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Original Article

EXPLORING ANTI-TYROSINASE AND PHOTOPROTECTIVE ACTIVITIES OF *Curcuma heyneana* (Val.) AND *Kaempferia galanga* (L.) ESSENTIAL OILS: IN VITRO AND IN SILICO APPROACHES

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ABSTRACT

The essential oils of *Curcuma heyneana* and *Kaempferia galanga* are known for their antioxidant, anti-inflammatory, and antibacterial biological activities. However, studies of their anti-tyrosinase activity and photoprotective effects are still limited. This study was conducted to identify metabolite profiles and assess the anti-tyrosinase activity and photoprotective effects of *C. heyneana* and *K. galanga* essential oils through *in vitro* and *in silico* approaches. The essential oils were extracted using hydrodistillation. The metabolite profiles of both essential oils were identified using GC-MS techniques. The major compounds of *K. galanga* essential oil are *trans*-ethylcinnamate, ethyl-*p*-methoxycinnamate, and borneol. The major compounds of *C. heyneana* essential oil are epicurzerenone, camphor, eucalyptol, camphene, and germacrone. The IC_{50} values of tyrosinase enzyme inhibition activity of *K. galanga* and *C. heyneana* essential oils were 3.991 and 6.423 mg/mL, respectively. The SPF values of *K. galanga* and *C. heyneana* essential oils were 39.77 (ultra category) and 5.99 (medium category), respectively. The *trans*-ethylcinnamate and ethyl-*p*-methoxycinnamate compounds are thought to be bioactive compounds that inhibit tyrosinase enzyme activity in *K. galanga* essential oil because they have a high affinity or docking score of -5.9 kcal/mol and -5.8 kcal/mol, respectively, and bind to the active site of tyrosinase. Germacrone has good affinity (-5.8 kcal/mol) but only interacts with residue VAL283, possibly forming a non-specific bond. The conclusion is that the essential oil of fresh rhizomes of *K. galanga* has more potential to be used as an active ingredient for brightening and skin protection.

KEYWORDS: *Curcuma heyneana*, *Kaempferia galanga*, Essential oil, Tyrosinase inhibition, Photoprotective.

1. Introduction

The skin is a protective organ of the body from the external environment. This primary function often exposes the skin to ultraviolet radiation, especially UVA and UVB from sunlight [1]. Skin being steadily exposed to the sun can result in photoaging [2]. Photoaging is skin aging caused by sun exposure and indicated by wrinkles, sagging skin, and hyperpigmentation [3].

Hyperpigmentation is a dermatological condition with a darkening of the skin color, such as melasma, post-inflammatory hyperpigmentation, lentigines, and ephelides. It occurs due to the formation of large amounts of melanin [4]. UVB radiation increases melanin production through the melanocortin-1 receptor (MC1R)/ pro-opiomelanocortin (POMC)/ cyclic adenosine monophosphate (cAMP)/ cAMP-response element binding protein (CREB) pathway [5]. This process involves tyrosinase as a key enzyme in melanin synthesis [6]. Therefore, inhibition of tyrosinase enzyme activity and measuring the Sun Protection Factor (SPF) value are the best strategies in developing active ingredients that brighten and protect the skin from UV radiation [7].

The currently used synthetic brightening and sunscreen active ingredients have the side effects of irritation and potential carcinogenicity [8]. This encourages the exploration of natural-based alternative active ingredients that are safer. Essential oils are one of the most widely researched natural ingredients for the treatment of skin issues, due to their bioactivities such as antioxidant, anti-inflammatory, and antibacterial properties [9]. Essential oils are complex combinations of volatile and aromatic compounds, such as terpenoids, phenolics, aldehydes, esters, ethers, ketones, and hydrocarbons [10][11]. The compounds are reported to

have antioxidant, anti-inflammatory, anti-aging, and antibacterial activities [12][13].

Curcuma heyneana and *Kaempferia galanga* of the *Zingiberaceae* family are plants that produce essential oils, mostly containing terpenoids and phenolic compounds [14]. Some essential oils from the *Zingiberaceae* family, such as *Curcuma mangga*, *Boesenbergia rotunda*, and *Zingiber purpureum* rhizome, have antioxidant and anti-inflammatory properties that can help inhibit the signs of aging[15].

Until now, research on exploring anti-tyrosinase and photoprotective activities of *C. heyneana* and *K. galanga* essential oils is still limited. Thus, this study aims to identify the metabolite profiles of *C. heyneana* and *K. galanga* essential oils using the GC-MS technique, evaluate the anti-tyrosinase activity through the tyrosinase enzyme inhibition test, the photoprotective effect through the SPF test, and determine the molecular interaction of major compounds on tyrosinase with the *in silico* approach.

2. Materials and Methods

2.1. Plan collection and identification

Fresh rhizomes of *C. heyneana* and *K. galanga* were each harvested as much as 10 kg from community gardens in August 2024 in West Sumatra province, Indonesia. Plant identification was carried out at the ANDA herbarium, Universitas Andalas. Specimen numbers of *C. heyneana*: K-ID711, *K. galanga*: K-ID303. The rhizomes of both plants are shown in Figure 1.

