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Full Length Article

Isolation of Coumarin Compound Derivatives from the Ethanol Extract of Kesambi (*Schleichera oleosa*) Leaves and their Activity as Anti-Tyrosinase

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Abstract

The skin is the outermost part of the human body, which plays an important role in protecting the body. One of the functions of the skin is to protect the body from exposure to UV rays. Based on these problems, a search was carried out for active secondary metabolites from the ethanol extract of kesambi leaves as inhibitors of the tyrosinase enzyme. In the future, these inhibitors can be developed as active ingredients in skin-lightening cosmetics that are relatively safe and effective. The dried kesambi leaf samples were extracted with ethanol (70% v/v) solvent by maceration. The extract of ethanol obtained was fractionated using radial chromatography. The fraction was then purified and characterized for its chemical structure using ^{1}H-NMR ^{13}C-NMR and Mass Spectroscopy. After being characterized, the isolate was tested for activity inhibiting the tyrosinase enzyme and molecular docking of the tyrosinase protein with the protein code 2Y9X. Based on the results obtained from this study, two types of compounds were isolated from ethyl acetate extract. The analysis using NMR found that the isolates obtained were scopoletin and umbelliferon, with the best activity in inhibiting the tyrosinase enzyme, namely umbelliferon compounds. Based on the results of our study, it can be concluded that in the ethyl acetate extract, there are scopoletin and umbelliferon compounds with good tyrosinase inhibitory activity.

Keywords: Anti-tyrosinase; Kesambi; Scopoletin; Umbelliferon

Introduction

The skin is the outermost part of the human body, which plays an important role in protecting the body. One of the functions of the skin is to protect the body from exposure to UV rays (Nur *et al.* 2023b). Exposure to UV rays continuously in the long term will cause skin hyperpigmentation. Hyperpigmentation is a skin pigment disorder caused by increased melanin production and uneven distribution of melanin. In this condition, it will cause darkening of the skin and the appearance of black spots on certain parts. One way to prevent hyperpigmentation is to inhibit the formation of melanin by inhibiting tyrosinase activity (Pintus *et al.* 2015; Pillaiyar *et al.* 2017; Lukitaningsih *et al.* 2020).

The tyrosinase enzyme is key in melanogenesis, so it can be used as a target to inhibit melanin production. This tyrosinase plays a role in catalyzing two main reactions in melanin biosynthesis, namely the hydroxylation of L-tyrosine to L-dopa and the oxidation of L-dopa to become dopaquinone, then dopaquinone polymerizes to form dopachrome, which then becomes melanin (Bae-Harboe and Park 2012; Otang-Mbeng and Sagbo 2020). Melanin is a pigment that protects the skin from exposure to UV rays. However, abnormalities in melanin production in the skin can cause abnormal skin pigmentation or hyperpigmentation and cause skin aesthetic problems. Hyperpigmentation is a disorder of skin pigment caused by an increase in the process of melanogenesis, causing the darkening of skin color (Pillaiyar *et al.* 2017; Yousef *et al.* 2024).

Tyrosinase enzyme inhibitors can be obtained chemically and naturally, each with certain weaknesses (Qian *et al.* 2020) including hydroquinone and kojic acid.

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The chemical Hydroquinone has the potential to cause dermatitis and skin irritation, while kojic acid is carcinogenic. The use of these two synthetic ingredients has a negative effect on the skin in long-term use (Couteau and Coiffard 2016). Currently, tyrosinase inhibitors derived from natural sources are attracting more attention, especially in cosmetic preparations, because they can inhibit skin hyperpigmentation with the concept of being healthy and safe. Tyrosinase inhibitors derived from natural sources can be obtained from plant secondary metabolites, such as phenolics/flavonoids, triterpenes, or alkaloids (Liu 2022). Flavonoids are polyphenol derivatives widely distributed in plant parts, such as leaves, seeds, skin, roots, and flowers of plants (Zengin *et al.* 2018; Uysal *et al.* 2019; Yılmaz *et al.* 2023). Various derivatives of flavonoids have been identified to protect against ultraviolet radiation (Nur *et al.* 2022; Sulistyowati *et al.* 2023), with the biggest role as a tyrosinase inhibitor. One type of plant that has been reported to be rich in flavonoid phenolic compounds is kesambi (*Schleichera oleosa*).

