HAYATI
Journal of Biosciences

Vol. 32 No. 5, September 2025 1109-1122
DOI:10.4308/hjb.32.5.1109-1122
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Secondary Metabolite Profiling, *In-Silico*, and *In Vitro* Study of *Acriopsis liliifolia* Roots as Active Cosmetic Ingredients

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ARTICLE INFO

Article history:
Received November 19, 2024
Received in revised form March 15, 2025
Accepted March 20, 2025

KEYWORDS:
Acriopsis liliifolia,
molecular docking,
roots,
secondary metabolites,
tyrosinase inhibitor

ABSTRACT

Beauty and health are currently topics of the global trend. Secondary metabolites from natural sources have become increasingly important for their potential application in cosmetics. This study aims to investigate the potential of *Acriopsis liliifolia* roots (ALR) as a source of active cosmetic ingredients through secondary metabolite profiling, *in silico*, and *in vitro* analysis. *A. liliifolia*, a medicinally valuable orchid species, was selected due to its rich phytochemical content, which could offer beneficial properties for skin, such as skin-brightening effects. The roots of *A. liliifolia* were subjected to microchemical assay and metabolite profiling using Liquid Chromatography High-Resolution Mass Spectrometry (LC-HRMS) to determine the bioactive compounds. Additionally, the identified compounds were evaluated through molecular docking studies to assess their interactions with key skin-related enzymes, such as tyrosinase. *In vitro* studies were conducted to confirm the activity of secondary metabolites of *A. liliifolia* root on the inhibition of tyrosinase. Microchemical results showed that ALR is positive for phenolics and alkaloids. Metabolite profiling revealed the presence of 125 compounds, then 14 of the most potential compounds were selected. The docking studies exhibit that 1,3-dilinolenoylglycerol had the lowest S-score of -9.54 kJ/mol and lower than kojic acid, suggesting that 1,3-dilinolenoylglycerol has the potential to inhibit tyrosinase. The *in vitro* studies showed that *A. liliifolia* roots extract at 250 mg/ml can inhibit tyrosinase (150 U/ml) by 42.56%. However, further research is required to ascertain its potential effect and safety assessment of cosmetics.

1. Introduction

Inflammation-related skin conditions are common in the natural aging process and normal skin physiology, often leading to unwanted skin darkening. Tyrosinase enzyme has an important role in skin pigmentation. One of the primary objectives of research aimed at preventing hyperpigmentation is to control melanin production by inhibiting the activity of tyrosinase (Sepehri *et al.* 2021; Niri *et al.* 2023). Tyrosinase (TYR) is a key enzyme that plays an important role in melanogenesis. It catalyzes two reactions, the hydroxylation of L-tyrosine into L-dihydroxyphenylalanine (L-DOPA) and the oxidation of L-DOPA to dopaquinone. This compound subsequently polymerizes to form dopachrome, ultimately leading to melanin pigment formation of the skin (Hosseinpoor *et al.* 2021; Alizadeh *et al.* 2022).

Cosmetics are products designed for external use on the body, intended to enhance appearance, maintain health, cleanse the skin, and address body odor (Salvioni *et al.* 2021). In modern society, the use of cosmetics has become a widespread trend. The global pandemic has led to changes in lifestyle, increasing the demand for cosmetics to promote healthy and beautiful skin (Choi *et al.* 2022). Cosmetics have become essential for body care, with skincare products used by both women and men, who increasingly recognize the importance of maintaining healthy and attractive skin. Skin brightening refers to using chemicals or other products

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with depigmenting properties to lighten skin tone or enhance complexion. This is accomplished by reducing melanin concentration and decreasing the skin's natural pigmentation (Nordin *et al.* 2021). New tyrosinase inhibitors are being developed and hold significant value for the pharmaceutical and cosmetic industries. Certain substances have been explored as tyrosinase inhibitors they have been discovered to have side effects, such as hydroquinone can cause melanin destruction, irritation, ochronosis, and dermatitis (Irfan *et al.* 2022), kojic acid due to its carcinogenicity (Ashooriha *et al.* 2019), and arbutin can release hydroquinone that may exerts allergy and cytotoxicity reactions (Li *et al.* 2024). Due to these negative effects, safer and more natural options are being explored for enhancing skin brightness. The discovery of novel tyrosinase inhibitors through High-Throughput Screening Techniques, QSAR Models, and Bioinformatics approaches facilitate the identification and characterization of compounds with improved specificity and sensitivity against tyrosinase (Zolghadri *et al.* 2019).

The interaction between active metabolites from plants and the human skin plays a crucial role, as the skin has a major impact on appearance and beauty. Orchids are one of the horticultural plants with high variation, with more than 28,000 species and 763 genera (Christenhusz & Byng 2016). Although orchids have been primarily known for their aesthetic values, they provide a rich source of secondary metabolites that are often underrated (Li *et al.* 2023). Recently, orchids have been a potential source of natural tyrosinase inhibitors, which aligns with the increasing demand in the pharmaceutical industry. Several orchid species, such as *Dendrobium sulcatum* Lindl, *Dendrobium hybrid, Malaxis acuminata*, and *Bletilla striata* have been shown for their active compounds that can inhibit tyrosinase activity (Bose *et al.* 2017; Rungsang *et al.* 2023; Swainson *et al.* 2023; Zhu *et al.* 2023). Rhizome of *Gastrodia elata* has been reported to have great potency for skin-whitening agents. Natural component isolated from *Gastrodia elata* roots shows a strong competitive inhibition of mushroom tyrosinase which is superior to kojic acid (Chen *et al.* 2015).