2.2. Essential oils extraction

The essential oil was obtained by hydrodistillation. Fresh rhizomes were washed and dried at room temperature. The rhizomes were then chopped to a thickness of 2-3 mm. A sample of 9.7 kg and 9.8 kg of *C. heyneana* and *K. galanga* rhizomes, respectively, was placed into a distillation flask equipped with a Clevenger apparatus. The distillation time was six hours. The essential oils obtained were added to anhydrous sodium sulfate to remove the remaining water. The essential oils were stored at 4 °C until used.

2.3. Physical characterization

Physical analysis

Physical analysis of essential oils includes appearance, color, smell, and yield percentage. The yield percentage of essential oils is calculated using the following formula [16]:

$$Yield\ (\%\ v/w) = \frac{Amount\ of\ oil\ (mL)}{Weight\ of\ plant\ material\ (g)} x\ 100$$

Specific gravity

Specific gravity is determined using a pycnometer Iwaki®. Before using it, the interior of the pycnometer must be thoroughly cleaned and dried. Weigh the empty pycnometer on an analytical balance and record its weight. The pycnometer is filled with distilled water, and its weight is recorded. The pycnometer is filled with the essential oil, and its weight is recorded. The specific gravity value can be calculated using the following formula [16]:

$$Specific\ gravity\ (g/mL) = \frac{w1 - w}{w2 - w}$$

Where: w = weight of empty pycnometer; w1 = weight of pycnometer containing essential oil; w2 = weight of pycnometer containing distilled water.

Refractive index

The refractive index is measured using an Abbe Refractometer®. Before measuring the refractive index, the prism is cleaned with alcohol moistened on a cotton swab, and the ambient temperature is recorded. Essential oil is dripped 1-2 drops on the lower prism and then covered. Adjust the prism light so the light and dark sides can be seen in the eyepiece lens. The refractive index value is read and recorded [17].

2.4. Analysis of essential oil by GC-MS

The constituents from the essential oil of *C. heyneana* and *K. galanga* were identified using GC-MS, with conditions analyzed in Table 1. Identification of constituents matched with the NIST14 library database on the GC-MS system with a quality value >90%, matched with literature, and the NIST Chemistry WebBook. The percentage relative is the percentage of the area of the total area.

2.5. Tyrosinase enzyme inhibition assay

The tyrosinase enzyme used was from fungal tyrosinase (Sigma-Aldrich® T3824-25KU). L-DOPA was used as a substrate, and kojic acid was used as a positive control or standard. Test solutions of each essential oil were prepared from a concentration range of 0.625 to 20 mg/mL using ethanol p.a. The testing was conducted using a 96-well plate. The total mixture volume was 200 µL, which included 80 µL of phosphate buffer (50mM, pH 6.5), 40 µL of tyrosinase enzyme, 40 µL of the test solution (or kojic acid), and 40 µL of L-DOPA. The dopachrome formation was measured with a Biorad® Microplate Reader. The experiments were performed in triplicate, and the percentage of inhibition and IC_{50} values were calculated.

2.6. Sun Protection Factor (SPF) determination

The test solution of 300 ppm was prepared from a 1000 ppm stock solution using ethanol p.a, and kojic acid 300 ppm as a positive control. Ethanol p.a was used as a blank. Absorbance measurement was conducted using a Shimadzu® UV-1800 spectrophotometer. The absorbance of each test and control solution was measured throughout the UVB range 290-320 nm at 5 nm intervals. All measurements were in triple repetition. SPF values were calculated according to Mansur's equation in the publication Sayre [18]:

$$SPF = CF\ x \sum_{\lambda=290}^{320} EE(\lambda)\ x\ I(\lambda)\ x\ Abs(\lambda), \quad \Delta\lambda = 5\ nm$$

Where: CF = correction factor = 10; EE = erythemal effect spectrum; I = solar intensity spectrum; Abs = sample absorbance. The EE (λ) x I (λ) values were obtained from reference data published by Sayre [18].

The calculation of UV resistance based on SPF values refers to the provisions in Regulation of the Indonesian Food and Drug Administration (Badan Pengawasan Obat dan Makanan, BPOM: No.30/2020) [19]:

$$Resistance\ (minute) = 10\ x\ SPF\ value$$

Table 1. The GC-MS condition

Specification	Information
Chromatography-Mass Spectrometry (GC-MS) system	
Instrument	GC (Agilent®) 7890A
Detector	MS (Agilent®) 5975C
Column	HP-5MS (Agilent®)
Gas carrier	Helium
GC condition	
Inlet	
Heaters	250°C
Pressure	11.7 psi
Split ratio	200: 1
Split flow	240 mL/minute
Volume of injection	1 µL
Column	
Speed genre gas	1.2 mL/minute
Pressure	11.7 psi
Oven	
Temperature column	The temperature used is a gradient of the total time of 45.5 minutes. At 80°C for 1 minute, 80-110°C at 2°C/minute, 110-140°C at 3°C/minute, 140-170°C at 4°C/minute, 170-200°C at 5°C/minute.
Aux heaters	
Temperature	280°C
MS condition	
MS source	230°C
MS quad	150°C
Tune type	EI
Tune EMV	1247

2.7. In silico (molecular docking simulation)

The *in silico* study was used to investigate the binding interactions between the major compounds from essential oils of *C. heyneana* and *K. galanga* with tyrosinase (PDB ID:2Y9X). The 3D structure of mushroom tyrosinase was downloaded from the Protein Data Bank.