Kesambi (*Schleichera oleosa*) is a plant from the Sapindaceae tribe which has been reported to have pharmacological benefits and effects. Previous studies have reported that kesambi leaves contain phytochemicals from alkaloids, saponins, phenolics, tannins, flavonoids, and phytosterols. Previous study reported that the ethanol extract of kesambi leaves contained total flavonoid compounds of 62.1 ± 0.98 mg EQ/g extract (Nursamsiar 2021). The total flavonoid content reported in the ethanol extract of kesambi leaves is large enough to play a role in treating various biological problems. The flavonoid component acts as an alternative enzyme substrate because it shows good affinity with the tyrosinase enzyme, so the formation of dopachrome can be prevented. Thus, the components of kesambi leaves are expected to provide the availability of materials or marker compounds to prevent skin pigmentation through their tyrosinase inhibitor activity. Based on these problems, this study will investigate secondary metabolites from the ethanol extract of kesambi leaves as markers for inhibiting tyrosinase activity so that they can be developed as active ingredients for skin-lightening cosmetics that are relatively safe and effective.

Materials and Methods

The chemicals used in this study were n-hexane, ethyl acetate, ethanol (70% v/v), chloroform, and DMSO with an analytical grade from Merck, Germany. Distillate water, L-Dopa (3-(3,4-Dihydroxyphenyl)-L-alanine), and Mushroom Tyrosinase were purchased from Sigma, Aldrich. Samples of kesambi leaves were obtained from Merdekayya Village, Takalar Regency, South Sulawesi, Indonesia (5°17'00.6"S 119°27'01.5" E). Specimens of kesambi plants have been determined at the Biology Research Center, Herbarium Bogoriense, National Research and Innovation Agency (BRIN), with specimen number B-132-DI-05.07.

Sample preparation

Kesambi leaves that had been collected as much as 2 kg were sorted wet, and then the samples were washed using running water. Samples that had been washed clean and drained were then carried out by the slicing process, then dried by placing them in an oven at 50˚C for 2 days. After drying, dry sorting was then carried out. The sample was then sieved using a 40 mesh sieve, and 500 g of kesambi leaf dry sample was obtained, and then the extraction process was carried out.

Extraction

The dry sample (500 g) was extracted using 5 L of ethanol (70% v/v) using the maceration method. The dry sample was put into the maceration container then enough solvent was added for the wetting process. The remaining solvent was added until all the dry sample was completely submerged and left in a place protected from sunlight for 3 days while stirring occasionally. The filtrate was filtered, and the residue was macerated again (re-maceration) with the same solvent as before. The filtrate was collected and evaporated using a rotary evaporator to obtain a thick extract.

Chlorophyll removal

The thick ethanol extract of Kesambi leaves was followed by separating the chlorophyll by weighing 10 g of the extract, then dissolving it in 100 mL of distilled water and stirring for 1 h at 50˚C. Then the extract was filtered while still hot, and the water fraction was concentrated using a rotary evaporator and continued with ovens. The weight of the water fraction obtained was 2.1857 g. The water fraction obtained was then purified by the radial chromatography method.

Separation by radial chromatography

Before radial chromatography separation (RC), the eluent's orientation was first carried out to see the best separation. The first eluent used n-hexane: ethyl acetate (5:5), the second eluent used n-hexane: ethyl acetate (6:4), and the third eluent used n-hexane: ethyl acetate: chloroform (5:3:2). The best eluent was obtained from the third eluent, namely n-hexane: ethyl acetate: chloroform (5:3:2), so this eluent was used in the separation process using RC. The stationary phase is silica gel, while the mobile phase is a mixture of organic solvents (eluents). The separation of compounds was monitored using a UV 366 lamp. Pure compounds were characterized by the results of separation that appear as a single spot-on observation with TLC using mobile phase n-hexane: ethyl acetate: chloroform in the ratio (5:3:2).

Characterization of compounds

The isolates resulting from separation and purification by

radial chromatography were further characterized using Nuclear Magnetic Resonance (NMR) to determine protons (^{1}H-NMR) and carbon (^{13}C-NMR) and the fragmentation of compounds by mass spectroscopy (MS) (Manivannan 2016).

Molecular docking

In silico analysis was carried out by molecular docking method using 2X9Y target protein (Tyrosinase). First, the target protein and native ligand were downloaded *via* the protein data bank (https://www.rcsb.org/). The 2X9Y protein was separated with a native ligand and then prepared according to the protocol from (Nursamsiar *et al.* 2020; Nur *et al.* 2023a).