Acriopsis is a small genus of Orchidaceae. It is native to Southeast Asia, including Myanmar, Vietnam, Malaysia, Thailand, Indonesia, and the Philippines (Lok *et al.* 2009). *Acriopsis liliifolia* is an epiphytic orchid with sympodial stem growth. This orchid has heteroblastic pseudobulbs, textured surfaces, green in color, and very close pseudobulb positions. *A. liliifolia* has green leaves with pointed leaf bases, flat leaf edges, lanceolate leaf structure, blunt leaf tips, smooth leaf surfaces, and thin. This type of orchid has roots with small, smooth surfaces and white (Yulia & Tarmudji 2007; Huda *et al.* 2022). The roots of *A. liliifolia* have been traditionally used to treat fever (Sulistiarini 2003). While the plant has been known for its medicinal properties, its use in cosmetic formulations remains largely unexplored. Previous studies of *A. liliifolia* only focuses on botanical (Setiawan *et al.* 2024) and ecological aspects (Sofiyanti 2014). Secondary metabolites exploration of *A. liliifolia* roots (ALR) can make them potential candidates for developing skin-lightening agents.

This study aims to explore the potential of *A. liliifolia* roots as a source of active ingredients for cosmetic applications. The phytochemical components of ALR will be analyzed using Liquid Chromatography High-Resolution Mass Spectrometry (LC-HRMS). The molecular docking study investigated the binding interaction affinity between the bioactive compounds in the extract and their inhibitory effect on the tyrosinase. The magnitude of inhibition was determined through *in vitro* studies based on the color formation reaction pure enzyme reaction.

2. Materials and Methods

2.1. Microchemical Test

Microchemical tests of the ALR commenced with preparing five cross-sectional slices using a razor blade: one control sample and four treated with different reagents (Faizul Huda *et al.* 2023). The testing involves applying specific reagents to identify particular groups of compounds: sitroborate reagent for flavonoids, $FeCl_3$ for phenolic compounds, a mixture of anisaldehyde and sulfuric acid for terpenoids, and Dragendorff for alkaloids. Each treated roots sample is then examined under a microscope, and any colour changes or other observable characteristics are noted as indicators of the presence of the tested compounds.

2.2. Chemical and Reagents

Solvents (water, methanol (MeOH), and acetonitrile) LC-MS grade were supplied by Fisher Scientific. Hydrochloric acid (HCl), formic acid (FA), acetone, ammonium bicarbonate ($(NH_4)HCO_3$), HPLC-grade solvent of methanol, and trifluoroacetic acid (TFA) were sourced from E. Merck. The Pierce LTQ Velos ESI positive and Pierce ESI negative calibration solutions were acquired from Thermo Fisher Scientific (Rockford, IL, USA).

2.3. *Acriopsis liliifolia* Roots Sample Collecting and Extraction

Acriopsis liliifolia was obtained from Purwosari, Kulon Progo. The fresh roots were finely ground to produce a uniform powder. The order of samples for omics-based analyses was randomized and prepared in three replicates. A total of 50 mg samples were analytically weighed and placed into microcentrifuge tubes. Each sample was extracted with 1 ml of hypergrade MeOH for liquid chromatography. Subsequently, samples were vortexed for 30 seconds and sonicated using an ultrasonic for 30 minutes at room temperature. The supernatant was centrifuged at 1,400×g for 5 minutes to separate the supernatant from the pellet. The supernatant was filtered through a PTFE filter (0.22 µm) and placed in the vial to prepare for LC-HRMS analysis. MS grade MeOH was also prepared as a blank sample (Windarsih *et al.* 2022).

2.4. Metabolomics Analysis

The untargeted metabolomics analysis used liquid chromatography with Vanquish Ultra High-Performance Liquid Chromatography (UHPLC) and Orbitrap high-resolution mass spectrometer established from Thermo Scientific, USA. An Accucore C-18 analytical column was employed for liquid chromatography, in which the samples were injected at 3 µL. The mobile phases consisted of MS-grade water (A) and MS-grade methanol (B) containing 0.1% formic acid for each mobile phase to optimize separation and reduce ion suppression, with a flow rate of 0.30 ml/min. Elution was carried out using the gradient technique: initially, the mobile phase was set at 5% B, then gradually increased to 90% B at 16 minutes, maintained at 90% for 4 minutes, and then returned to 5% B (25 minutes). During the analyte separation, the column temperature was maintained at 40°C. The sample was injected at 10 µL for each measurement, both in blank and in samples. The HRMS parameters for sheath gas, auxiliary gas, and sweep gas were set at 32, 8, and 4 arbitrary units (AU), respectively. Electrospray ionization (ESI) was used in positive and negative modes. Both ESI positive and ESI negative was calibrated using an ESI calibration solution before the analysis. The temperature for capillary was set at 320°C, followed by a gas heater temperature of 30°C during the analysis. The compounds were scanned at mass ranges from 66.7 to 1,000 m/z. The system was managed by XCalibur 4.4 software (Thermo Scientific) (Windarsih *et al.* 2022).

2.5. Secondary Metabolites Profiling

The Compound Discoverer 3.2 (Thermo Scientific, USA) software was used to identify the metabolites present in ALR from the raw data. It was called total ion chromatogram (TIC) and included the blank. Annotation was done by comparing m/z and fragmentation patterns between experimental and theoretical results using databases include Chemspider, mzCloud, and Predicted Compositions (Sapozhnikova & Nuñez 2022). Then, the resulting compounds were filtered based on their names, best-matched fragmentation pattern to MzCloud, and data-dependent acquisition for preferred ions.

2.6. Molecular Docking

2.6.1. ALR Ligand Preparation

The ligands used *in silico* studies were compounds detected in the ALR sample. The three-dimensional (3D) conformations were generated using Molecular Operating Environment (MOE) software by the builder module (Khan *et al.* 2023). Energy minimization was performed on the compounds using the MOPAC system, utilizing PM3 as the potential energy function to assign charges. The optimized structures were then stored in mdb format.