The segregation between receptor and Tropolone as a native ligand was performed using Biovia Discovery Studio 2021. Preparation of the receptor and Tropolone was carried out using AutoDockTools 1.5.7 software. This process involves deleting water, adding hydrogen, adding a Gasteiger charge, and adding a hydrogen merge to non-polar.

The compounds tested in this study are all major compounds from each essential oil. The 3D structure of the test ligand was downloaded from the PubChem database in SDF format, then converted into PDB format using Biovia Discovery Studio 2021. The molecular preparation included optimizing ligand (tested compounds) geometry and applying the MMFF94 force field using Avogadro software. The molecular docking protocol was validated by superimposing the ligand co-crystal (Tropolone) with the docked ligand. The docking simulation was performed using AutoDockVina 1.1.2 software. Grid center: x = -9.985, y = -28.541, z = -43.490. Grid size: x = 30, y = 30, z = 30. Receptor-ligand binding interaction was determined based on binding affinity energy (kcal/mol). Visualizing interactions using the Discovery Studio Visualizer.

3. Results

3.1. Physical characteristics of essential oils

The results of the physical characteristics of *C. heyneana* and *K. galanga* essential oils are summarized in Table 2. The hydrodistillation method extracted a colorless essential oil from *K. galanga*, yielding 0.69% v/w. This is more than *C. heyneana* oil, which is only 0.29% v/w, brownish colored. Both essential oils have a unique and intense aroma. The physical form of the essential oils can be seen in Figure 1.

3.2. Metabolite profile of essential oils

The GC-MS spectrum of *C. heyneana* and *K. galanga* essential oil can be seen in Figure 2. The chemical compounds are detailed in Table 3. According to the analysis results, 26 compounds accounted for approximately 86.56% of the total composition of *C. heyneana* essential oil. Most of them are composed of oxygenated monoterpenes (39.58%) and oxygenated sesquiterpenes (31.78%), the rest are monoterpene hydrocarbons (6.87%), sesquiterpene hydrocarbons (3.13%), and others (5.20%). Major compounds are epicurzerenone (25.74%), camphor (25.47%), eucalyptol (9.25%), camphene (3.78%), and germacrone (3.70%). On the essential oil of *K. galanga*, 19 compounds were obtained, which accounted for 99.99% of the total oil composition. These consisted of phenolics (84.68%), monoterpenes hydrocarbons (10.34%), and oxygenated monoterpenes (4.50%). The major compounds are *trans*-ethylcinnamate (46.14%), ethyl-*p*-methoxycinnamate (37.85%), and berneol (3.76%). The structures of these major compounds are shown in Figure 3.

3.3. Anti-tyrosinase activity

The enzyme tyrosinase inhibitory activity is presented in Table 4. The results indicate that *K. galanga* essential oil has a lower IC_{50} value of 3.991 mg/mL, compared to the IC_{50} value of *C. heyneana* essential oil, which is 6.423 mg/mL. The results of this study indicate that *K. galanga* essential oil has substantial activity in inhibiting the tyrosinase enzyme. However, when compared with kojic acid as a standard or positive control, these two essential oils are still classified as weak.

3.4. Photoprotective effects

The SPF values of *K. galanga* and *C. heyneana* essential oils are presented in Table 5. *K. galanga* essential oil showed the highest photoprotective activity with an SPF value of 39.77, including in the ultra category, with a resistance to UV radiation for 397.70 minutes, equivalent to 6.6 hours.

This value indicates that *K. galanga* essential oil provides a potent photoprotective effect compared to *C. heyneana* essential oil, which is only able to withstand UV radiation for 59.93 minutes with an SPF value of 5.99, and better than kojic acid as a positive control with an SPF value of 12.95, which can withstand UV radiation for

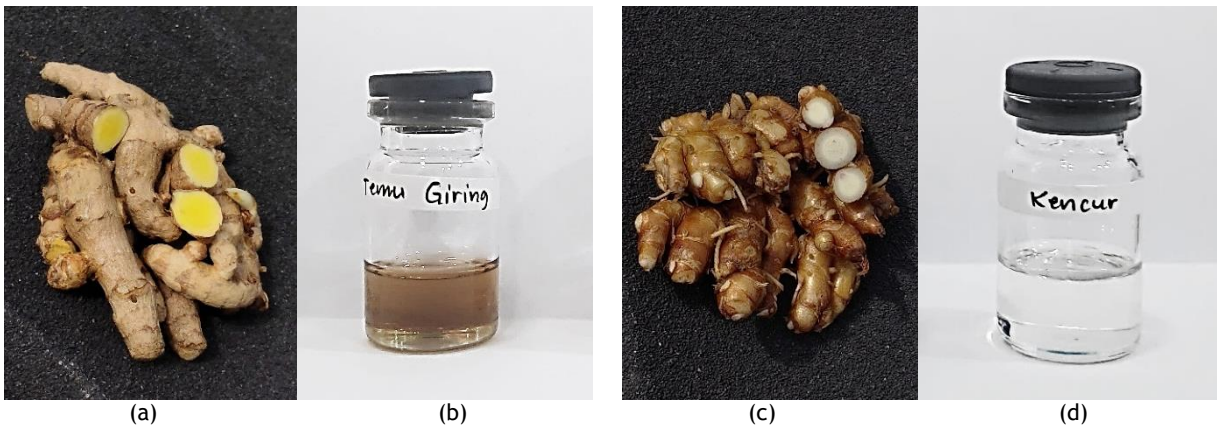
129.53 minutes or 2.2 hours.

