Minoxidil was redocked to the androgen receptor to generate a conformity shift of 2.21 Å from the crystallography, as can be seen in Figure 11. Table 4 presents the molecular docking predictions of the best conformation of the compounds in Fractions 2 and 4 with the binding energy ratio.

As shown in Table 4, there were three bioactive compounds in Fractions 2 and 4 of *Petrocelinum crispum* Mill. leaves that scored lower in the docking than the native ligand, and one compound that scored higher than the native ligand. The lower the binding energy generated, the greater the stability of the ligand-receptor complex. The visualization of the molecular interaction is shown in Table 5.

Androgen receptor (AR), testosterone (T), and the metabolite 5-dihydrotestosterone (DHT) are among the important targets in the proper development and functioning of male reproductive organs, such as the prostate and epididymis, as well as non-reproductive organs, such as the muscles, hair follicles, and brain.[15,16] DHT is the stronger androgen that comes from testosterone metabolism through the action of 5-alpha reductase enzyme. Compared with testosterone, DHT binds more strongly to the androgen receptor in hair follicles, resulting in improved genetic regulation that is responsible for the gradual transformation of terminal hair follicles into mini hair follicles.[3] The AR inhibition based on molecular docking was also investigated to predict the bioactive compound of *Petrocelinum crispum* Mill. with potential anti-alopecia activity.

The molecular interaction analysis from the minoxidil docking study was used as a positive control compound against the androgen receptor. It was revealed that minoxidil formed hydrogen bonds at the GLU793 (3.38 Å) residue in the pyrimidine ring of the androgen receptor, as well as hydrophobic interactions at the LYS861 (4.67 Å) residue in the piperidine ring, which stabilizes the ligand-receptor binding.[17] All the compounds bound to the androgen receptor, most of which were by hydrophobic interactions with TYR857, GLN858, LYS861, GLU793, TRP796, LEU797, SER865, and HIS789 residues. Here we illustrate the compound interactions based on the docking scores and compared them against minoxidil.

The compounds paeonilactone C1, 3-(3,4-dihydroxybenzylidene)-7-hydroxychroman-4-one, and d-cathecin are flavonoids. Paeonilactone C1 formed hydrogen bonds (H-bonds) with SER865 (2.40 Å) and LYS861 (2.24 Å), as well as a hydrophobic interaction with GLU793 at a 4.50 Å distance. The compound 3-(3,4-dihydroxybenzylidene)-7-hydroxychroman-4-one had a hydrogen bond interaction with GLN858 at a 2.06 Å distance, as well as a dipole-dipole interaction with LYS861 at a 1.50 Å distance. The compound digiprolactone formed an amino acid interaction bond with SER86 at a 3.33 Å distance and had three amino acid residues, LYS861, TYR865, and LEU797 with 4.27 Å, 4.87 Å, and 4.29 Å apart, respectively. The compound 2,4,4,6-tetrahydroxy-benzophenone belongs to the benzophenone class,[18] has an inhibitory effect on DHT androgenic activity. This compound interacted with the same amino acid as minoxidil native ligand.

The compounds aviprin and xanthotoxin are compounds in the coumarin class. It has been reported that coumarins are frequently used as topical preparations.[19] Aviprin formed a hydrogen bond (H-bond) with SER865 (3.32 Å) and hydrophobic interactions with LYS861 (4.27 Å) and LEU797 (4.29 Å). The amino acids SER (serine) and LYS (lysine) are predicted to play an important role in the binding with the androgen receptor in alopecia.[16,20] Xanthotoxin had two hydrogen bonds with SER865 and LEU862, each at 2.93 Å and 3.42 Å, as well as GLU793, TYR 857, and LYS861 residues. The compound d-catechin is a flavonoid, and it had three hydrogen bonds with GLU793, GLN858, and LYS861, each at 2.51Å, 2.33 Å, and 2.98 Å. The amino acid GLU (glutamic acid) has a role in promoting hair growth and keratinocyte proliferation on new skin signaling pathways with neurotransmitter mediation.[21] The compound digitopurpone belongs to the quinone class, and it had a hydrogen bond with HIS789 at 2.19 Å, as well as GLU793, LEU797, and TRP796 residues, each with hydrogen bond at distances of 3.32 Å, 4.72 Å, and 3.78 Å.