Tyrosinase inhibitory activity

Testing the activity of inhibiting the tyrosinase enzyme was done using the method (Nur *et al.* 2017) with a few modifications. The anti-tyrosinase activity was carried out using mushroom tyrosinase as an enzyme, L-Dopa as a substrate, kojic acid as a positive control, and extracts and isolates from kesambi leaves. 40 μL of each sample with a certain concentration was put into well-plate 96, which previously contained phosphate buffer solution (0.1 *M*, pH 6.8), then added 40 μL (4 m*M*) of L-Dopa solution and then incubated (25°C for 10 min). Then 40 μL of tyrosinase enzyme solution (75 units/mL) was added and incubated (25°C for 30 min). After that, the absorbance was measured using a UV-Vis spectrophotometer at λ 490 nm. Measurements were also made on the blank and positive control of kojic acid. The inhibitory activity of the tyrosinase enzyme was expressed in the IC_{50} value (Pintus *et al.* 2015; Saleem *et al.* 2021).

Results

Extraction process and chlorophyll removal

The extraction of dried Kesambi leaves with 70% (v/v) ethanol solvent yielded 21.4%. These results indicate that the solvent and extraction method effectively withdraw the compounds in the Kesambi leaves. The solvent used affects the number of active compounds contained in the extract. According to the concept of like dissolves like, polar compounds will dissolve in polar solvents, and non-polar compounds will dissolve in non-polar solvents (Nur *et al.* 2022, 2021). Based on the yield results obtained, it shows that most of the compounds from Kesambi leaves are soluble in polar solvents.

The ethanol extract of kesambi leaves was identified to contain chlorophyll which could interfere with separating the active compounds. In this study, the chlorophyll compound contained in the kesambi ethanol extract was separated by dissolving the extract in distilled water which was stirred at a temperature of 50°C and then extracted liquid-liquid using ethyl acetate solvent to separate the active components and chlorophyll compounds bound to the compound. To ensure the clean chlorophyll extract was identified by TLC, which was observed under 366 nm UV light. Fig. 1 shows extracts free of chlorophyll and compared with extracts without experiencing chlorophyll separation. The results of the chlorophyll separation showed that the residue (A) and the unseparated extract (C) had similar profiles, and there was chlorophyll with a red spot on UV 366. Different results were obtained from extracts (B) that had gone through a separation process which showed the presence of active compounds which has been cleaned of chlorophyll. In the process of separating chlorophyll from the extract, ethyl acetate solvent is used. This is because chlorophyll compounds tend to be attracted to semipolar solvents such as ethyl acetate. The procedure for removing chlorophyll compounds from natural product extracts has been reviewed by Kim *et al.* (2020).

Separation by radial chromatography

Fractionation of compounds from kesambi ethanol extract was carried out using radial chromatography and separated using the mobile phase n-hexane: ethyl acetate: Chloroform in the ratio (5:3:2). The TLC results from the separation of compounds by radial chromatography are shown in Fig. 2. Fig. 2 shows the TLC results from the separation of compounds by using radial chromatography, where several fractions with the same stain pattern are combined. A single spot pattern was shown by fractions 4,5,6 and 7 for compound one and 11, 12, 13, 14 and 15 for compound 2. The TLC results of the two compounds are shown in Fig. 3.

Characterization of isolated compounds

Umbelliferon compound: Yellowish white crystalline powder with NMR characterized: ^{1}H-NMR (500 MHz, DMSO d_6) δ ppm 6.20 (*d*, 9.5 Hz, 1H, H-3), 7.93 (*d*, 9.5 Hz, 1H, H-4), 7.53 (*d*, 8.5 Hz, 1H, H-5), 6.78 (*dd*, 8.5 Hz & 2.8 Hz, 1H, H-6), 6.71 (*d*, 2 Hz, 1H, H-8). ^{13}C-NMR (100 MHz, DMSO d_6) δ ppm 160.9 (C-2), 112.8 (C-3), 143.8 (C-4), 112.3 (C-4'), 130.5 (C-5), 113.5 (C-6), 158.2 (C-7), 102.7 (C-8), and 157.2 (C-8'). *m/z* $[M-H]^-$ 191.07 with molecule formula $C_{10}H_7O_4$. The NMR spectrum of compound 1 can be seen in Fig. 4.

Scopoletin compound: White crystalline powder with NMR characterized: ^{1}H-NMR (500 MHz, DMSO d_6) δ ppm 6.15 (*d*, 9.2, 1H, H-3), 7.85 (*d*, 9.2, 1H, H-4), 7.11 (*s*, 1H, H-5), 6.77 (*s*, 1H, H-8), 3.90 (*s*, 3H, H-10). ^{13}C-NMR (100 MHz, DMSO d_6) δ ppm 164.3 (C-2), 113.0 (C-3), 146.1 (C-4), 109.9 (C-5), 147.3 (C-6), 151.4 (C-7), 104.9 (C-8), 152.9 (C-9), 112.5 (C-10), and 56.8 (C-6-OMe). *m/z* $[M-H]^-$ 161,24 m/z with molecule formula $C_9H_5O_3$. with molecule formula $C_{10}H_7O_4$. The NMR spectrum of compound 1 can be seen in Fig. 5.