3.5. *In silico* evaluation

Validation of the docking method obtained an RMSD value of 2.9 Å. The structure of the native ligand (Tropolone), the tyrosinase receptor, and the superimposition of Tropolone with the docked ligand are shown in Figure 4. The docking simulation scores of the major compounds from the essential oils of *C. heyneana* and *K. galanga* are presented in Table 6. Visualization of the binding interaction can be viewed in Figure 5. The molecular docking simulation results indicate that *trans*-ethylcinnamate and ethyl-*p*-methoxycinnamate compounds have the highest affinity with docking scores of -5.9 and -5.8 kcal/mol, respectively, and are closest to Tropolone -6.0 kcal/mol, interacting with residues HIS263, VAL283, and ALA286, which are also found in Tropolone (native ligand) and kojic acid (standard). Germacrone also has a good affinity of -5.8 kcal/mol, but only interacts with the VAL283 residue.

Table 2. Physical characterization of essential oils of *C. heyneana* and *K. galanga*

Spesies	Part of plant	Yield (% v/w)	Colour	Specific gravity (g/mL)	Refractive index (at 25 °C)
Curcuma heyneana Val.	Fresh rhizome	0.29	Brownish	0.9864	1.4964
Kaempferia galanga L.	Fresh rhizome	0.69	Colorless	1.0304	1.5488



(a) (b) (c) (d)

Fig 1. (a) Rhizome of *C. heyneana*, (b) Essential oil of *C. heyneana*, (c) Rhizome of *K. galanga*, and (d) Essential oil of *K. galanga*

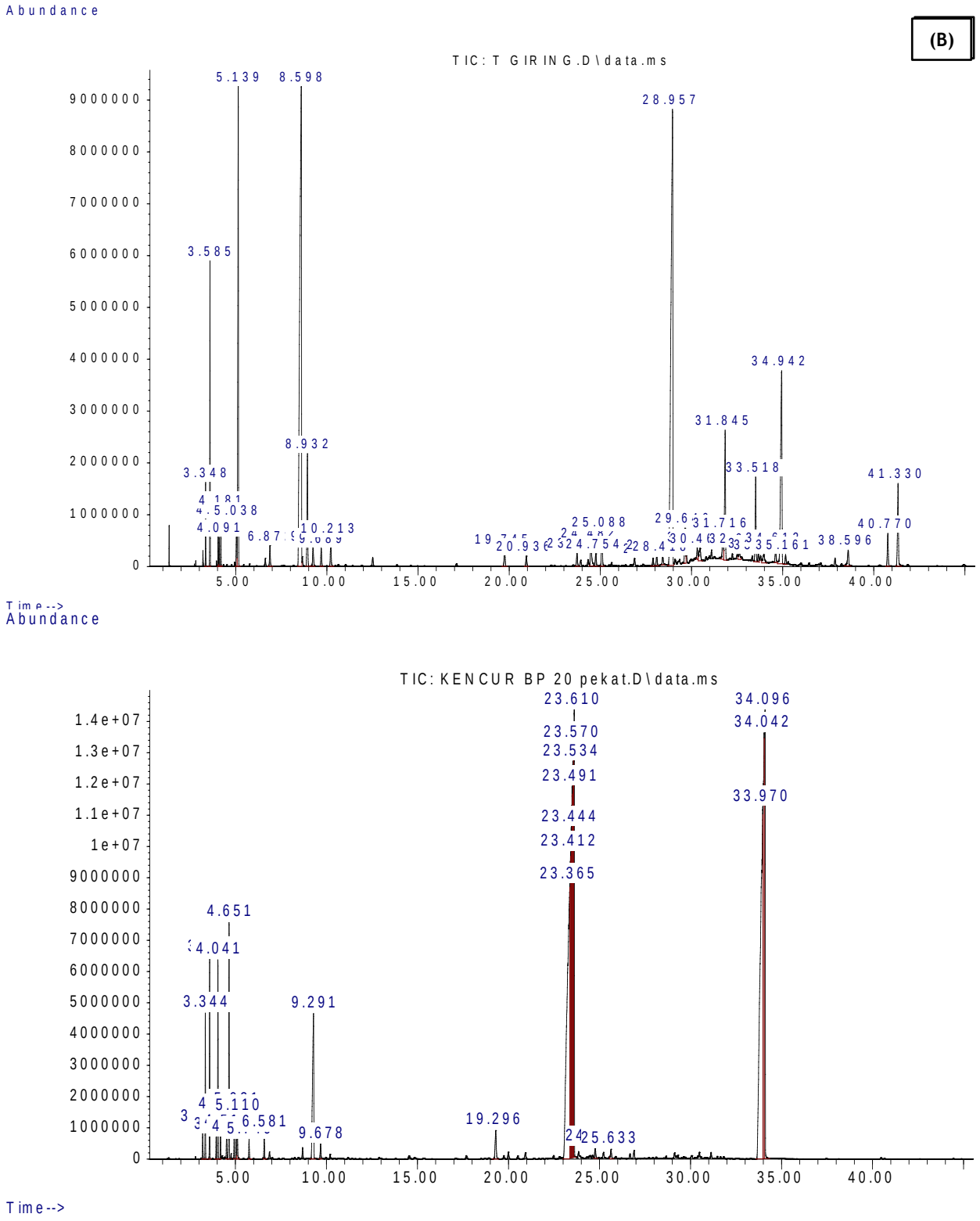


Fig 2. The GC-MS spectrum of (A) *C. heyneana* essential oil; (B) *K. galanga* essential oil