2.6.2. Protein Preparation

The non-mutated crystal structure of the tyrosinase (PDB ID: 2Y9X) was initially downloaded in PDB format from the Protein Data Bank (Berman *et al.* 2000) and then imported into MOE software (Chemical Computing Group ULC 2022). The protein crystal was treated by selecting one chain, removing water molecules, and adding hydrogen atoms to the structure according to their standard geometry. Water molecules were eliminated because the water stabilized the tetramer chain, which was removed. Then, energy minimization was carried out using an Amber99 force field (Manh Khoa *et al.* 2023; Khojah *et al.* 2024).

2.6.3. Pose Validation

The co-crystallized native ligand were redocked into the active sites using the default docking protocol in MOE for software validation. Root mean square deviation (RMSD) is a parameter used to compare the

ligand with its related crystal structure (Khan *et al.* 2024). The conformation with the lowest energy after minimization was chosen for further analysis.

2.6.4. Molecular Docking

The validated docking method was used to elucidate the mechanism of compounds in binding with tyrosinase enzyme. The protein crystal structure was refined, and the ligand was linked to the active site of the target (Song *et al.* 2020). The analysis of docking results includes evaluating the best pose, score, and the interactions between the compounds and the amino acid residues within the active site of the tyrosinase enzyme (Apsari *et al.* 2024).

2.7. Determination of Tyrosinase Inhibitory Activity

The mushroom tyrosinase inhibition assay was performed in a 96-well microplate using a spectrophotometer microplate reader (Thermo Scientific). Initially, 20 µL of potassium phosphate buffer (pH 6.5), 20 µL of the tested sample with varying concentrations (0.00005—250 mg/ml), and 60 µL of mushroom tyrosinase (150 U/ml) were placed into a 96-well microplate. After a preincubation of 10 minutes at room temperature, 100 µL L-DOPA (10 mM) was added in a total reaction volume of 200 µL followed by an incubation at room temperature for 20 minutes (kojic acid) and 80 minutes (sample). After an incubation period, the absorbance of dopachrome was spectrophotometrically measured at 482 nm. Kojic acid was used as a standard inhibitor (0.00005—5 mg/ml). Each concentration was conducted in triplicate and the results were presented as the mean value. The percent of inhibition was calculated according to this formula: inhibition (%) = {[C - (S - B)] / C} × 100, where C, S, and B are the absorbance for the control, sample, and blank..

3. Results

Microchemical testing was determined for preliminary testing. Microchemical assays are effective tools for rapidly screening and characterizing substances across various fields. These assays allow for quick identification and quantification of materials, often requiring minimal sample volumes and equipment. The following sections detail their applications, advantages, and limitations. Microchemical tests are widely utilized for screening drugs, explosives, and biological materials, providing presumptive results through color changes or microcrystal formation. The microchemical testing results showed that the ALR sample was positive for containing phenolic marked by purplish-green color changes and alkaloids for the formation of yellow color (Figure 1).

Acriopsis liliifolia roots were analyzed with liquid chromatography (LC) connected to a high-resolution mass spectrometer (HRMS). The most likely molecular formula was determined based on its structure using the ChemSpider and PubChem databases. Then, compounds were classified by superclass, class, and subclass, as shown in Figure 2-4. The most abundant superclass, class, and subclass of secondary metabolites in *Acriopsis liliifolia* roots were lipids and lipid-like molecules (37.1%); fatty acyls (21.8%); and amino acids, peptides, and analogues (11.3%).

The content of chemical compounds in the extracts of roots of *A. liliifolia* was summarized into 14 compounds based on the number of hydrogen bonds, then presented in Table 1. The fourteen compounds were selected by the most hydrogen bonds according

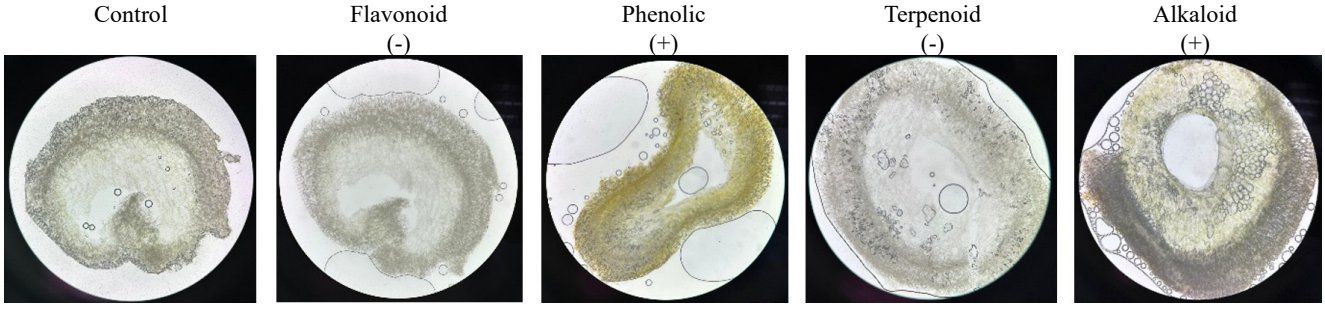


Figure 1. Microchemical assay results of ALR samples as control and tested for flavonoid, phenolic, terpenoid, alkaloid