Binding affinity indicates the degree of stability of the chemical bond between the ligand and the receptor. A ligand-receptor complex with lower binding energy, indicates that the complex is stable and the ligand and receptor form a strong bond. Negative affinity values indicate that the compound can spontaneously interact with the androgen receptor. There were eight bioactive compounds from *Petroselinum crispum* that had better binding affinity to the androgen receptors when compared to minoxidil. Several other studies related to androgen receptor and its binding site, as well as molecular docking and simulation showed that several important amino acids including LYS861, GLN858, and GLU7933 play a role in hair growth. Compounds that binds to these amino acids produced low binding energy values and form more stable drug-receptor complex.[22]

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Table 2: Rabbits hair lengths during treatment with *Petroselinum crispum* leaf ethyl acetate fractions

	Hair length (cm)						
Treatment	**Day-3**	**Day -6**	**Day -9**	**Day -12**	**Day -15**	**Day -18**	**Day -21**
P1	0±0	0.15±0.01	0.27±0.26	0.37±0.01	0.47±0.03	0.63±0.01	0.84±0.01
P2	0±0	0.15±0.03	0.33±0.01	0.47±0.05	0.6±0.01	0.9±0.01	1.11±0.02
P3	0.13±0	0.26±0.02	0.37±0.02	0.43±0.05	0.68±0.04	0.79±0.02	0.96±0.03
P4	0.25±0	0.35±0.01	0.46±0.0	0.64±0.02	0.79±0.01	1.06±0.03	1.23±0.01
P5	0.24±0	0.39±0.01	0.58±0.01	0.71±0.01	0.85±0.02	0.98±0.01	1.10±0.01
P6	0.31±0.01	0.35±0.02	0.39±0.03	0.53±0.02	0.67±0.04	0.75±0.02	0.85±0.01
P7	0.28±0.0	0.20±0.03	0.31±0.03	0.49±0.07	0.56±0.10	0.83±0.02	1.05±0.01
KNF	0.16±0.01	0.27±0.01	0.43±0.04	0.52±0.01	0.53±0.04	0.73±0.01	0.85±0.02
KP	0.15±0.01	0.37±0.01	0.57±0.01	0.66±0.01	0.86±0.02	1.03±0.02	1.16±0.02
KN	0.12±0.0	0.32±0.02	0.46±0.03	0.55±0.26	0.63±0.02	0.81±0.01	0.85±0.01

Values are Mean ± SD

Table 3: Rabbits hair weights after treatment with *Petroselinum crispum* leaf ethyl acetate fractions

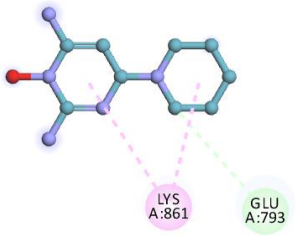
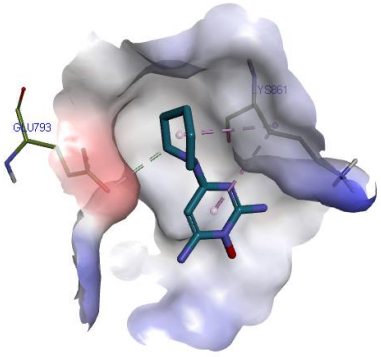
Hair weights (g)									
P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**KNF**	**KP**	**KN**
0.087± 0.01	0.125± 0.02	0.057 0.02	0.10± 0.02	0.075± 0.02	0.035± 0.02	0.073± 0.02	0.02± 0.01	0.91± 0.01	0.07± 0.01

Values are Mean ± SD

Table 4: Binding energy ratio of native ligands and fraction 4 compounds to the target androgen receptor