Table 3. Chemical composition of *C. heyneana* and *K. galanga* essential oils

No.	Retention time (min)	Chemical compound	Percentage of Relative Area (%)		Molecular weight	Molecular formulas
			C. heyneana	*K. galanga*		
1	3.348	α-Pinene	0.90	1.49	136.125	$C_{10}H_{16}$
2	3.585	**Camphene**	**3.78**	1.80	136.125	$C_{10}H_{16}$
3	4.037	Β-Pinene	0.61	1.99	136.125	$C_{10}H_{16}$

4	4.091	6-Methyl-5-heptene-2-one	0.37	-	126.104	$C_8H_{14}O$
5	4.181	ß-Myrcene	0.74	0.41	136.125	$C_{10}H_{16}$
6	4.511	α-Phellandrene	-	0.28	136.125	$C_{10}H_{16}$
7	4.651	3-Carene	-	2.76	136.125	$C_{10}H_{16}$
8	4.931	p-Cymene	-	0.23	134.110	$C_{10}H_{14}$
9	5.038	D-Limonene	0.84	0.58	136.125	$C_{10}H_{16}$
10	5.096	ß-Sabinene	-	0.23	136.125	$C_{10}H_{16}$
11	5.139	**Eucalyptol**	**9.25**	0.48	154.136	$C_{10}H_{18}O$
12	5.749	γ-Terpinene	-	0.24	136.125	$C_{10}H_{16}$
13	6.581	Terpinolene	-	0.36	136.125	$C_{10}H_{16}$
14	8.598	**Camphor**	**25.47**	-	152.120	$C_{10}H_{16}O$
15	8.932	Isoborneol	2.76	-	154.136	$C_{10}H_{18}O$
16	9.237	**Borneol**	0.58	**3.76**	154.136	$C_{10}H_{18}O$
17	9.689	Terpinen-4-ol	0.44	0.25	154.136	$C_{10}H_{18}O$
18	10.213	Terpineol	0.72	-	154.136	$C_{10}H_{18}O$
19	19.296	Cinnamic acid	-	0.69	162.068	$C_{10}H_{10}O_2$
20	20.936	Caryophyllene	0.32	-	204.188	$C_{15}H_{24}$
21	23.365	***trans*-ethylcinnamate**	-	**46.14**	176.084	$C_{11}H_{12}O_2$
22	23.721	Germacrene D	0.39	-	204.188	$C_{15}H_{24}$
23	24.482	Isogermafurene	0.99	-	216.151	$C_{15}H_{20}O$
24	24.754	ß-Elemene	0.39	-	204.188	$C_{15}H_{24}$
25	24.769	Pentadecane	-	0.25	212.250	$C_{11}H_{12}O_2$
26	25.088	α-Farnesene	1.04	-	204.188	$C_{15}H_{24}$
27	25.633	δ-Cadinene	-	0.22	204.188	$C_{15}H_{32}$
28	28.092	ß-Panasinsene	0.33	-	204.188	$C_{15}H_{24}$
29	28.957	**Epicurzerenone**	**25.74**	-	230.131	$C_{15}H_{18}O_2$
30	29.646	Isospathulenol	1.02	-	220.183	$C_{15}H_{24}O$
31	30.317	ß-Eudesmol	0.33	-	222.198	$C_{15}H_{26}O$
32	30.464	ß-Selinene	0.65	-	204.188	$C_{15}H_{24}$
33	31.845	**Germacrone**	**3.70**	-	218.167	$C_{15}H_{22}O$
34	33.518	Naphtho[2,3-b]furan-4(6H)-one	2.28	-	230.131	$C_{15}H_{18}O_2$
35	33.970	**ethyl-*p*-methoxycinnamate**	-	**37.85**	206.094	$C_{12}H_{14}O_3$
36	34.612	Furanodienon	0.46	-	230.131	$C_{15}H_{18}O_2$
37	41.330	Zederone	2.47	-	246.126	$C_{15}H_{18}O_3$
38	-	Unknown	13.45	-	-	-
	Total components (%):		**100**	**100**		

