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Article

Melanogenesis inhibition activity of ethyl acetate fraction from *Curcuma zedoaria* in B16 cell line

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Curcuma zedoaria is a traditional medicine used for various purposes in human life. The organic solvent extracts of this plant also exhibit antioxidant, antibacterial, anti-inflammatory, anti-cancer, anti-necrosis, and hepatoprotective properties. However, the prospect of employing this plant extract for skin whitening, particularly its molecular mechanism, has not been reported yet. Therefore, in this study, the total polyphenolic content, antioxidant activity, cytotoxicity, tyrosinase inhibitory, melanin inhibitory, and target gene expression using the ethyl acetate fraction of ethanolic extract from *Curcuma zedoaria*, were all examined. The TPC result of the sample was 42.18±2.25 mg GAE/g extract with impressive antioxidant activity. Half maximal inhibitory concentration value of 2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay and Ferric reducing antioxidant power FRAP value at a concentration of 2.5 µg/mL were 49.06±0.29 µg/mL and 116±50 µM Fe^{2+}/L, respectively. The extract had no cytotoxicity at 25 µg/mL, inhibited melanin production for 74.02±3.05% and tyrosinase for 137.45±1.38%. In particular, the extract demonstrated the ability to block the expression levels of four melanogenesis-related genes. Moreover, GCMS analysis results also detected 28 different compounds, with two new compounds accounting for a high proportion. These results suggested that *C. zedoaria* has a significant potential for application in skin-pigmentation treatment cosmetics.

Keywords: *Curcuma zedoaria*. Tyrosinase inhibitory. Melanin inhibitory. Antioxidant activity.

INTRODUCTION

Melanin, synthesized in melanosomes of melanocytes, is vital in mammalian skin pigmentation because it protects the skin and eyes from light, absorbs damaging drugs as well as toxins, prevents brain neurons from iron-induced oxidative damage, and also responds to oxidation that results from stress process and inflammation (Brenner, Hearing, 2008). Many distinct genes affect melanin production, including TRP-1, TRP-2, TYR, and MITF (Videira, Moura, Magina, 2013). Excessive melanin or an irregular distribution of pigment on the skin produces cosmetic issues or dermatological conditions. With the growing demand for skin whitening, it is critical to identify novel whitening active compounds with safe qualities (Bhattacharyya *et al.*, 2021).

Tyrosinase (TYR) is one of the crucial enzymes which turn L-tyrosine into L-dihydroxyphenylalanine

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(L-DOPA) and then successively oxidized to dopaquinone to create melanin polymers (Varghese *et al*., 2021). By activating the genes involved in melanin production, such as tyrosinase-related protein-1 (TRP-1) and TRP-2, the microphthalmia-associated transcription factor (MITF) is also strongly linked to the synthesis of melanin (Jeon *et al*., 2022).

Due to their efficiency and safety, natural antioxidant products from plants with an excessive concentration of polyphenolic chemicals which block the production of melanin have recently received much research (Zolghadri *et al*., 2019). The amount of phenolics has been strongly correlated to their antioxidant activity, as reported in many previous studies (Aryal *et al*., 2019; Kumar, Sandhir, Ojha, 2014). Moreover, the ability to inhibit tyrosinase is a property of many plant extracts with strong antioxidant capacity (Athipornchai, Jullapo, 2018; Fu *et al*., 2014). The expression of the TRP-1, TRP-2, TYR, and MITF genes is one of the targets of skin-whitening substances (Pillaiyar, Manickam, Namasivayam, 2017; Shim, 2021).

Curcuma zedoaria is an Asian traditional medicine used for various purposes within human life (Khuntia *et al*., 2023). Antioxidant compounds such as curzerene and epicurzerene have been found in its rhizome oil. The organic solvent extracts of this plant also exhibit antioxidant, antibacterial, anti-inflammatory, anti-cancer, anti-necrosis, and hepatoprotective properties (Gharge *et al*., 2021). To the best of our knowledge, the prospect of employing this plant extract for skin whitening, particularly its molecular mechanism, has not been reported yet. Therefore, in this study, ethyl acetate (EtOAc) fraction of ethanolic extract of *C. zedoaria* was used to investigate polyphenolic concentration, antioxidant activity, tyrosinase, and melanin synthesis inhibitory activity, as well as the influence on the expression of melanogenesis-related genes.