Molecular docking interaction

The inhibition capacity of the umbelliferone compound with scopoletin was also confirmed through a molecular docking approach to the tyrosinase target protein (PDB id: 2Y9X). Before re-docking the ligand molecule to the target protein, a validation analysis was conducted to determine the bond distance between the ligand and the protein < 2 Å, calculated based on the Root Mean Standard Deviation (RMSD). The results showed that the RMSD value of re-docking the native ligand in the tyrosinase protein pocket was 1.908 Å. An RMSD value of < 2 Å indicates the closeness of the test results to the crystallographic results of the native ligand so that the docking method used is valid and meets measurement suitability (Adianingsih *et al.* 2022; Nur *et al.* 2023a). The results of the molecular docking analysis between the umbelliferone and scopoletin compounds for the 2Y9X protein can be seen in Fig. 6.

The analysis showed that the umbelliferone and scopoletin compounds had more negative bond-free energy values of -5.99 and -5.84 kcal/mol, respectively, compared to the native ligand (-4.45 kcal/mol). Predicted inhibition constants (IC) of native ligand, umbelliferone, and scopoletin were 542.7, 40.54 and 52.67 uμM, respectively. These results indicate that the umbelliferone compound has a stronger inhibiting ability than scopoletin. This can be seen from the bond-free energy of the umbelliferone compound, which is more negative than scopoletin. In addition, the amino acid residues that interact with hydrogen, namely the ASN260 amino acid in the umbelliferone compound, are similar to the interactions in the native ligand.

Anti-tyrosinase activity

The umbelliferone and scopoletin compounds were evaluated for their bioactivity as antityrosinase *in vitro* and compared to ethanol extract of kesambi leaves. The results of activity testing can be seen in Table 1. The results of the anti-tyrosinase activity test showed that the umbelliferone compound had very strong activity in inhibiting the action of the tyrosinase enzyme with an IC_{50} value of 18.55 µg/mL. This differs from the scopoletin compound, which exhibits weak inhibitory activity on tyrosinase with an IC_{50} value of > 200 μg/mL. The tyrosinase inhibitory activity of the umbelliferone compound was also stronger than the ethanol extract of kesambi leaves. The ethanol extract of kesambi leaves obtained an IC_{50} value of 141.42 μg/mL, categorized as medium activity (Table 1).

Discussion

This research was conducted to study active secondary metabolites that could be developed as anti-melanocytes through *in vitro* and silico tyrosinase inhibition approaches. Melanocytes are skin disorders due to hyperpigmentation which can be a serious problem. Hyperpigmentation occurs due to the accumulation of melanin in the cells.

Table 1: The anti-tyrosinase activity of ethanol extract and compounds isolated from kesambi leaves

Concentration (μg/mL)	Inhibition (%) of Sample		
	Ethanol extract	Umbelliferone	Scopoletin
1.5625	18.6 ± 2.08	4.94 ± 1.38	7.48 ± 1.39
3.125	27.7 ± 1.59	9.52 ± 4.12	8.44 ± 0.78
6.25	35.2 ± 3.79	9.26 ±0.50	8.22 ± 1.13
12.5	47.3 ± 3.05	19.21 ± 7.15	10.14 ± 0.41
25	59.7 ± 2.64	27.14 ± 6.80	12.49 ± 1.16
50	71.2 ± 1.55	39.32 ± 3.70	16.99 ± 0.34
100	80.2 ± 0.91	80.33 ± 3.72	24.06 ± 2.21
200	86.1 ± 2.35	97.93 ± 0.40	34.47 ± 3.36
IC_{50} (μg/mL)	141.42	18.55	> 200

Fig. 1: TLC profile of the extract that has been cleaned up from chlorophyll. (**A**) Residue, (**B**) extract with chlorophyll separation, (**C**) without chlorophyll separation

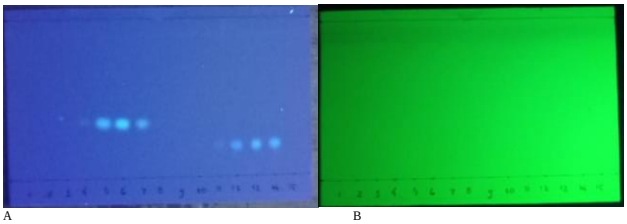

Fig. 2: TLC results from separating compounds using radial chromatography; mobile phase n-hexane: ethyl acetate: chloroform (5:3:2). Fig. 2a is a visualization under UV 366 and 2b at UV 254. On UV366, it is visualized for candidate compounds 1 and 2