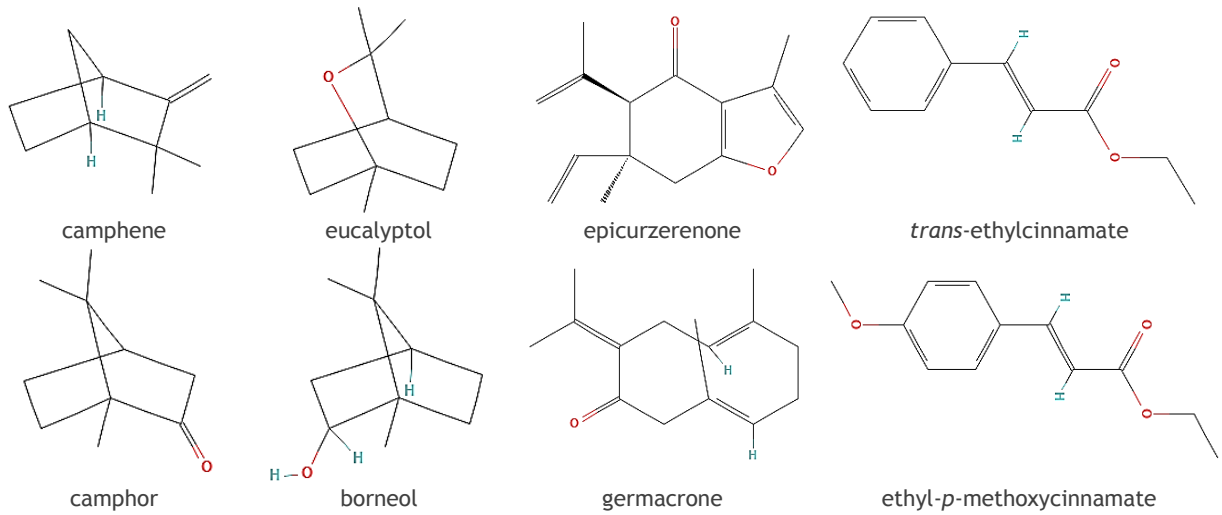


Fig 3. The chemical structure of major compounds of *C. heyneana* and *K. galanga* essential oils (source: PubChem)

Table 4. Tyrosinase inhibitory activity of *C. heyneana* and *K. galanga* essential oils

Sample	Tyrosinase Enzyme Inhibitory Activity

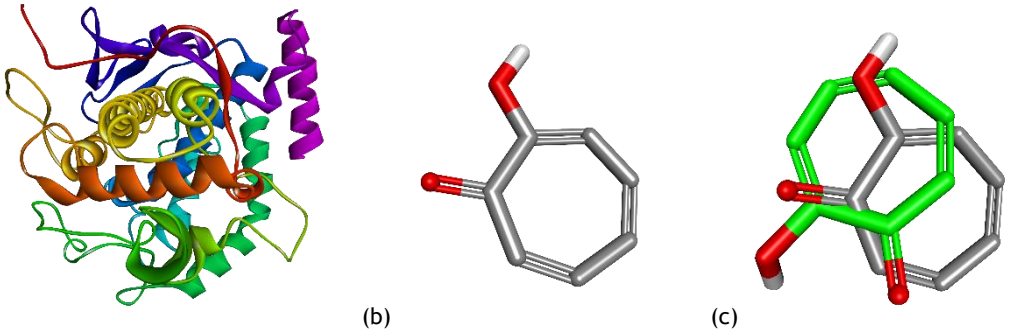
	IC_{50} (mg/mL)
Curcuma heyneana Val.	6.423 ± 0.0
Kaempferia galanga L.	3.991 ± 0.2
Kojic acid	0.093 ± 0.0

All data are shown as mean ± SD (n = 3), where n indicates the number of observations, with kojic acid used as a positive control.

Table 5. SPF value of *C. heyneana* and *K. galanga* essential oils

Sample	SPF value ± SD	SPF categories	Resistance UV (minutes)
Curcuma heyneana Val.	5.99 ± 0.4	Medium	59.93
Kaempferia galanga L.	39.77 ± 0.2	Ultra	397.70
Kojic acid	12.95 ± 0.0	Maximum	129.53

All data are shown as mean ± SD (n = 3), where n indicates the number of observations, with kojic acid used as a positive control.



