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Skin-whitening mechanism of cumin (*Cuminum cyminum* L.) extract

Ya Wang[1,2,†], Guo-Ying Du[1,2,†], Tao Guo[1,3,*], Hong-Mei Zou[1] and Dan Jia[1]

[1]School of Life Science and Engineering, Lanzhou University of Technology, Lanzhou, China
[2]Key Laboratory of Drug Screening and Deep Processing for Traditional Chinese and Tibetan Medicine of Gansu Province, Lanzhou, China
[3]Department of Pharmacy, Henan University of Chinese Medicine, Zhengzhou, China

Abstract: Skin-whitening effect is closely linked with the melanogenesis inhibitory activity and free radical scavenging capacity. The purpose of the present study was to evaluate the skin-whitening effect of cumin (*Cuminum cyminum* L.) extract. The whitening activity was evaluated by cell-free mushroom tyrosinase assay, free radical scavenging assay, cell viability assay, cellular tyrosinase assay and melanin content assay using B16F10 murine melanoma cells. The results showed that cumin extract exhibited concentration-dependent inhibitory effect on both monophenolase and diphenolase activities of mushroom tyrosinase (IC_{50} values of 1.027mg/mL and 0.977mg/mL, respectively). Kinetic study on diphenolase showed that the cumin extract was a reversible mixed-type inhibitor, and the inhibition constant (K_I) was determined to be 0.62mg/mL. In addition, cumin extract significantly suppressed melanin production and cellular tyrosinase activity of B16F10 melanoma cells in a concentration and time dependent manner without cytotoxicity. Moreover, cumin extract exerted strong scavenging capacity on DPPH, hydroxyl and superoxide anion radicals. Taken together, these results strongly suggest that cumin is a potential skin-whitening agent for the cosmetic industry.

Keywords: Cumin extract, melanin synthesis, tyrosinase activity, free radical scavenging activity, kinetic study.

INTRODUCTION

Melanin is produced by the melanocytes of the basal layer of skin epidermis, whose color changes from yellow to black. Melanin has diverse physiological functions, for instance, protecting skin against UV-induced injury and scavenging free radicals (Solano *et al*., 2006; Hwang *et al.*, 2018). However, excessive production or accumulation of melanin may cause hyper pigmentation, such as chloasma, freckles and melanosis. This kind of disease can be prevented by limiting the formation or accelerating the decomposition of melanin (Seo *et al.*, 2003; Liu *et al*., 2017). In other words, developing melanogenesis inhibitors is the key strategy for preventing skin pigmentation disorders. Tyrosinase (EC 1.14.18.1) is a copper-containing oxidoreductase, which plays the role of key enzyme and rate-limiting enzyme in melanogenesis and is widely distributed in plants, microorganisms and animals (Wang and Hebert, 2006). Tyrosinase participated in the first two steps in the biosynthesis of melanin, hydroxylating L-tyrosine to L-DOPA and oxidizing L-DOPA to dopaquinone (Olivares *et al.*, 2001; Ghanem and Fabrice, 2011; Bae *et al*., 2016). Free radicals, caused by environmental pollution, ultraviolet light and a variety of other factors, could promote the skin ageing process (Kim *et al*., 2013). Therefore, scavenging free radicals can suppress the oxidation reaction of melanin synthesis, and may be one of the ways of skin whitening (Wang *et al*., 2015).

Cumin (*Cuminum cyminum* L.) is an annual or biennial herbaceous plant and belongs to Apiaceae family. Cumin is widely cultivated in India, Iran, Turkey, China and other countries. In China, it is mainly produced in Xinjiang, Gansu and Inner Mongolia (Ma *et al*., 2015). Cumin is one of the world's most popular spices, and more attention is paid to it owing to its medical and edible values (Sowbhagya, 2013; Mnif and Aifa, 2015). According to the theory of traditional Chinese medicine, cumin is smelled sweet with the effects of warming stomach spleen, promoting digestion and dispelling cold (Li and Jiang, 2004). Modern research showed that cumin seeds had valuable antibacterial, anticancer and analgesic activities (Moghaddam and Pirbalouti, 2017). Tyrosinase inhibitors, for instance, hydroquinone, kojic acid, linoleic acid and arbutin, have been used as skin-whitening agents, but some of them have recently been found to exhibit side-effects (Kim *et al*., 2017). Additionally, a previous research showed that many plant extracts have the inhibitory effect on melanin synthesis (Jin *et al*., 1999). So numerous researchers have focused on searching safe and natural skin-whitening agents from plants, cumin is a potential candidate.