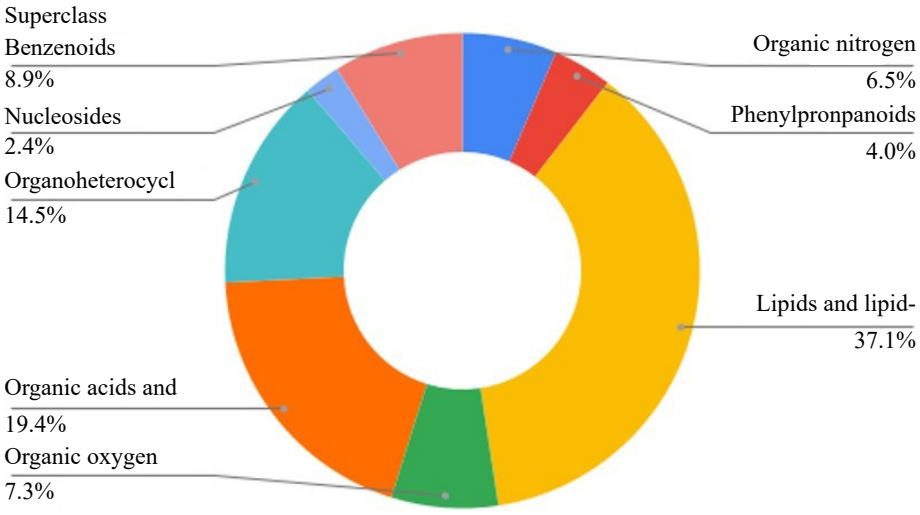


Figure 2. Chemical taxonomy (Superclass) of secondary metabolites of *Acriopsis liliifolia* roots

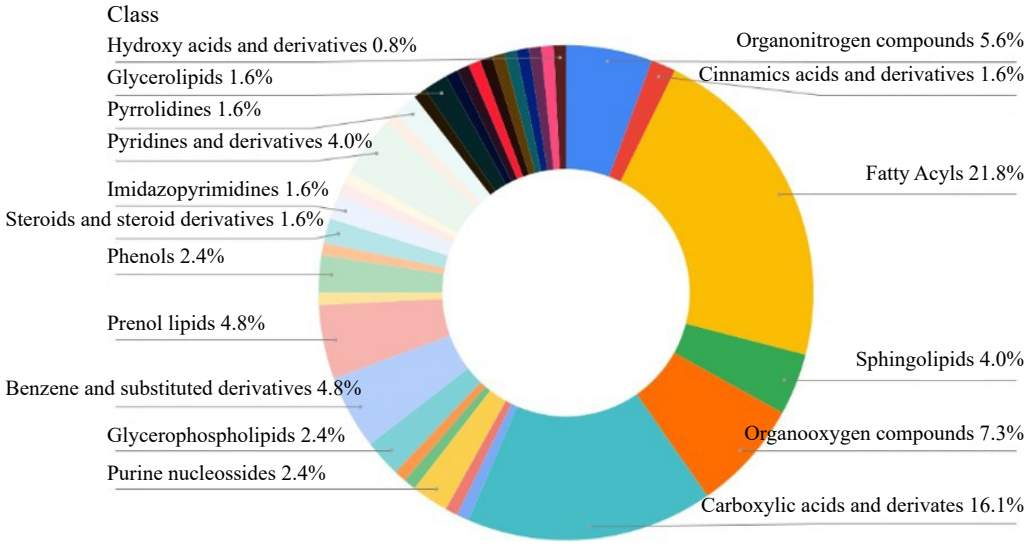


Figure 3. Chemical taxonomy (Class) of secondary metabolites of *Acriopsis liliifolia* roots

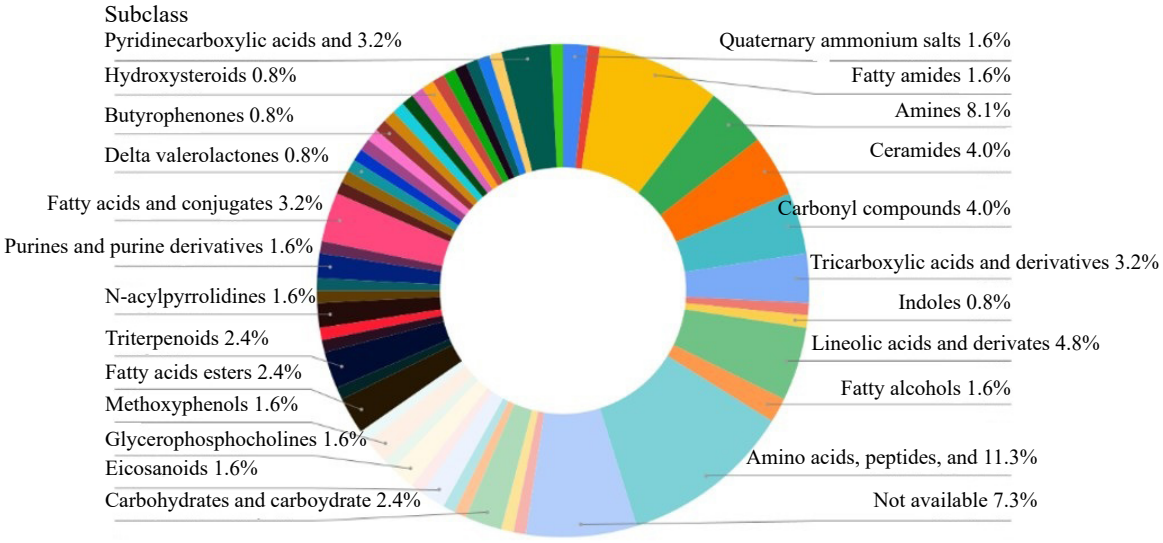


Figure 4. Chemical taxonomy (Subclass) of secondary metabolites of *Acriopsis liliifolia* roots

Table 1. List of compounds detected in ALR sample, co-crystal ligand, and S-score

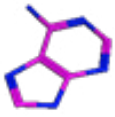
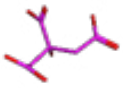
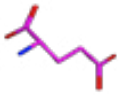
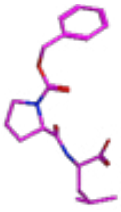
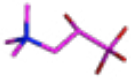
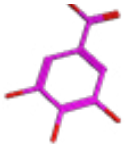
Compound	Co-crystal structure	S-score (kJ/mol)
1,3-dilinolenoylglycerol		-9.54
1-Linoleoyl glycerol		-7.04
3-hydroxydodecanoylcarnitine		-6.79
Adenine		-4.86
Citric acid		-4.96
L-Glutamic acid		-5.90
Prolylleucine		-6.73
Hydrogen [1-hydroxy-2-(trimethylammonio)ethyl] phosphonate		-5.11
Gallic acid		-6.45
Phytosphingosine		-5.68

Table 1. Continued

Compound	Co-crystal structure	S-score (kJ/mol)
2-Amino-1,3,4-octadecanetriol		-6.18
Choline		-4.13
D-Sphingosine		-6.93
N,N-Bis(2-hydroxyethyl)dodecanamide		-7.09

to residues and one compound with the lowest S-score. Hydrogen bonds have the most important specific interactions that can stabilize the ligands-receptor complex.