MATERIAL AND METHOD

Plant material and extraction

Fresh *C. zedoaria* rhizomes were collected in An Giang Province (Vietnam) and identified by MSc. Cao Ngoc Giang, Research Center for Ginseng and Medicinal Materials in Ho Chi Minh City. A voucher specimen (MCE0063) has been deposited at the Division of Medicinal Chemistry, Faculty of Chemistry, University of Science, VNU-HCM. The air-dried powder rhizomes of *C. zedoaria* (1.0 kg) were exhaustively extracted in a Sohxlet extractor with ethanol. The crude extract was then fractionated with n-hexane (2.0 L) and ethyl acetate (2.0 L) to yield n-hexane (40.3 g) and ethyl acetate (35.6 g) fractions, respectively.

Total phenolic content (TPC)

The sample (extracts) or standard (gallic acid) was combined with 2 mL of Folin-Ciocalteu reagent (1:10 in water), followed by the addition of 2.5 mL of 10% (w/v) Na_2CO_3 and 10 mL of distilled water. The mixture was allowed to react for 30 minutes in the dark at room temperature. At a wavelength of 737 nm, absorbance was measured with a UV-Vis spectrophotometer, and TPC was determined using gallic acid as a standard calibration curve. TPC was calculated in milligram equivalents of gallic acid (GAE) per gram of extract (Blainski, Lopes, De Mello, 2013). The same solution used for diluting the extract at various concentrations was used as negative control.

Antioxidant assay

2,2-Diphenyl-1-picrylhydrazyl radical scavenging method (DPPH assay) and ferric reducing antioxidant power (FRAP) methods were used to evaluate the antioxidant activity of the sample (Thaipong *et al*., 2006). First, 20 µL of varied concentrations were pipetted into 980 µL of DPPH solution (1.98 mg/mL in methanol) for the DPPH experiment. The results of the reactions were measured with a spectrophotometer at a wavelength of 517 nm after 30 minutes of incubation. Ascorbic acid was positively controlled, whereas the negative control was a solution without an extract sample. By decreasing the absorbance of DPPH, antioxidant activity was demonstrated.

For the FRAP experiment, solutions containing 10 mM TPTZ, 20 mM $FeCl_3.6H_2O$, and 300 mM acetate

buffer (pH 3.6) were prepared in a 1:1:10 volume ratio at 37 °C. The reaction mixture included 900 µL of FRAP reagent and 100 µL of varied concentrations of the sample. The ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex was reduced to the ferrous (Fe^{2+}-TPTZ) complex after 30 minutes which has an intense blue color and absorption at 593 nm. Using the absorbance of varying concentrations of $FeSO_4$, the calibration curve was plotted.

Cytotoxic assay on B16 melanoma cell

The B16 cells were cultured in high glucose-Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum, 100 mg/mL streptomycin, and 100 U/mL penicillin. The MTT assay were used to determine the viability of B16 cells in the sample extract (Ye *et al.*, 2010). Cells were seeded in 96-well plates at a density of 1×10^4 cells/100 µL/well before incubation for 24 hours in a culture medium. Fresh medium with different sample concentrations (0 to 800 µg/mL) was added to each well. Plates were incubated for 3 hours with MTT (0.5 mg/mL) after 48 hours and formazan crystals were solubilized in DMSO. The microplate reader (Thermo Multiskan Ascent 96) at 550 nm was used to identify the darker solution. The percentage of inhibition was estimated using the equation below: (%) inhibition = 100*OD1/OD0. The OD of the control is OD1 while the OD of the sample is OD0.

Melanin inhibition and intracellular tyrosinase inhibition

The melanin content was determined as reported (Ha *et al.*, 2023), with a few modifications. B16 cells (2×10^5/well) were seeded in a 6-well plate. The medium was changed after 24 hours with the new one containing either varying sample concentrations or arbtutin with 3-isobutyl-1-methylxanthine (IBMX) (100 nM) in 48 hours. Cells were washed in cold PBS after culture and lysed in 20 mM sodium phosphate (pH 6.8), phenylmethylsulfonyl fluoride (PMSF), and 1% Triton X-100. After being frozen and thawed for 20 minutes, the lysates were centrifuged at 14768 g. The melanin pellets were ultrasonically solubilized in 150 µL NaOH 1N (containing 10% DMSO) for 45 minutes at 80 °C. A microplate reader (Thermo Multiskan Ascent 96) was used to measure the melanin solutions at 450 nm. Using the following equation to calculate the proportion of melanin content: Melanin content (percentage) = 100*OD1/OD0. The OD of the control and sample are OD1 and OD, respectively.

The supernatants were then incubated for 10 minutes at 37 °C for tyrosinase-specific activity evaluation (Tran *et al.*, 2021). After 10 minutes, L-DOPA (10 mM) was added to the microplate and the microplate reader (Thermo Multiskan Ascent 96) detected absorbance at 492 nm. Using BSA as the protein standard, the Bradford evaluation was used to determine protein concentration in the supernatant.