No	Compound Name	Binding Energy (Kcal/mol)
1	Minoxidil (native ligand)	-4.7
2	Paeonilactone C1	-6.6
3	3-(3,4-dihydroxybenzylidene)-7-hydroxychroman-4-one	-5.8
4	Digitopurpone	-5.7
5	2,4,4,6-tetrahydroxy-benzophenone	-5.7
6	Aviprin	-5.4
7	d-catechin	-5.3
8	Xanthotoxin	-4.9
9	Digiprolactone	-4.5

Table 5: Visualization of the molecular interaction of compounds with the androgen receptor active site

No	Compound	2D Visualization	3D Visualization
1	Minoxidil (Native Li-gan)		

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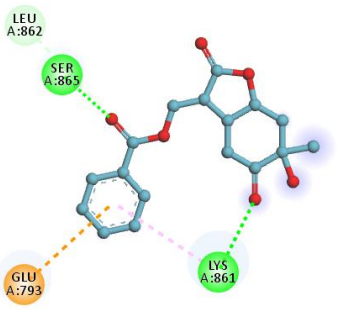
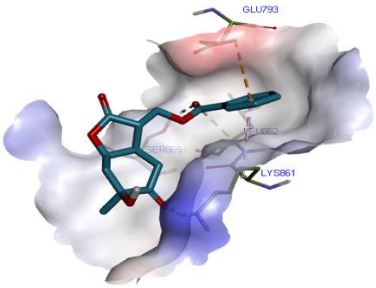
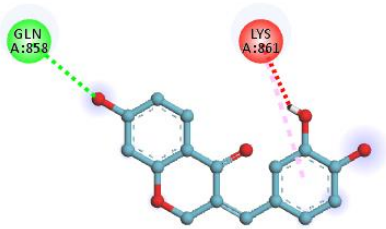
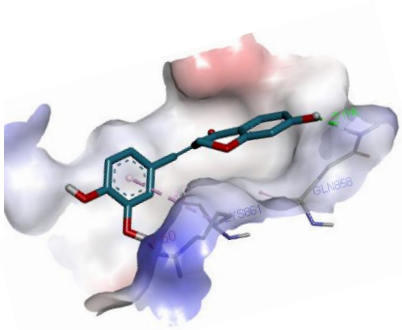
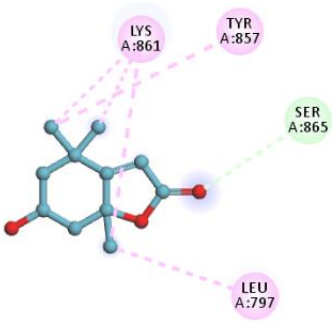
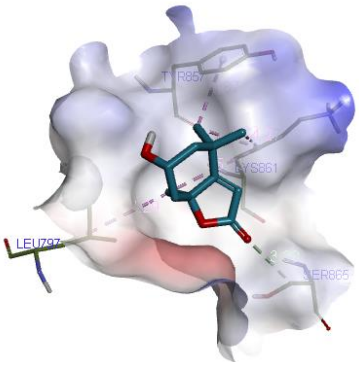
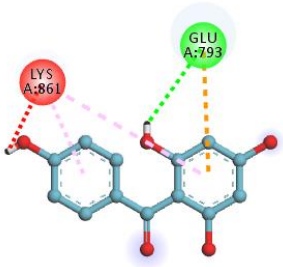
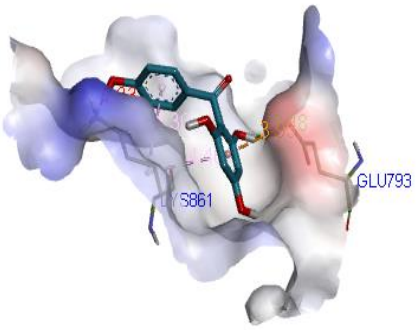
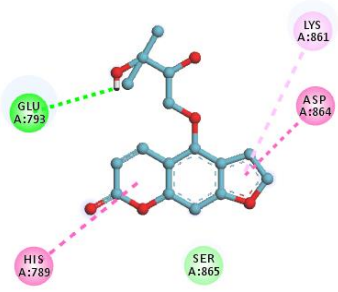
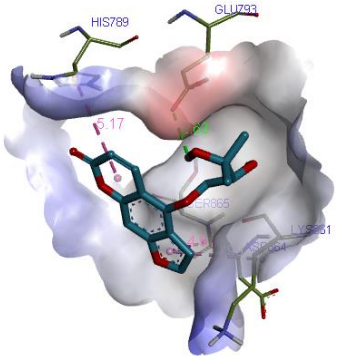
2	Paeonilactone C1		
3	3-(3,4-Dihydroxybenzylidene)-7-hydroxychroman-4-one		
4	Digiprolactone		
5	2,4,4,6-Tetrahydroxy-benzophenone		
6	Aviprin		