In silico docking study was performed using the Molecular Operating Environment (MOE) to provide the binding energy analysis of *A. liliifolia* roots compounds with tyrosinase enzyme. Investigating these molecular interactions employs a validated docking simulation that effectively replicates the binding conformation of *A. liliifolia* roots compounds to the active site of tyrosinase, achieving an RMSD value of 0.584 Å. Superimposition was performed between the crystallized native ligand and the redocked ligand on the receptor (Figure 5).

The analysis showed that 1,3-dilinolenoylglycerol exhibits the lowest binding energy at -9.54 kJ/mol against tyrosinase. At the same time, the binding

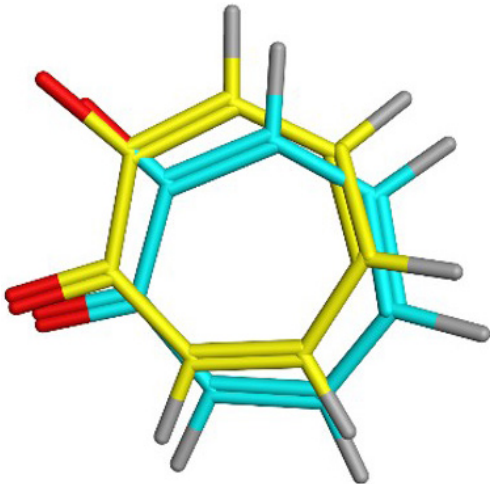


Figure 5. The alignment of the crystallized native ligand (green) with the re-docked native ligand (yellow) with the RMSD value of 0.584 Å

energy value of kojic acid was -5.05 kJ/mol. Lower binding energy indicates a stronger interaction/affinity between the compound and the tyrosinase enzyme, suggesting that the compound could more effectively inhibit tyrosinase enzyme activity. Visualization of the interaction between the best-scoring ligand and receptor is shown in Figure 6. The comparison of the interaction between 1,3-dilinolenoylglycerol and kojic acid is shown in Figure 7. The hydrogen bond pointed at arginine indicates a strong ligand-receptor binding affinity. The interaction of the other compounds is shown in Figure 8. In contrast, compounds 5, 4 and 12 have binding energies of -4.96 kJ/mol, -4.86 kJ/mol, and -4.13 kJ/mol, respectively (Table 1).

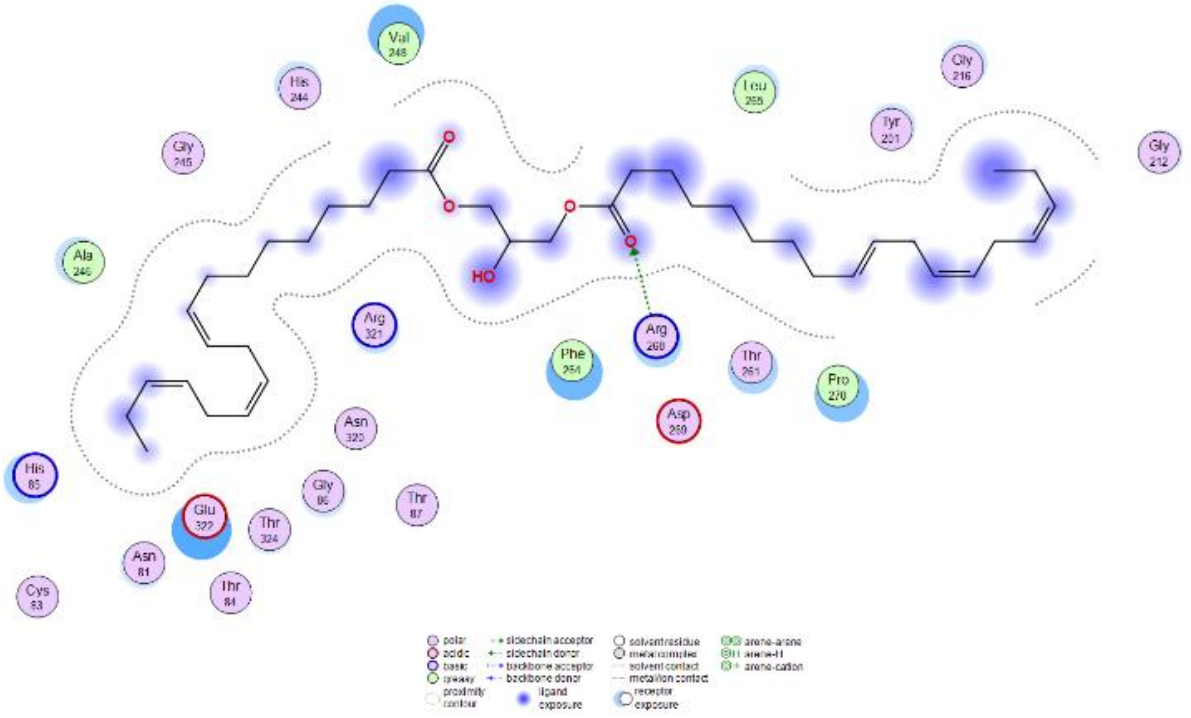


Figure 6. The lowest energy 2-dimensional of 1,3-dilinolenoylglycerol docked poses within the binding pocket of tyrosinase. The key amino acid residues are shown as spheres, while hydrogen bond interactions are illustrated with green dashed lines