Total RNA isolation and Real-Time PCR

Similar to the intracellular tyrosine extraction procedure, RNA was extracted using Trisure in lieu of Triton X-100. Isolated RNAs were stored in 20 µL of water. Complement DNAs and real-time PCR products were synthesized using SensiFAST™ cDNA and SensiFAST™ HRM kits (Bioline), respectively. Primers sequences for melanogenesis-related genes are as follows: For β-actin, 5'-CGACCAGCGCAGCGATATC-3' (reverse) and 5'-CTTCTTTGCAGTCCTTCGTTG-3' (forward) (Gyamfi *et al.*, 2009); for *tyr*, 5'-CATCGCATAAAACCTGATGGC -3' (reverse) and 5'-GTCGTCACCCTGAAAATCCTAACT-3' (forward) (Sun *et al.*, 2017); for *mitf*, 5'-GTGAGATCCAGAGTTGTCGT-3' (reverse) and 5'-AGTACAGGAGCTGGAGATG-3' (forward) (Zang, Niu, Aisa, 2019); for *trp1*, 5'-AAGACGCTGCACTGCTGGTCT-3' (reverse) and 5'-GCTGCAGGAGCCTTCTTTCTC-3' (forward) (Yang *et al.*, 2006); for *trp2*, 5'-GGGAATGGATATTCCGTCTTA-3' (reverse) and 5'-TTATATCCTTCGAAACCAGGA-3' (forward) (Sun *et al.*, 2017).

GCMS analysis

The extract was analyzed using GC-MS apparatus GCMS QP2010 SE Shimadzu according to the procedure described in a previous investigation (Gomathi *et*

al., 2015). The apparatus has a DB-5MS UI with the following dimensions of 30m length × 0.25m internal diameter × 0.25m film thickness. Helium is the carrier gas with a minimum flow rate of 1.0 mL/min. The injector temperature was set to 250 degrees Celsius, and the oven temperature was programmed as follows: 50 °C for two minutes, then 5 °C/min to 100 °C, 10 °C/min to 150 °C, and 25 °C/min to 250 °C. The total duration of the chromatography was 22 minutes. Using the NIST 2005 library of mass spectra, the relative percent of each component was determined by comparing its average peak area to the total area of identification of the constituents.

Statistical analysis

At least three independent repetitions of each experiment were conducted. GraphPad Prism 9 analyzed the data, which were presented as means, standard deviations, and error bars. One-way analysis of variance (ANOVA) was used in combination with Tukey's multiple comparison test to determine the statistical significance. Differences at $p < 0.05$ were considered significant.

RESULTS

Total phenolic content and Antioxidant assay

The total polyphenolic content of the EtOAc fraction of *C. zedoaria* extract was 42.18±2.25 mg GAE/g extract. There are various methods used to analyze antioxidant capacity based on contents that present different mechanisms for their antioxidant capacities. In this study, DPPH assay and FRAP assay were selected to evaluate the antioxidant activities of *C. zedoaria* extract in vitro. As shown in Table I, IC50 value of free radical scavenging antioxidant and FRAP value of EtOAc with ascorbic acid as the control was determined. The extract was determined high DPPH scavenging ability and IC50 value of 49.06±0.29 µg/mL. At a concentration of 2.50 µg/mL, *C. zedoaria* extract and ascorbic acid showed reducing capacity as 116±5 µM Fe2+/ and 211088±1300 µM Fe2+/L, respectively. The higher the amount of reduced Fe^{2+} expressed, the higher the reducing power.

TABLE I - IC50 value (µg/mL) of DPPH assay and FRAP value (µM Fe^{2+}/L) at a concentration of 2.50 µg/mL *C. zedoaria* extract

IC50 value of DPPH assay		FRAP value	
Sample	Ascorbic	Sample	Ascorbic acid
49.06±0.29	0.12±0.00	116±50	211088±1300

Cytotoxic assay on B16 melanoma cell

Figure 1 demonstrated that the cytotoxicity on B16 melanoma cells of the sample increased as the concentration of the extract increased. There was no statistical difference between concentrations of 6.25, 12.5, 25 µg/mL, and the control, whose percent cell growth was 108.53±2.68%, 106.79±0.72%, and 105.25±0.66%, respectively. Therefore, at lower concentrations of 25 µg/mL, the extract was not deleterious to B16 cells.