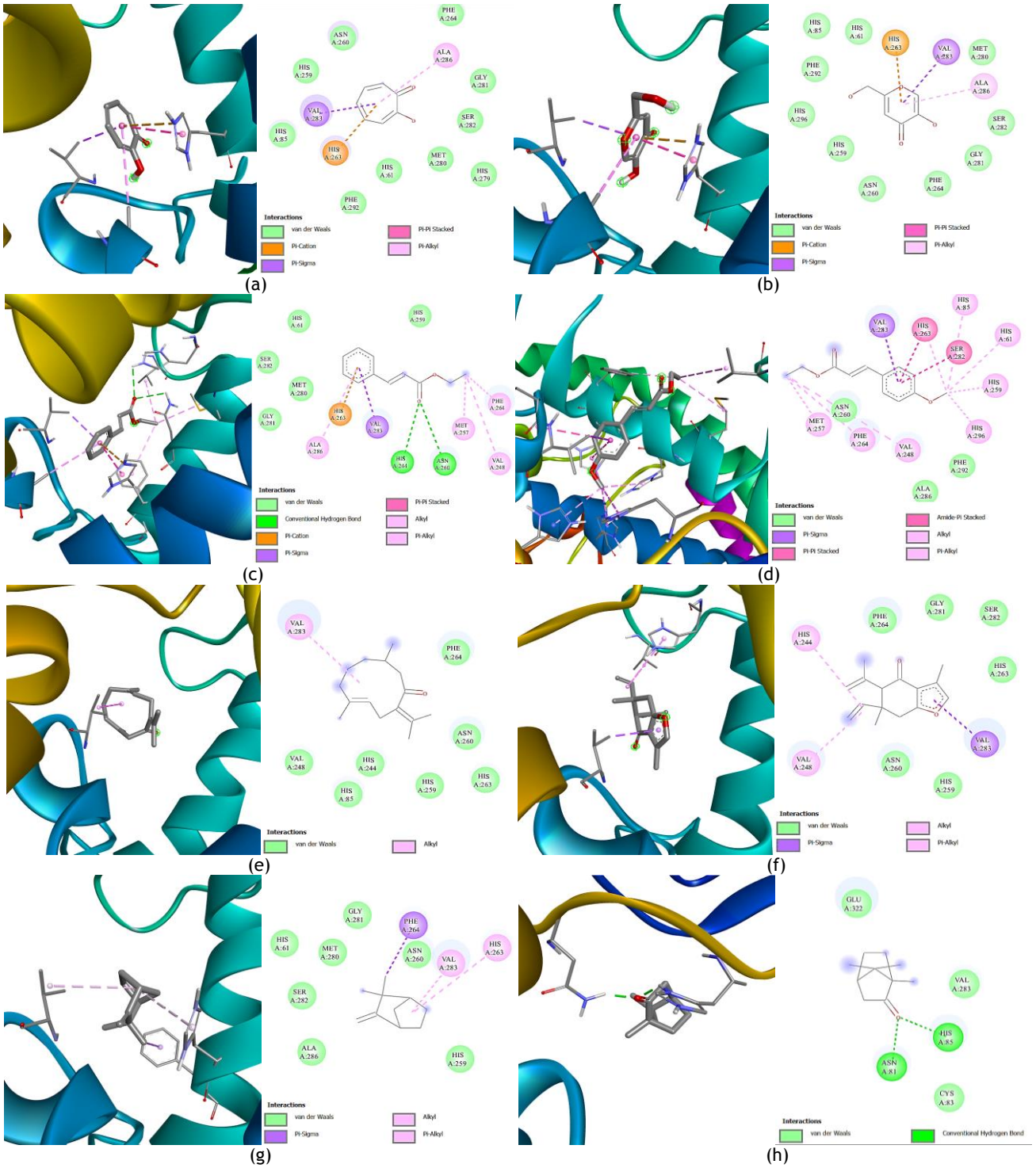
(a) (b) (c)

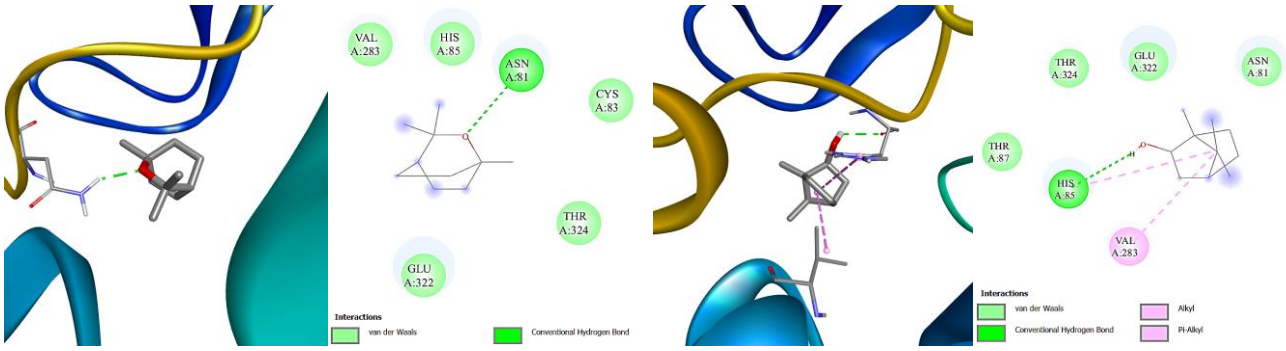
Fig 4. (a) Tyrosinase reseptor (PDB ID: 2Y9X); (b) The 3D structure of native ligand (Tropolone); (c) Superimposition of tropolone with the docked ligand

Table 6. Docking simulation result of native ligand, major compound of *C. heyneana* and *K. galanga* essential oils

No.	Ligand	Affinity (kcal/mol)	Residue interaction
Native ligand (PDB ID: 2Y9X)			
(a)	Tropolone (0TR)	-6.0	HIS263, VAL283, ALA286
(b)	Kojic acid (standard)	-5.8	HIS263, VAL283, ALA286
K. galanga			
(c)	*trans*-ethylcinnamate	-5.9	HIS263, VAL283, ALA286, HIS244, ASN260, MET257, VAL248, PHE264
(d)	ethyl-*p*-methoxycinnamate	-5.8	HIS263, VAL283, SER282, HIS85, HIS61, HIS259, HIS296, MET257, PHE264, VAL248
(j)	Borneol	-4.0	VAL283

C. heyneana			
(e)	Germacrone	-5.8	VAL283
(f)	Epicurzerenone	-5.6	HIS244, VAL248, VAL283
(g)	Camphene	-5.3	HIS263, PHE264, VAL283
(h)	Camphor	-4.3	ASN81, HIS85
(i)	Eucalyptol	-4.2	ASN81





(i) (j)