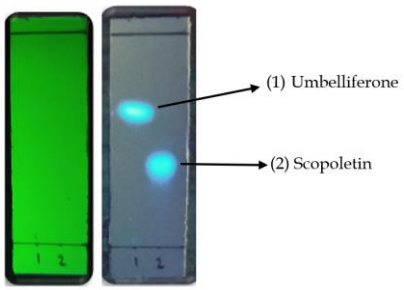

Fig. 3: TLC results of compounds 1 (umbelliferone) and 2 (scopoletin), the mobile phase of n-hexane: ethyl acetate (5:5)

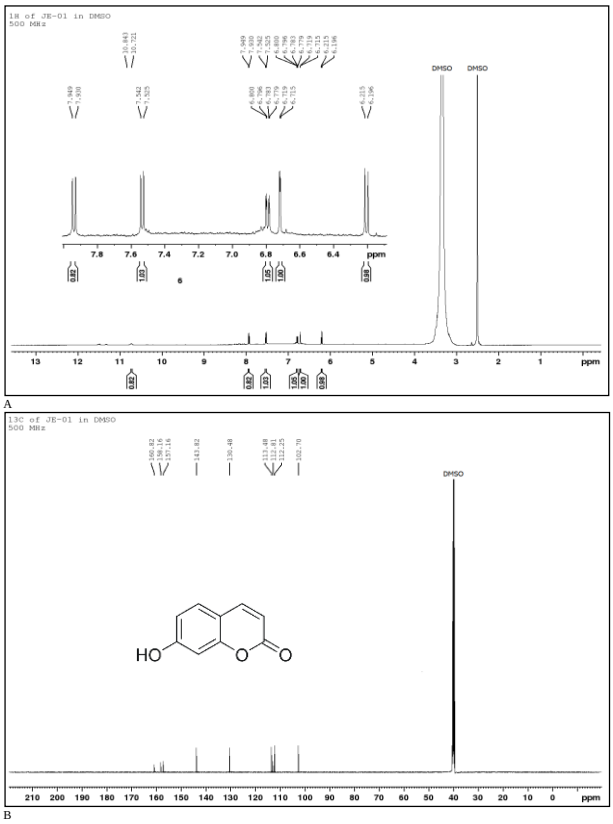

Fig. 4: H-NMR (4A) and C-NMR (4B) spectrum of compound 1

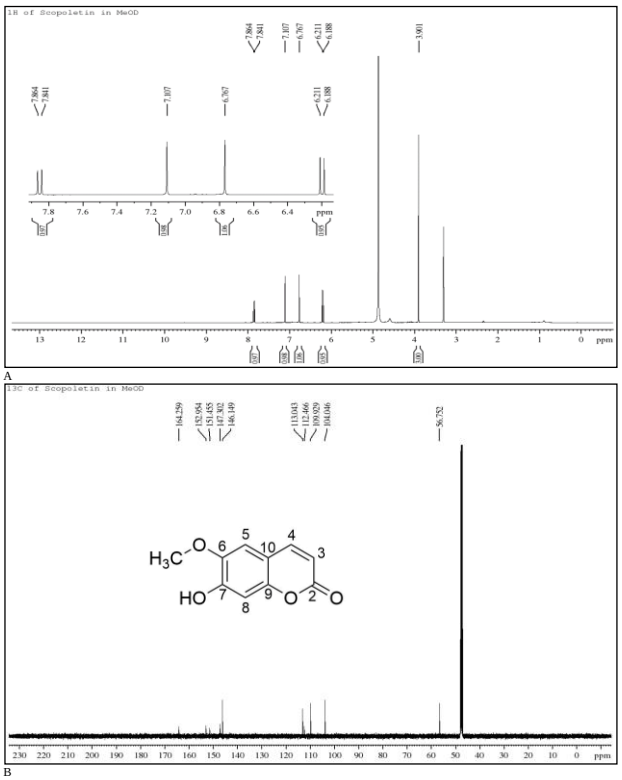

Fig. 5: H-NMR (4A) and C-NMR (4B) spectrum of compound 2

Melanin pigment or melanogenesis formation is the most important photoprotective response to ultraviolet radiation from the sun and carcinogenesis (Pintus *et al.* 2015; Li *et al.* 2023). So the loss of melanin and abnormal depigmentation can be serious facial aesthetic and dermatological problems in humans. Conversely, increased melanin synthesis and accumulation of excess pigment, known as hyperpigmentation, can also cause various skin disorders, including melasma (Couteau and Coiffard 2016; Zolghadri *et al.* 2019).