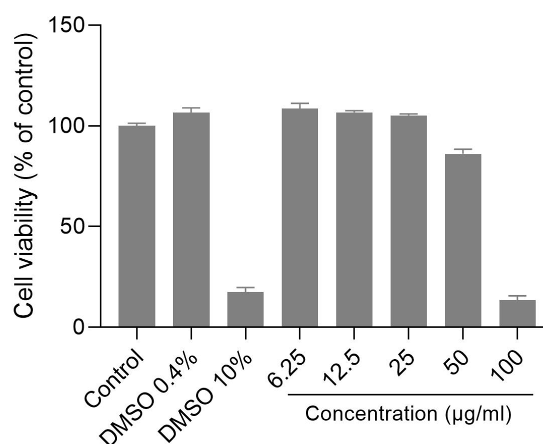

FIGURE 1 - Cytotoxicity at different concentrations of *C. zedoaria* extracts on B16 melanoma cell at concentrations lower than 25 µg/mL.

Melanin inhibition and intracellular tyrosinase inhibition

Melanin-stimulating IBMX at the concentration of 100 nM stimulated the production of melanin in B16 cells (Figure 2). The supplemented arbutin subdued the effect of IBMX. Figure 2 shows that *C. zedoaria* extracted inhibits melanin production, the number of melanin

gradually decreases with increasing concentration. At a concentration of 6.25 µg/mL and 12.5 µg/mL, the inhibition was substantially lower than arbutin, which percentage of melanin was 98.86±8.40% and 84.88±3.29%7, respectively. Especially, melanin content at arbutin and 12.5 µg/mL of the extract was no statistical difference, which was 74.02±3.00% and 62.72±0.38%, respectively. This suggested that 25 µg/mL samples inhibited melanin synthesis of more than 6.25 µg/mL and 12.5 µg/mL samples.

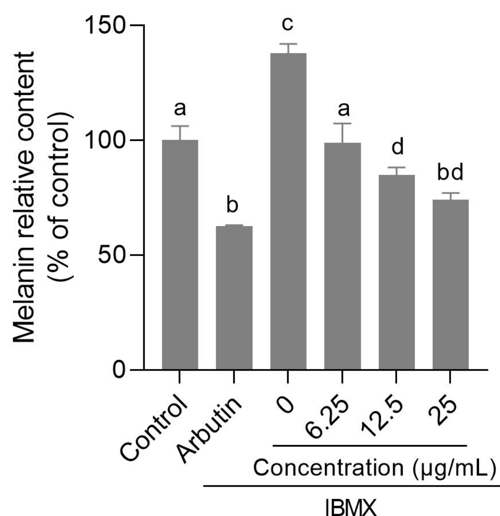

FIGURE 2 - The effect of *C. zedoaria* extracts on intracellular melanin content. Different letters were significantly different ($p < 0.05$).

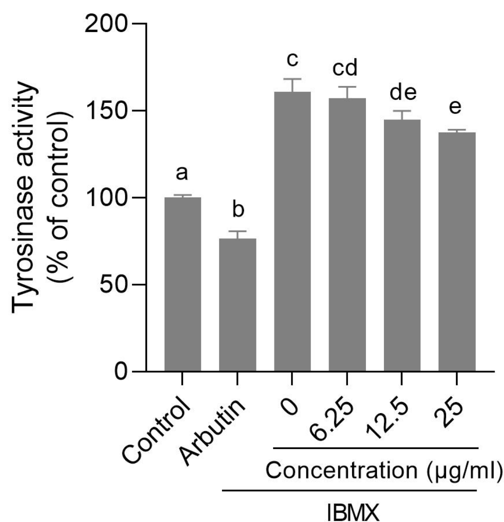

FIGURE 3 - Intracellular tyrosinase activity at different concentrations of *C. zedoaria* extracts on B16 melanoma cell. Different letters were significantly different ($p < 0.05$).

The evaluation of intracellular tyrosinase is an important experiment to study inhibited melanin production of extract because tyrosinase is a vital factor in melanin synthesis pathway. As shown in Figure 3, sample concentrations of 12.5 and 25 µg/mL led to a decrease in tyrosinase enzyme activity (Figure 3). The tyrosinase activity of arbutin was 1.80 times lower than that of EtOAc at 25 µg/mL (76.39±4.29%). This result suggested that the inhibition of tyrosinase intracellular activity may be the one of mechanisms by which the extract reduced the melanin concentration of B16 cells (Figure 2).

Melanogenesis-related gene expression

Sample extracts at concentrations of 6.25, 12.5, and 25 µg/mL were used to evaluate the expression of the *tyr, trp1, trp2,* and *mitf* genes involved in the inhibition of melanin synthesis (Figure 4).