Fig 5. The visualization interactions of (a) native ligand; (b) kojic acid (standard); (c) *trans*-ethylcinnamate; (d) ethyl-*p*-methoxycinnamate; (e) germacrone; (f) epicurzerenone; (g) camphene; (h) camphor; (i) eucalyptol; (j) borneol

4. Discussion

The essential oils of *C. heyneana* and *K. galanga* extracted by hydrodistillation have different physical characteristics. The essential oil of *C. heyneana* has a distinctive smell and is brownish, while the oil of *K. galanga* is colorless. The yield of the two essential oils also differs. The yield of *C. heyneana* in this study was 0.29% v/w with a chemical composition (major compounds) of epicurzerenone (25.74%), camphor (25.47%), eucalyptol (9.25%), camphene (3.78%), and germacrone (3.70%). The results differ from previous studies that reported the essential oil yield of *C. heyneana* of 0.43% v/w, with major compounds being curcumanolides (19.6%), dehydrocurcumin (17.1%), isocurcumenol (16.5%), curcumenol (13.7%), and curcumenone (6.4%) [20].

Furthermore, the essential oil yield of *K. galanga* in this study was 0.69% v/w with major compounds being *trans*-ethylcinnamate (46.14%), ethyl-*p*-methoxycinnamate (37.85%), and berneol (3.76%). These results are consistent with a study in India, which reported that the essential oil yield of *K. galanga* ranged from 0.3-1.9% v/w [21]. The major compounds of *K. galanga* essential oil in this study are also consistent with the Chinese study, which reported that the major compounds of *K. galanga* essential oil were *trans*-ethyl-*p*-methoxycinnamate (32.01%), *n*-pentadecane (29.14%), and *trans*-ethylcinnamate (19.50%) [22]. In the research of natural products, differences in plant varieties, climate, geographical location, harvest time, plant parts used, and extraction methods significantly affect essential oils' yield and chemical composition [23].

The best activity of the tyrosinase enzyme inhibitor is determined based on the IC_{50} value. The IC_{50} value refers to the concentration of a sample that can inhibit 50% of the activity of the enzyme tyrosinase. The lower the IC_{50} value, the stronger the inhibition potential [24]. The lower IC_{50} value in *K. galanga* essential oil is thought to be due to *trans*-ethylcinnamate and ethyl-*p*-methoxycinnamate compounds. Those compounds are a class of phenolic cinnamic acid derivatives with a chemical structure that can interact with the active side of tyrosinase and contribute to tyrosinase inhibition [25][26].

The ethyl-*p*-methoxycinnamate compound can reduce the expression of Microphthalmia-associated Transcription Factor (MITF). This major transcription factor controls tyrosinase expression, affecting melanocyte viability and reducing melanogenesis activity in the skin [27]. Furthermore, the high IC_{50} value of *C. heyneana* essential oil could be due to the possibility of a reduced quantity of bioactive compounds or compounds that do not have tyrosinase enzyme inhibitory activity in large quantities.

The high SPF value in *K. galanga* essential oil is thought to be affected by *trans*-ethylcinnamate and ethyl-*p*-methoxycinnamate compounds. Besides tyrosinase enzyme inhibitors, this cinnamic acid derivative compound is also reported to have vigorous UVB absorption activity [28][29]. On the other hand, the low SPF value of *C. heyneana* essential oil may be influenced by chemical components that are less supportive of photoprotective effects.

An *in silico* approach supported the in vitro tyrosinase inhibition assay results. The RMSD value of 2.9 Å indicates that the docking protocol was valid, thus accurately describing the binding interaction between tyrosinase and the test compound [30]. Determining the best ligand depends on the value of the free energy of binding or the binding affinity (ΔG value). The more negative the binding affinity value, the more stable the receptor-ligand complex and the stronger the binding affinity [31]. *Trans*-ethylcinnamate and ethyl-*p*-methoxycinnamate compounds from *K. galanga* essential oil are the best ligands because they have docking simulation scores close to tropolone. *Trans*-ethylcinnamate and ethyl-*p*-methoxycinnamate interact with HIS263, VAL283, and ALA286 residues in Tropolone and kojic acid. A very similar interaction may indicate competitive ability towards the tyrosinase catalytic site. This study is in line with *in silico* studies, which reported that cinnamate derivatives generally formed strong π-cation and hydrophobic interactions at the active site of tyrosinase. [32].

5. Conclusions

Thus, it can be concluded that *K. galanga* essential oil has more potential as an active ingredient for skin lightening and protection than *C. heyneana* essential oil. This is evidenced by the lower IC_{50} value indicating more potent inhibition, high SPF value indicating extreme protection effectiveness, and the main compounds *trans*-ethylcinnamate, ethyl-*p*-methoxycinnamate in *K. galanga* have the best affinity, close to Tropolone.

The *in vitro* and in silico approaches to testing anti-tyrosinase and photoprotective activities are limitations in this study, as these approaches cannot directly

describe the effects on the skin. In addition, essential oils contain complex compounds, so it was impossible to ascertain the active compounds involved in the anti-tyrosinase activity and photoprotective effect. Although these findings have limitations, they can provide a scientific basis for developing natural active ingredients in skin care products. Further research is recommended to conduct *in vivo* or clinical trials, isolate active compounds, and conduct formula stability tests to support their clinical and commercial applications.

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