Melanin biosynthesis in cells involves a central or rate-limiting enzyme, namely tyrosinase. Melanin biosynthesis can be reduced by inhibitor compounds inhibiting rate-limiting enzyme activity in the melanogenesis metabolic pathway (Zolghadri *et al.* 2019; Bursal *et al.* 2021). Compounds commonly used to regulate tyrosinase activity or tyrosinase inhibitors include kojic acid, hydroquinone, arbutin, and mercury. These compounds are compounds with the greatest inhibitory activity as antimelanosic. However, kojic acid, hydroquinone, mercury, and arbutin are known to be carcinogenic, so their use is limited and even prohibited in cosmetic preparations (Sarkar *et al.* 2013; Boo 2021). Therefore, searching for compounds from natural materials, including kesambi leaves, is an alternative for exploring anti-melanocyte active ingredients.

This study found two secondary metabolites, coumarin derivatives, identified using the proton and carbon NMR approach (^{1}H-NMR and ^{13}C-NMR). In compound 1, based on the 1H NMR spectrum, it is known that there are five Sp^2 protons consisting of a methine Sp^2 group (CH-Sp^2) which is known based on the peaks that appear at chemical shifts of 6–7 ppm. H3 and H4 are doublet multiplicity with a coupling constant (*J*) of 9.5, indicating they are bound to each other or are called protons that are neighbors with the ortho position. In H6, H5, and H8, the double doublet (*dd*) multiplicity with chemical constants of 8.5 and 2.8 indicates that they are ortho and meta neighbors. The ^{13}C-NMR spectrum data showed nine signal peaks at the δC chemical shift of 100–200 ppm, which is the type of Csp^2 bond. One of the peaks is at the chemical shift δC 160–170 ppm, indicating the presence of a C = O bond. One of the peaks is at the chemical shift δC 150–160 ppm, indicating the presence of the ArC-O bond (Fig. 4). From the ^{13}C and ^{1}H spectra results, it can be estimated that isolate 1 is an Umbelliferone compound from the coumarin group with the IUPAC name of 7-Hydroxycoumarine. This is supported based on the results of MS analysis. Based on the results of the MS isolate, compound JE-01 has m/z [M-H]- with the molecular formula $C_9H_5O_3$ is 161.24 (Fig. 7). Then m/z for [M] is 162.24 with the molecular formula $C_9H_6O_3$. According to Mazimba (2017), Umbelliferon's mass spectral analysis peaked at m/z 162 [M]+.

In compound 2, based on the proton NMR spectrum, it is known that there are four *sp2* protons and one *sp3* proton. Four *sp2* protons consist of four CH-*sp2* groups