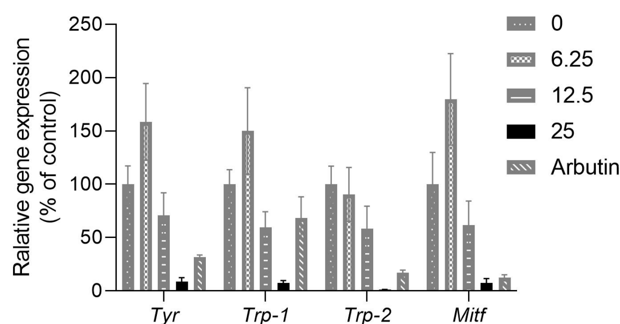

FIGURE 4 - The results of the gene encoding *tyr, trp1, trp2,* and *mitf* expression in B16 cells.

Among the examined concentrations, 6.25 µg/mL and 12.5 µg/mL of EtOAc fraction did not show a suppression ability on gene expressions in this study, except that the concentration of 25 µg/mL suppressed the expression level of *trp1*. In contrast, 25 µg/mL had strong effects and inhibited expression of *tyr, trp1, trp2,* and *mitf* gene was comparable to arbutin. Thus, at a concentration of 25 µg/mL, EtOAc had the potential to significantly inhibit the expression of melanogenesis-related genes in B16 cells, thereby reducing their capacity to produce melanin.

GCMS analysis

GC-MS analysis revealed the extract contained 28 different compounds, as shown in Table II.

TABLE II - GC-MS analysis of the extract of *C. zedoaria*

No	Compound	Peak area %
1	Eucalyptol	0.35
2	L-camphor	6.10
3	Isoborneol	1.07
4	Borneol	0.25
5	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	0.19
6	Pichtosine	0.28
7	delta-Elemene	1.95
8	beta-Elemene	0.18
9	**alpha-Elemene**	**10.69**
10	Cyclohexene, 3,4-diethenyl-1,6-dimethyl	0.25
11	gamma- Elemene	4.45
12	beta-Gurjunene	0.37
13	alpha-Caryophyllene	2.43
14	alpha-Gurjunene	2.62
15	Eudesma-4(14),11-diene	4.10
16	alpha-Selinen	2.95
17	delta-Cadinene	3.02
18	**Eremophilene**	**13.62**
19	**Eudesma-3,7(11)-diene**	**13.23**
20	**Germacrone**	**20.02**
21	(3,8,8-Trimethyl-1,2,3,4,5,6,7,8-octahydro-2-napthalenyl)methyl acetate	2.17
22	Spathulenol	1.97
23	Ar-tumerone	1.14
24	Epiglobulol	0.91
25	Aromadendrene, dehydro-	0.83
26	beta-Maaliene	1.11
27	cis-Verbenone	1.54
28	Juniper camphor	2.21

DISCUSSION

Recently, there has been a rise in demand for natural anti-melanogenic compounds for cosmetic purposes and other treatments. Modulating melanogenesis is a fascinating area of study, thus screening for new plants, fractions, extracts, or compounds that inhibit melanin production or melanogenesis-related genes, is a challenging yet exciting quest. We have concentrated on identifying novel agents regulating melanogenesis from natural resources due to the negative impacts of synthetic medications such as arbutin (Yang *et al.*, 2013), 3,4-dihydroxyacetophenone, phospholipase D1, phospholipase D2 (Akaberi *et al.*, 2018).

In this study, the TPC concentration of the EtOAc fraction is higher than that of the hexane extract (40.2±1.2 mgGAE/g), but lower than that of the methanol (122.12±1.6 mgGAE/g), chloroform (61.15±1.1 mgGAE/g), and ethyl acetate (141.31±1.5 mgGAE/g) extracts of the same species (Rahman *et al.*, 2014). This suggests that the polyphenolic content of the extract is affected by the solvent used for extraction (Do *et al.*, 2014).

On the basis of quantitative TPC results, antioxidant activity was measured. The antioxidant capacity of EtOAc was 1.72, and 1.13 times greater than that of the total ethanol extract (84.53±0.49 µg/mL) and the ethyl acetate fraction (55.35±0.11 µg/mL) of *C. zedoaria* (Lan, Linh, 2022). Also, in the above-mentioned study, n-hexane and dichloromethane extracts of *C. zedoaria* demonstrated potent antioxidant activity. This is due to the composition of the plant, which contains many antioxidant-rich compounds, including curcumin, ethyl p-methoxycinnamate, β-turmerone, β-eudesmol, zingiberene, dihydrocurcumin, furanodiene, α-phellandrene, 1–8 cineole, β-elemense, and germacrone (Gharge *et al.*, 2021). For the FRAP method, the EtOAc fraction concentration was substantially distinct from the ascorbic acid concentration. In the study conducted by Tanvir *et al.,* (2017), an ethanol extract of turmeric (*Curcuma longa*) isolated in Khulna, Bangladesh, has a FRAP antioxidant capacity of 4204.46±74.48 µM Fe^{2+}/L in 100 g fresh samples (Tanvir *et al.*, 2017), which is 1103.7 times less than that of EtOAc fraction. Also, in the same investigation, the aqueous extract of yellow

turmeric is collected from Khulna, Bangladesh, for the FRAP antioxidant capacity of 1015.52±3.11μM Fe^{2+}/L in 100g of fresh samples, which is 4571.4 times less than the EtOAc fraction (Tanvir *et al*., 2017). The results for the DPPH method indicate that a low concentration of *C. carsia* extract has excellent antioxidant capacity, while results for the FRAP method indicate an opposite tending. In both methods of assessing antioxidant capacity, the ascorbic acid control demonstrated a superior antioxidant capacity to the EtOAc fraction.