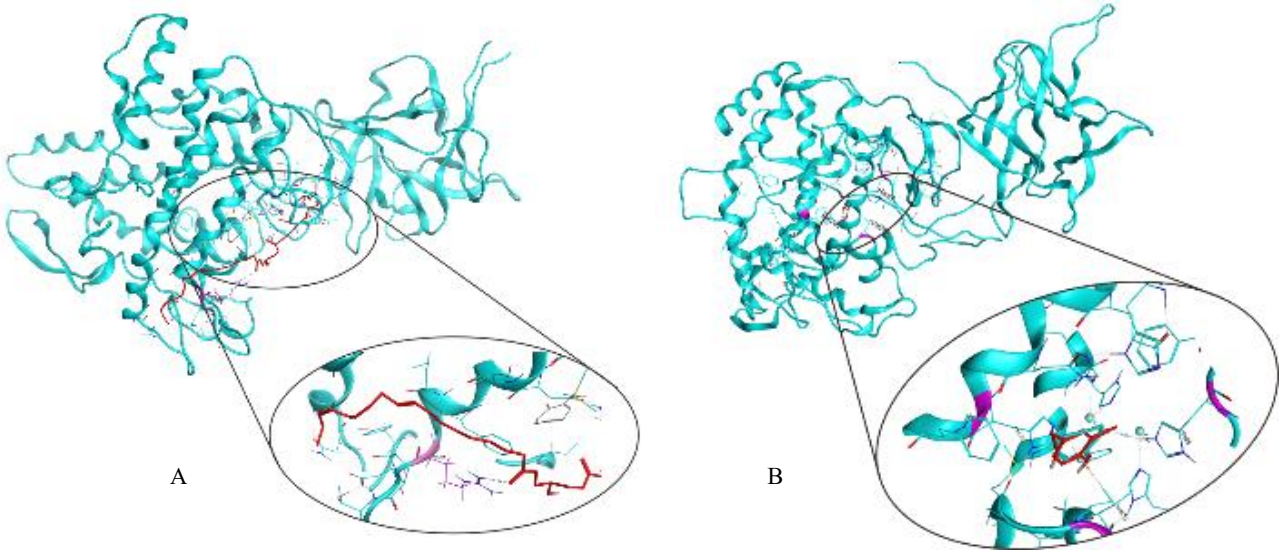


Figure 7. Lowest energy 3-dimensional docked poses of 1,3-dilinolenoylglycerol (A) and kojic acid (B) within the binding pocket of tyrosinase

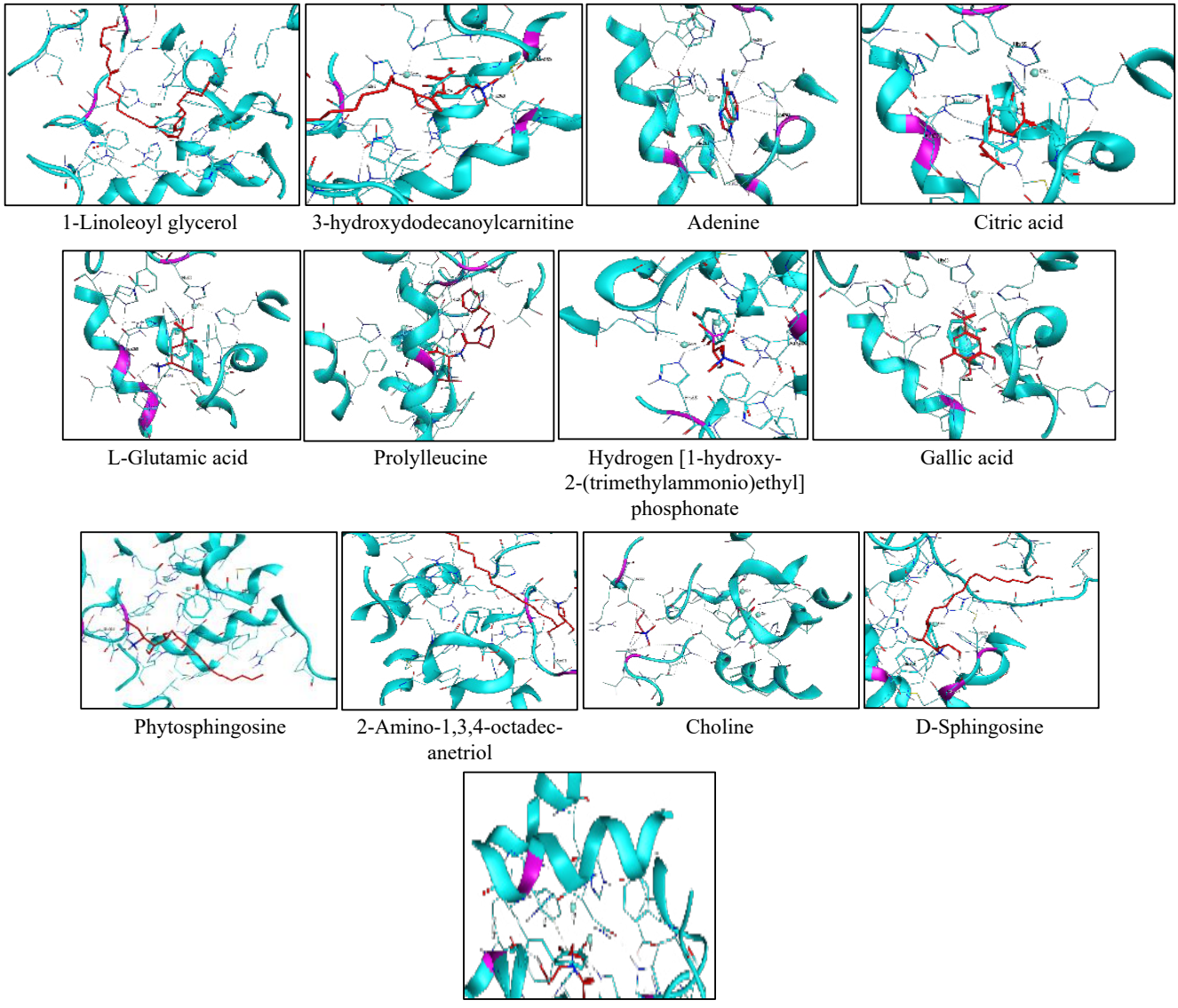


Figure 8. Three-dimensional visualization of the interaction *Acriopsis liliifolia* roots compounds with amino acid residues from tyrosinase enzyme receptor