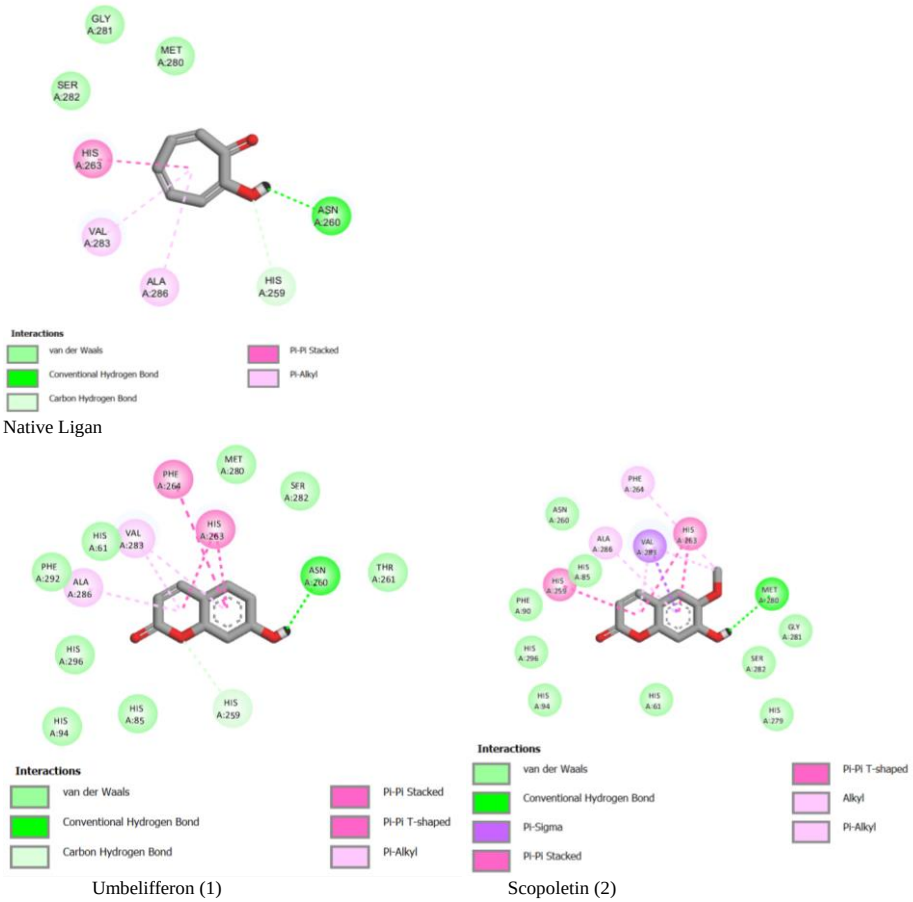

Fig. 6: Results of molecular docking analysis of umbelliferone and scopoletin compounds against the 2Y9X target protein

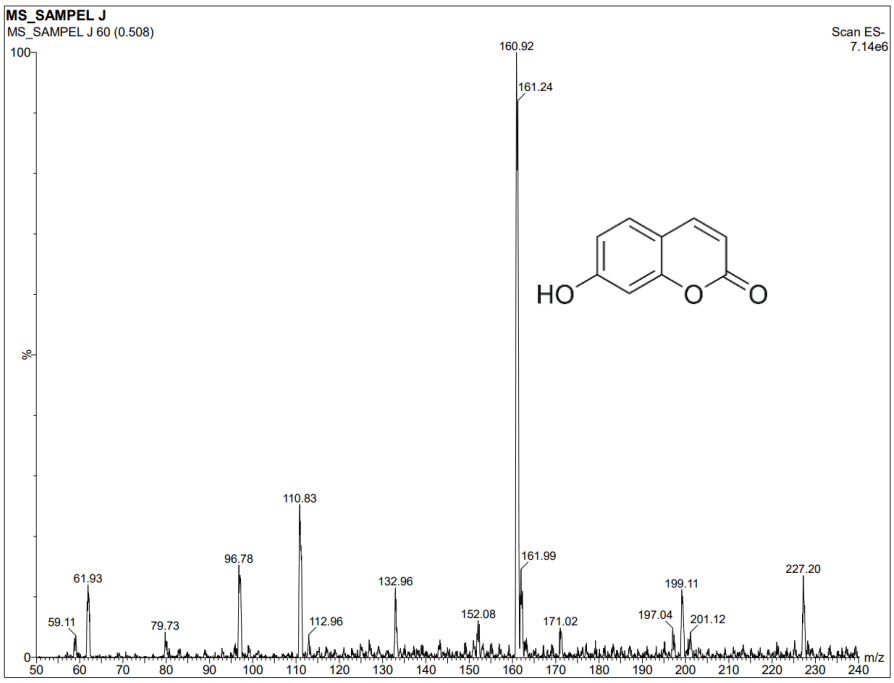

Fig. 7: MS spectrum of compound 1

which are known based on chemical shifts of 6–7 ppm (Bienz *et al.* 2021). Then 1 methyl oxy/methoxy is at a chemical shift of 3–4 ppm. C-3 and C-4 are known to have a multiplicity (*d*) with a coupling constant (*J*) of 9.2 which indicates that C-3 and C-4 are bound to each other. For C-5 and C-8, they are in a para position with each other. Therefore, the spectral peaks of C-5 and C-8 have multiplicity (*s*) or are independent. The group OCH_3 also has a multiplicity (*s*) because it is not tied to anything else. The ^{13}C NMR spectrum shows 10 signal peaks which are divided into two types of bonds, one signal, which is in the chemical shift δC 50–100 ppm, is the type of C*sp3* bond, and 9 signals, which are in the chemical shift δC 100–200 ppm is the type of C*sp2* bond (Fig. 5). From the ^{13}C and ^{1}H spectra results, it can be estimated that isolate 2 is a scopoletin compound from the coumarin group with the IUPAC name 7-hydroxy-6-methoxychromen-2-one. This is supported based on the results of MS analysis. Based on the MS results, the scopoletin compound has m/z [M-H]- with the molecular formula $C_{10}H_7O_4$ of 191.07. Then m/z for [M] is 192.07 with the molecular formula $C_{10}H_8O_4$ (Fig. 8).