As the concentration of *C. zedoaria* extract progressively increased, so did the cytotoxicity of EtOAc fraction. Thus, at concentrations below 25 μg/mL, the sample extract was non-toxic to B16 cells. In investigations on the turmeric family, essential oils extracted from turmeric exhibites cytotoxicity ranges from 13.96 to 135.97 μg/mL on B16 cells (Zhang *et al*., 2017). Danciu *et al*., (2015) find that the B16 cell inhibition index of turmeric and ginger ethanol extracts at 100 μg/mL concentrations is 38±35 and 17±16, respectively. At a concentration of 25 μg/mL of EtOAc fraction, the presence of melanin was reduced by 1.48 times (109.90±8.32%) which is more than ethanol extract at a concentration of 500 μg/mL in galangal (*Alpinia conchigera*) (33). Also found to inhibit melanin production in the study by Ujang *et al.,* 2013, were two compounds extracted from the galangal plant: trans-cinnamaldehyde and chavicol glucopyranoside at a concentration of 4.9 μg/mL. Current research on *C. zedoaria* with tyrosinase inhibitory activity is extremely limited. In contrast, according to one study, the curcumin compound found in *C. zedoaria* can inhibit melanin formation by inhibiting the enzyme tyrosinase. However, its precise mechanism of action is still being studied (Zolghadri *et al*., 2019).

The molecular mechanisms of cells which can provide in-depth explanations of gene expression, are currently the subject of research. Since then, investigations on compounds or groups of active constituents related to the expression of TYR, TRP-1, TRP-2, and MITF gene groups have progressively garnered more interest. The aromatic (ar)-turmerone compound isolate from turmeric inhibits melanin synthesis and tyrosinase activity more effectively than curcumin (Park *et al*., 2011), according to a study. The research also demonstrates that aromatic(ar)-turmerone inhibited the expression of the *tyr, trp1 and trp2* genes. Two curcumin derivatives, dimethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), are evaluated for their capacity to inhibit melanin production in seahorse embryos and B16 cells were reported (Jeon *et al*., 2023). Four target genes have their expression significantly reduced by BDMC and curcumin, according to a previous study. However, curcumin is toxic to seahorse embryos at a concentration of 5 μM (20). Jinpeng *et al.,* (2021) identify a chemically modified derivative of curcumin (J147) that inhibits melanin production and tyrosinase enzyme activity. In this study, J147 is found to be responsible for the degradation of MITF as well as a reduction in the expression of tyrosinase, TRP-1, TRP-2, Myosin Va, Rab27a, and Cdc42, ultimately resulting in the inhibition of melanin synthesis and melanosome transport (Lv *et al*., 2021).

The results of the analysis of volatile compounds in our extract found that germacrone was the highest percentage (20.02%) compound, followed by Eremophilene (13.62%), Eudesma-3,7(11)-diene (13.23%), and alpha-Elemene (10.69%). The two main compounds Eremophilene, and Eudesma-3,7(11)-diene were not previously reported in this species compared with the following studies. The results of Purkayastha *et al.* and our study in both indicate that the main volatile compound of essential oils or the EtOAc fraction of *C. zedoaria* is germacrone. Due to its multiple pharmacological applications, this flexible chemical entity has gained attention in recent years. Germacrone causes cell cycle arrest and apoptosis in a variety of malignancies via modulating multiple cell signaling molecules and pathways involved in cancer growth (Riaz *et al*., 2020). It was possible that germacrone is a vital compound that contributes antioxidant capacity as well as inhibits the formation of melanin pigment. On the other hand, the two main volatile compounds Eremophilene, and Eudesma-3,7(11)-diene have not been clearly reported for biological activity.