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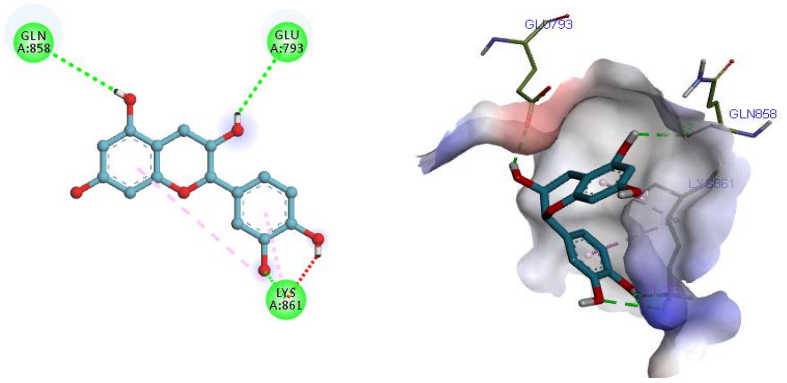
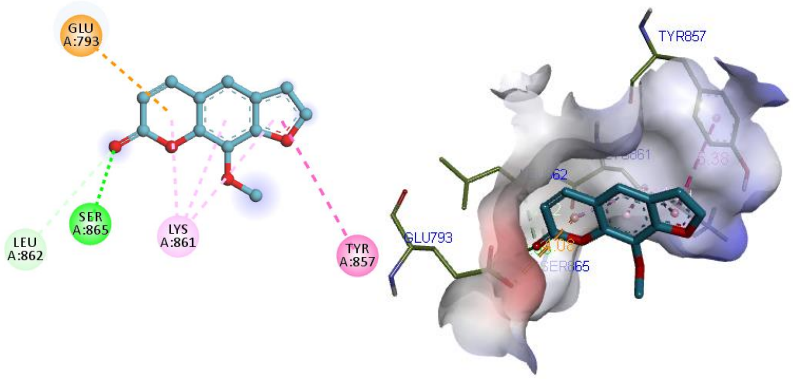
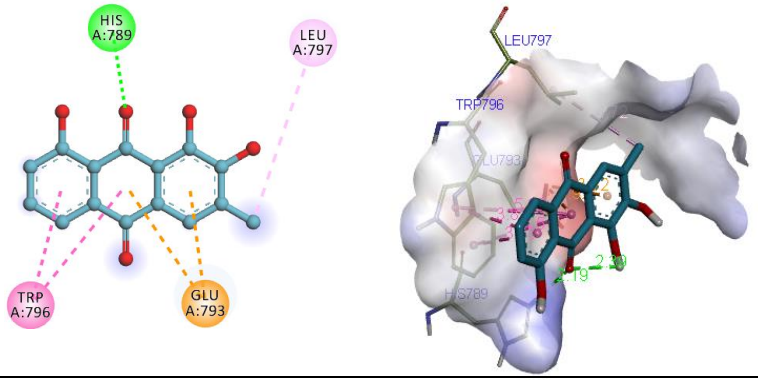
7	d-Catechin	
8	Xanthotoxin	
9	Digitopurpone	