The inhibitory activity of *A. liliifolia* roots was evaluated by using a commercially available tyrosinase. Unfortunately, the half maximal inhibitory concentration (IC_{50}) could not be determined even when the concentration of the ALR extract was increased to 250 mg/ml. Based on the percent inhibition, *A. liliifolia* roots showed low inhibition of tyrosinase compared to the standard kojic acid (Figure 9). This extract activity is categorized as weak. This is because the concentration of compounds that act as tyrosinase inhibitors is very low. However, the findings from this research could open up opportunities for further synthesis of active compounds in orchids, especially *A. liliifolia*.

4. Discussion

Based on the microchemical assay, the positive result detected the phenolic compound. Phenolic compounds, including coumarins and chlorogenic acid, exhibit significant tyrosinase inhibition, contributing to their potential use in pharmaceutical and cosmetic applications for skin whitening and antioxidant effects (Kanbolat *et al.* 2022; Nunes *et al.* 2023). Dehydroevodiamine, an alkaloid isolated from *Evodia rutaecarpa*, directly inhibits tyrosinase activity, reducing melanin synthesis without cytotoxicity (Luo *et al.* 2010). This result showed that ALR compounds have the potential for tyrosinase

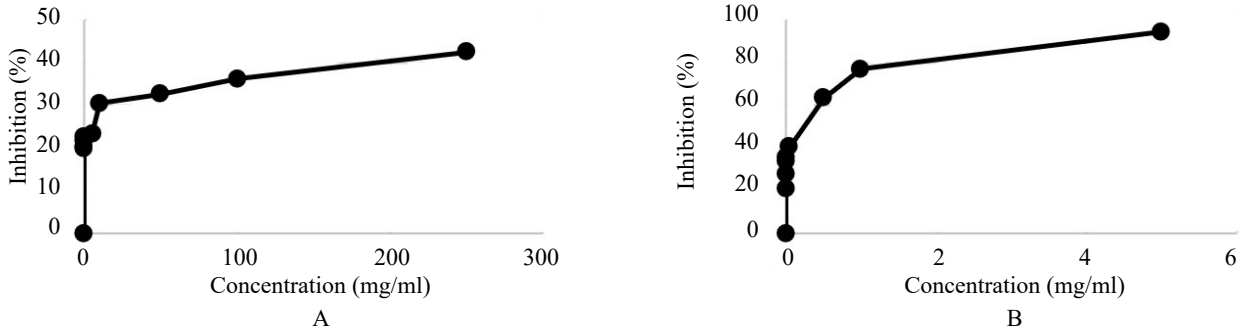


Figure 9. Effect of ALR extract (A) and kojic acid (B) against mushroom tyrosinase

inhibitors. The secondary metabolites using LC-HRMS detected phenolic compounds in the ALR sample, such as 5-pentyl resorcinol, eugenol, and nonivamide. Eugenol has shown tyrosinase inhibitory activity through competitive inhibition, where hydroxyl groups and conjugated carbonyls enhance their binding affinity to the enzyme (Li *et al.* 2023; Vaezi 2023).

The secondary metabolites of ALR were classified by superclass, class, and subclass, a structured approach to organizing entities based on shared characteristics and hierarchical relationships. This method enhances the understanding of complex data by allowing for the modeling of dependencies among classes and subclasses, which is particularly useful in multi-dimensional classification tasks. The most abundant superclass compounds were lipid and lipid-like molecules. This molecule type can act as an antimicrobial and antiinflammation (Fischer 2020).

The results of metabolite profiling using LC-HRMS confirmed the results of microchemical tests that ALR contains phenolic and alkaloid compounds. This match can be seen in the subclass of compounds. Phenolic compounds include Hydroxycinnamic acids and derivatives (0.8%), Hydroxyisoflavonoids (0.8%), Methoxyphenols (1.6%), Benzenediols (0.8%), O-methylated flavonoids (0.8%), Cinnamic acids (0.8%). Alkaloid compounds include Indoles, Amines (4.0%), Quaternary ammonium salts (1.6%), Pyridine carboxylic acids and derivatives (3.2%), Phenylmethylamines, N-acyl pyrrolidines (1.6%).

Several of the best S-score compounds of *A. liliifolia* roots have important functions. Adenine plays a role as a signaling molecule. Adenine influences processes such as seed germination and root growth (Liu *et al.* 2023). Citric acid can facilitate adenosine triphosphate (ATP) production and enhances plant growth and yield by improving photosynthetic rates and reducing reactive oxygen species (Wang *et al.* 2017; Tahjib-Ul-Arif *et al.* 2021). Proline, a key component of prolyl leucine, is associated with increased tolerance to environmental stresses by stabilizing proteins and scavenging reactive oxygen species (ROS). It supports reproductive development and modulates root size that impacts plant growth (Kishor *et al.* 2015; Renzetti *et al.* 2024). Phytosphingosine plays a multifaceted role, primarily functioning as a signaling molecule involved in plant defense and stress responses (Seo *et al.* 2021; Glenz *et al.* 2022). Choline acts as a precursor for phosphatidylcholine, a major component of cell membranes, and plays a role in membrane integrity and signaling. Additionally, choline is implicated in the stress response that is important in plant growth and development (Paper *et al.* 2018; Zhang *et al.* 2021). Sphingolipids, including D-Sphingosine, regulate cellular homeostasis, stress responses, and immunity by mediating defense signaling and influencing cell death during pathogen interactions (Liu *et al.* 2021; Zeng & Yao 2022).