CONCLUSION

The TPC, DPPH, and FRAP study results revealed that the ethyl acetate fraction of *C. zedoaria* extract

had considerable antioxidant activity. It was not only non-toxic to B16 cells at a concentration of 25 µg/mL, but also had a remarkable capacity to inhibit melanin production, tyrosinase, and group gene targets (*tyr, trp1, trp2,* and *mitf*) expression also. The GCMS analysis revealed 28 distinct volatile compounds, with two new compounds accounting for a large fraction of the total. This data suggests that the extract of *C. zedoaria* has outstanding possibilities for application in cosmetics beauty products for skin pigmentation therapy. Hence, further investigation should involve the isolation and assessment of the melanogenesis inhibitory potential of bioactive compounds localized in the ethyl acetate fraction of *C. zedoaria*.

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

ETHICS APPROVAL

Not applicable.

DATA AVAILABILITY

Data available within the article or its supplementary materials.

REFERENCES

Akaberi M, Emami SA, Vatani M, Tayarani-Najaran Z. Evaluation of antioxidant and anti-melanogenic activity of different extracts of aerial parts of n. Sintenisii in murine melanoma b16f10 cells. Iran J Pharm Res: IJPR. 2018;17(1):225.

Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western nepal. Plants. 2019;8(4):96.

Athipornchai A, Jullapo N. Tyrosinase inhibitory and antioxidant activities of orchid (dendrobium spp.). S Afr J Bot. 2018;119:188-192.

Bhattacharyya J, Dash MK, Hewege C, Balaji M, Lim WM. Social and sustainability marketing: A casebook for reaching your socially responsible consumers through marketing science: CRC Press; 2021.

Blainski A, Lopes GC, De Mello JCP. Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from limonium brasiliense l. Molecules. 2013;18(6):6852-6865.

Brenner M, Hearing VJ. The protective role of melanin against uv damage in human skin. J Photochem Photobiol. 2008;84(3):539-549.

Danciu C, Vlaia L, Fetea F, Hancianu M, Coricovac DE, Ciurlea SA, et al. Evaluation of phenolic profile, antioxidant and anticancer potential of two main representants of zingiberaceae family against b164a5 murine melanoma cells. Biol Res. 2015;48:1-9.

Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of limnophila aromatica. J Food Drug Anal. 2014;22(3):296-302.

Fu R, Zhang Y, Guo Y, Chen F. Antioxidant and tyrosinase inhibition activities of the ethanol-insoluble fraction of water extract of sapium sebiferum (l.) roxb. Leaves. S Afr J Bot. 2014;93:98-104.

Gharge S, Hiremath SI, Kagawad P, Jivaje K, Palled MS, Suryawanshi SS. Curcuma zedoaria rosc (zingiberaceae): A review on its chemical, pharmacological and biological activities. Futur J Pharm Sci. 2021;7(1):1-9.

Gomathi D, Kalaiselvi M, Ravikumar G, Devaki K, Uma C. Gc-ms analysis of bioactive compounds from the whole plant ethanolic extract of evolvulus alsinoides (l.) l. J Food Sci Technol. 2015;52:1212-1217.

Gyamfi MA, Tanaka Y, He L, Klaassen CD, Wan Y-JY. Hepatic effects of a methionine–choline-deficient diet in hepatocyte rxrα-null mice. Toxicol Appl Pharmacol. 2009;234(2):166-178.

Ha HT, Tran-Van H, Van Tran T, Nguyen HTN, Phan DTA. Study on chemical compositions, antioxidants and intracellular anti-melanogenic activities of varieties of ganoderma lucidum in vietnam. Int J Food Sci Technol. 2023;58(8):4127-4135.

Jeon G, Ro H-S, Kim G-R, Lee H-Y. Enhancement of melanogenic inhibitory effects of the leaf skin extracts of aloe barbadensis miller by the fermentation process. Ferment. 2022;8(11):580.

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Jeon H-J, Kim K, Kim C, Lee S-E. Antimelanogenic effects of curcumin and its dimethoxy derivatives: Mechanistic investigation using b16f10 melanoma cells and zebrafish (danio rerio) embryos. Foods. 2023;12(5):926.

Khuntia S, Lenka J, Dash M, Sahoo BC, Kar B, Sahoo S. Bioactivity screening of thirty black turmeric (curcuma caesia roxb.) essential oils against free radicals and mdr isolates. Pharmacogn Mag. 2023;19(3):615-625.

Kumar S, Sandhir R, Ojha S. Evaluation of antioxidant activity and total phenol in different varieties of lantana camara leaves. BMC Res Notes. 2014;7:1-9.

Lan NTÁ, Linh TC. Điều tra sơ bộ về hoạt tính chống oxy hóa, chống đái tháo đường và chống viêm in vitro của các cao chiết từ thân củ nghệ đen (curcuma zedoaria). UD-JST. 2022:81-86.

Lv J, Yang Y, Jia B, Li S, Zhang X, Gao R. The inhibitory effect of curcumin derivative j147 on melanogenesis and melanosome transport by facilitating erk-mediated mitf degradation. Front Pharmacol. 2021;12:783730.