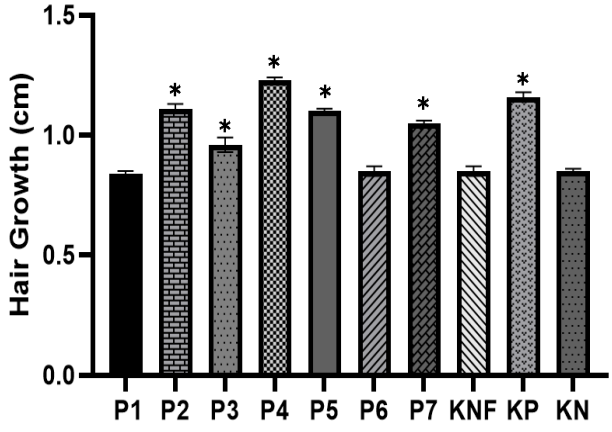


Figure 9: Hair lenght of rabbits on day 21 of treatment with *Petroselinum crispum* leaf ethyl acetate fraction. (P1) Fraction 1, (P2) Fraction 2, (P3) Fraction 3, (P4) Fraction 4, (P5) Fraction 5, (P6) Fraction 6, (P7) Fraction 7, (KNF) Normal Control, (KP) Positive control minoxidil, (KN) negative control Na-CMC (*significant difference from negative control and normal control)

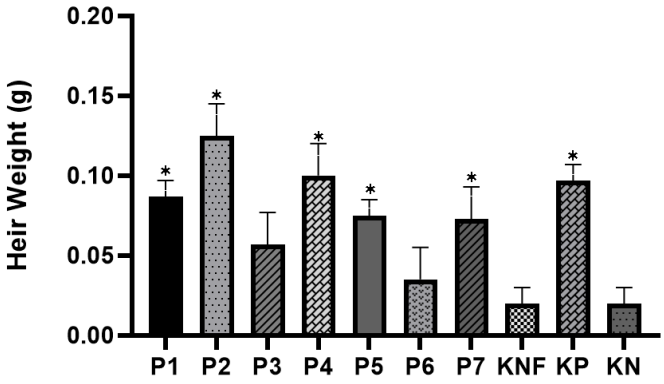


Figure 10: Hair weights of rabbits after treatment with *Petroselinum crispum* leaf ethyl acetate fraction. (P1) Fraction 1, (P2) Fraction 2, (P3) Fraction 3, (P4) Fraction 4, (P5) Fraction 5, (P6) Fraction 6, (P7) Fraction 7, (KNF) Normal Control, (KP) Positive control minoxidil, (KN) negative control Na-CMC (*significant difference from negative control and normal control)

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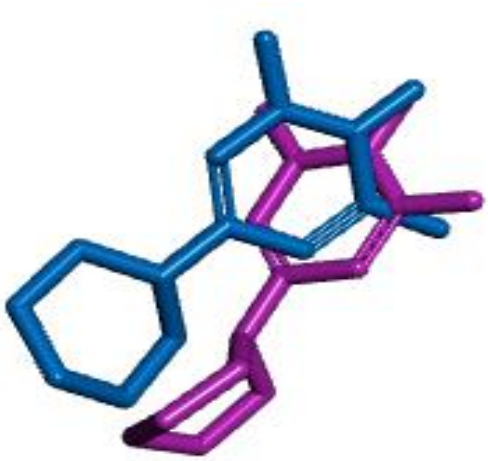

Figure 11: Visualization of two minoxidil molecules. The best pose of minoxidil redocking (blue) overlapped with natural minoxidil ligand before validation (purple).

Conclusion

The ethyl acetate fraction of *Petroselinum crispum* has been shown to have hair growth promoting activity with fractions 2 and 4 exhibiting the highest hair growth promoting activity comparable to that of minoxidil, a standard anti-alopecia drug. LC-MS analysis identified a number of compounds from fractions 2 and 4, among which the compound paeonilacton C_1 demonstrated the greatest binding energy with the androgen receptor *in silico*. *Petroselinum crispum* leaf fraction could be developed into hair tonic for use in the treatment and/or prevention of alopecia.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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