In this study, secondary metabolites were obtained as umbelliferone and scopoletin compounds. Both of these compounds belong to the coumarin group, which *in vitro* were known to have anti-melanocyte activity through the tyrosinase inhibition approach. Umbelliferone was identified as having very strong activity with an IC_{50} value of <20 ug/mL equivalent to 1.75 mM. The activity obtained was 8 times more active than in the ethanol extract of kesambi leaves. Meanwhile, the scopoletin compound was found to have weak inhibition with an IC_{50} value of > 200 µg/mL. Umbelliferone and scopoletin are both coumarin derivatives. Structurally, the difference between the two lies in the methoxy group attached to the aromatic carbon at the C6 position, namely scopoletin, whereas in umbelliferon, there is no methoxy group. A methoxy group in the scopoletin compound allows it to inhibit interaction with the tyrosinase enzyme. This makes scopoletin have low solubility, so its permeability is also low. As a result, *in vitro*, the scopoletin compound is less able to inhibit the action of tyrosinase under enzyme pH conditions (Chang and Chiang 1995).

The umbelliferone compound with a concentration of 1.75 m*M in vitro* inhibited tyrosinase activity by up to 50%. The very strong activity of the umbelliferone compound is also supported by its strong *in silico* interaction with target proteins. The umbelliferone compound has the most negative bond-free energy value of -5.99 kcal/mol (Ki = 40.54 *µM*). There is a hydrophilic interaction on the ASN260 amino acid residue of protein tyrosinase (2Y9X) with the -OH group of umbelliferone and has similarities to the binding pocket interaction of the native ligand (Fig. 6). Support for phi and phi-alkyl interactions on the HIS263 and PHE264 amino acid residues which provide bond stability so that the interaction occurs properly between umbelliferone ligands and proteins. Several previous studies

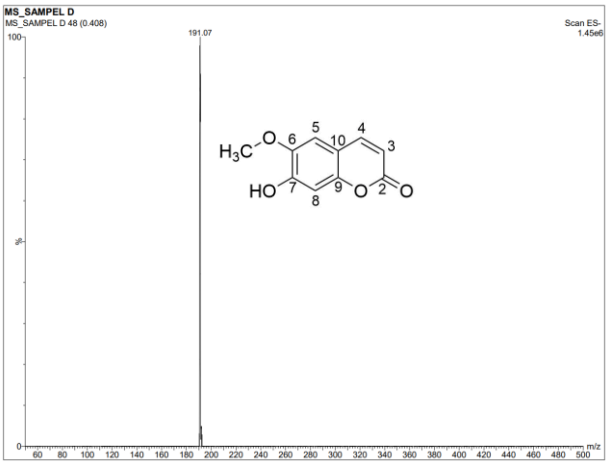

Fig. 8: MS spectrum of compound 2

stated that the histidine amino acid residue (HIS263) is the catalytic site of tyrosinase. Hydrophilic interactions and interactions with tyrosinase catalytic sites between umbelliferone to protein strongly inhibit tyrosinase (Nur *et al.* 2023a). *In silico*, the scopoletin compound (-5.84 kcal/mol) also has a binding effect on protein tyrosinase which is more negative than the native ligand (-4.65 kcal/mol). But *in vitro*, it gives weak activity (IC_{50} >200 ug/mL). The steric effect of the adjacent methoxy on the -OH group of the scopoletin compound allows it to have a role in the instability of the interaction between the ligand and the active site of tyrosinase (Fig. 6). So that this can lead to a reduced inhibitory effect (Nursamsiar *et al.* 2022; Nur *et al.* 2023b).

This study provides information that compounds isolated from kesambi leaves include umbelliferone *in vitro* and in silico, providing a very strong inhibition of tyrosinase. This allows the development of the umbelliferone compound as a marker from kesambi leaf extract so that it can be used as an anti-tyrosinase candidate in future research. The findings of this study have been supported by previous research data (Fais *et al.* 2009; Ashraf *et al.* 2015; Lu *et al.* 2023) which showed that several coumarin derivatives such as umbelliferon were identified as having the ability to act as tyrosinase inhibitors.

Conclusion

From Kesambi's leaves ethanol extract, the compounds scopoletin and umbelliferon, which are coumarin derivatives, were successfully isolated. The umbelliferone compound was identified as having strong antityrosinase activity. Therefore, the umbelliferone compound can be used as a marker for future research development.

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Author Contributions

NS: Conceptualization and data analysis, FJS: Planned experimental, Methodology and Characterization, HK: Methodology and Interpretation of the research, MW: Experimental and data analysis, and SN: Data interpretation and Manuscript preparation.

Conflicts of Interest

The authors declare that they are not aware of any competing financial interests or personal relationships that might appear to have influenced the work described in this article.

Data Availability

Data presented in this study will be available at the corresponding author's fair request.

Ethics Approval

Not applicable to this paper

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