Park SY, Jin ML, Kim YH, Kim Y, Lee S-J. Aromatic-turmerone inhibits α-msh and ibmx-induced melanogenesis by inactivating creb and mitf signaling pathways. Arch Dermatol Res. 2011;303:737-744.

Pillaiyar T, Manickam M, Namasivayam V. Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. J Enzyme Inhib Med Chem. 2017;32(1):403-425.

Purkayastha J, Nath SC, Klinkby N. Essential oil of the rhizome of curcuma zedoaria (christm.) rose. Native to northeast india. J. Essent. Oil Res. 2006;18(2):154-155.

Rahman A, Afroz M, Islam R, Islam KD, Hossain MA, Na M. In vitro antioxidant potential of the essential oil and leaf extracts of curcuma zedoaria rosc. J Appl Pharm Sci. 2014;4(2):107-111.

Riaz A, Rasul A, Kanwal N, Hussain G, Shah MA, Sarfraz I, et al. Germacrone: A potent secondary metabolite with therapeutic potential in metabolic diseases, cancer and viral infections. Curr. Drug Metab. 2020;21(14):1079-1090.

Shim J-H. Inhibitory effects of cycloheterophyllin on melanin synthesis. mol. 2021;26(9):2526.

Sun L, Guo Y, Zhang Y, Zhuang Y. Antioxidant and anti-tyrosinase activities of phenolic extracts from rape bee pollen and inhibitory melanogenesis by camp/mitf/tyr pathway in b16 mouse melanoma cells. Front Pharmacol. 2017;8:104.

Tanvir E, Hossen MS, Hossain MF, Afroz R, Gan SH, Khalil MI, et al. Antioxidant properties of popular turmeric (curcuma longa) varieties from bangladesh. J Food Qual. 2017;2017.

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of abts, dpph, frap, and orac assays for estimating antioxidant activity from guava fruit extracts. J Food Compost Anal. 2006;19(6-7):669-675.

Tran TV, Nguyen-Ho Q-N, Nguyen-Thien T-V, Ton-That Q, Tran TL, Tran-Van H. Melanogenesis inhibitory effects of ethanol extract of perilla frutescens's leaves on b16 melanoma cells. Iran J Pharm Sci. 2021;17(4):65-72.

Ujang ZB, Subramaniam T, Diah MM, Wahid HB, Abdullah BB, Abd Rashid AHB, et al. Bioguided fractionation and purification of natural bioactives obtained fromalpinia conchigera water extract with melanin inhibition activity. SCIRP. 2013.

Varghese PK, Abu-Asab M, Dimitriadis EK, Dolinska MB, Morcos GP, Sergeev YV. Tyrosinase nanoparticles: Understanding the melanogenesis pathway by isolating the products of tyrosinase enzymatic reaction. Int J Mol Sci. 2021;22(2):734.

Videira IFdS, Moura DFL, Magina S. Mechanisms regulating melanogenesis. An Bras Dermatol. 2013;88:76-83.

Yang C-H, Chang N-F, Chen Y-S, Lee S-M, Lin P-J, Lin C-C. Comparative study on the photostability of arbutin and deoxy arbutin: Sensitivity to ultraviolet radiation and enhanced photostability by the water-soluble sunscreen, benzophenone-4. Biosci Biotechnol Biochem. 2013;77(5):1127-1130.

Yang Jy, KOO Jh, SONG Yg, KWON Kb, LEE Jh, SOHN Hs, et al. Stimulation of melanogenesis by scoparone in b16 melanoma cells 1. Acta Pharmacol Sin. 2006;27(11):1467-1473.

Ye Y, Chu J-H, Wang H, Xu H, Chou G-X, Leung AK-M, et al. Involvement of p38 mapk signaling pathway in the anti-melanogenic effect of san-bai-tang, a chinese herbal formula, in b16 cells. J Ethnopharmacol. 2010;132(2):533-535.

Zang D, Niu C, Aisa HA. Amine derivatives of furocoumarin induce melanogenesis by activating akt/gsk-3β/β-catenin signal pathway. Drug Des Devel Ther. 2019:623-632.

Zhang L, Yang Z, Chen F, Su P, Chen D, Pan W, et al. Composition and bioactivity assessment of essential oils of curcuma longa l. Collected in china. Ind Crops Prod. 2017;109:60-73.

Zolghadri S, Bahrami A, Hassan Khan MT, Munoz-Munoz J, Garcia-Molina F, Garcia-Canovas F, et al. A comprehensive review on tyrosinase inhibitors. J Enzyme Inhib Med Chem. 2019;34(1):279-309.

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