The molecular docking analysis offered insights into the structural interactions occurring within the active site pockets of tyrosinase. Before running the docking, pose validation was needed. The RMSD value was 0.584 Å. RMSD values are calculated to compare geometries over time, with lower values indicating stability and accuracy in molecular computation (Saber Iraji & Ameri 2016). The molecular docking results highlighted the importance of oxygen and terminal hydroxyl groups in forming hydrogen bonds with the amino acid residues of tyrosinase, leading to energetically favorable conformations (Farooq *et al.* 2020).

Scoring functions (SFs) are integral to predicting binding affinities and conformations in molecular docking (Ashtawy & Mahapatra 2018). The S-score is parametric of the compound's ability to bind to a receptor (Hamzah *et al.* 2017). Lower S-scores correlate with stronger receptor-ligand interactions, which are crucial

for effective drug exploration (Ambarwati *et al.* 2017). Kojic acid was used as standard because previous studies evaluated kojic acid as a natural tyrosinase inhibitor that has been clinically utilized to treat skin hyperpigmentation (Ashooriha *et al.* 2020; Wang *et al.* 2022; Peng *et al.* 2023). This study evaluated the S-score of kojic acid, and the results obtained were -5.05 kJ/mol. It indicated that 1,3-dilinolenoylglycerol had a stronger binding to tyrosinase enzyme than kojic acid.

The results showed that 1,3-dilinolenoylglycerol had one hydrogen bond with Arg268 (2.99 distance) and 5 Van der Walls forces. A hydrogen bond indicates a specific, directional interaction where a hydrogen atom from the ligand forms a bond with an electronegative atom, contributing to molecular stability and structure (Portela & Fernández 2021). Van der Walls forces indicate multiple weak, non-covalent interactions from temporary charge distribution fluctuations. Van der Waals interactions are weaker than hydrogen bonds but are essential for stabilizing the ligand-receptor complex, especially when they occur in large numbers (Tantardini *et al.* 2020). This structure showed that 1,3-dilinolenoylglycerol can provide stable and potent inhibition of tyrosinase. On the other hand, kojic acid-tyrosinase interaction had one hydrogen bond with His85, one arene-hydrogen, and two ionic bonds. However, there are more bonds in the kojic acid interaction; the receptor exposed more amino acids in the interaction with a ligand of 1,3-dilinolenoylglycerol. This phenomenon could stabilize the structure and enable other forces leading to bonds.

Other compounds, such as Glutamic acid, are known for their melanin production inhibitory by inducing acidification and binding, leading to tyrosinase inactivation, which also inhibits L-DOPA auto-oxidation (Zhong *et al.* 2024). A previous *in vitro* study declared that Gallic acid exhibited significant inhibitory activity on tyrosinase (Xiong *et al.* 2023). Besides that, Gallic acid and its derivatives, such as pentagalloylglucose, can chelate copper ions essential for inhibiting tyrosinase function. Gallic acid-benzylidenehydrazine hybrids reduce it to Cu^{2+} into Cu^{1+}, enhancing anti-tyrosinase activity (Peng *et al.* 2022).

Tyrosinase facilitates the conversion of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) through hydroxylation, followed by the oxidation of L-DOPA into dopaquinone. This compound subsequently undergoes a series of non-enzymatic polymerization reactions to produce melanin. Accumulation of melanin can lead to hyperpigmentation. As a result, the development of safe and effective tyrosinase inhibitors has become essential for cosmetic industry to control melanin overproduction and promote skin whitening. The tyrosinase inhibitory activity of kojic acid was 94.43% at 5 mg/ml. However, the half maximal inhibitory concentration (IC_{50}) of ALR extract could not be determined and showed lower inhibitory activity than kojic acid, with 42.56% at 250 mg/ml. This low efficacy is partially due to the small concentration of antityrosinase components in the ALR.

According to secondary metabolites profiling and molecular docking study, 1,3-dilinolenoylglycerol had the potency as a lead compound for tyrosinase inhibitors. However, ALR showed low activity of tyrosinase inhibition compared to kojic acid. Further studies, such as animal experiments (*in vivo*), are needed to assess its safety for living bodies and humans. The series of analyses was able to confirm its effectiveness and safety for active cosmetic ingredients. Also, isolating the active compounds is needed to evaluate their bioproduction potential and assess the activity of the purified compound in both *in vitro* and *in vivo* tests.

This study enhances the significance of *A. liliifolia* in drug exploration while ensuring sustainable use. Future research related to cultivation through tissue culture techniques should be conducted to provide an efficient way to produce plant material without overharvesting from the natural population. Identifying lead compounds from *A. liliifolia* can open new opportunities for synthetic biology or biotechnological approaches, enabling large-scale production of bioactive compounds without relying on wild sources. Further research-related pharmacological studies are needed to validate its application in modern therapeutics. These efforts will support both scientific progress and the responsible utilization of plant-based utilization.

Acknowledgements

This research process was fully supported by the National Research and Innovation Agency (BRIN) through RIIM Biology Structure Batch I Grant No. 22/II.7/HK/2024/53.3.2.3, and also thank you for the support funding provided by Universitas Gadjah Mada Grant No. 4230/UN1/DITLIT/PT.01.03/2024. Also, part of the research was presented at the 2nd International Conference on Agricultural Nutraceutical and Food Science (ICANFS) on October 2024.

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