From our preliminary research, we found that cumin had the ability of inhibiting tyrosinase activity (Wang *et al*., 2015). Furthermore, according to a previous report, Cuminaldehyde (p-isopropylbenzaldehyde) from cumin had potential effect on anti-tyrosinase activity (Kubo and Kinst-Hori, 1998). The purpose of this study was to evaluate the skin-whitening effect of cumin extract by

**Corresponding author:* e-mail: gt010010@163.com
†These authors contributed equally to this work and should be considered co-first authors.

measuring the inhibitory mechanism of cell-free mushroom tyrosinase and melanin synthesis in melanocyte and free radicals scavenging activities. This paper discusses about the anti-melanogenesis and tyrosinase inhibition mechanism of cumin extract. The anti-tyrosinase and anti-melanogenesis effect of the main constituents of cumin will be study in the future.

MATERIALS AND METHODS

Reagents and cell lines

B16F10 murine melanoma cells were obtained from Shanghai cell bank (Shanghai, China). DMEM (Dulbecco's modified Eagle's medium), mushroom tyrosinase, kojic acid, arbutin, DPPH (2, 2-Diphenyl-1-picrylhydrazyl), ascorbic acid, L-tyrosine and L-DOPA (L-3, 4-dihydroxyphenylalanine) were purchased from Sigma-Aldrich (Shanghai, China). Cell counting kit-8 (CCK-8) was purchased from Beijing Wobisen Technology Co. (Beijing, China). Triton X-100 was purchased from Hangzhou Keyi Biotechnology Co. (Hangzhou, China).

Plant material collection and extraction

The seeds of cumin (*Cuminum cyminum* L.) were obtained from Gansu, China in 2016. 5.00g of cumin powder was extracted twice with 100mL of 80% ethanol at 80^{o}C for 2 hours. Then, the filtrate was collected and concentrated under reduced pressure. Finally, the extract was freeze-dried to yield 0.75g cumin powder and stored at -20^{o}C prior for further determination.

Determination of monophenolase and diphenolase activities of mushroom tyrosinase

The cumin extract was first dissolved in phosphate buffer saline (50mM, pH 6.8) at a concentration of 1mg/mL and then diluted to different concentrations using phosphate buffer saline (50mM, pH 6.8). Cumin extract was tested from 0.10 to 1.00mg/mL. In a 96-well micro plate, 100µL of L-tyrosine (2mM) or L-DOPA solution (1mM) in phosphate buffer saline (50mM, pH 6.8) was mixed with 40µl of test sample and pre-incubated at 37^{o}C for 10 min. Then 40µL of mushroom tyrosinase was added to the reaction mixture. When measuring the monophenolase and diphenolase activities, tyrosinase concentration was 40 and 10µg/mL, respectively. Then immediately determined the initial rate of increase in absorbance at 492nm, using a micro plate reader. Kojic acid was used as the positive control. IC_{50} value of cumin extract was determined by plotting the relative activities against the sample concentrations (Kim *et al*., 2013).

Kinetic analysis of the inhibition of mushroom tyrosinase

Usually, enzyme inhibitors can be divided into two types, reversible and irreversible. In order to find the inhibitory pattern of cumin extract, the concentration of L-DOPA was maintained at 1mM, while the concentration of tyrosinase was changed. At each tyrosinase concentration, different concentrations of cumin extract were added into reaction system to determinate the effect of cumin extract on the relationship between the catalytic reaction rate and the concentration of tyrosinase.

To explore the inhibitory type of cumin extract, remaining tyrosinase concentration at 10µg/mL and changing the concentration of L-DOPA from 0.5 to 2.0mM. The inhibitory type can be obtained from Line weaver-Burk plots by plotting 1/V versus the substrate concentrations with different concentrations of cumin extract. And the inhibition constant can be obtained from the Dixon plots by plotting 1/V versus the concentrations of inhibitor with varying concentrations of substrate (Si *et al*., 2017).

Cell culture

B16F10 murine melanoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100U/mL of penicillin and 0.10mg/mL of streptomycin at 37^{o}C in a 5% CO_2 incubator.

Cell viability assay

Cell viability was tested by the previously described method with slight modification (Roh *et al*., 2015). The cells (1×10^5 cells/well) were seeded in a 96-well plate and incubated for 24h, then using 100µL of cumin extract (0.01, 0.05, 0.10, 1.00mg/mL) to treat the cells at 37^{o}C with 5% CO_2 for 24, 48 and 72h. After incubation, 10µL of cell counting kit-8 (CCK-8) solution was directly added to wells and incubated for 4h. Then detecting the absorbance at 450nm using a micro plate reader. Arbutin, a good tyrosinase inhibitor, was used as a reference. The viability of the cells was analyzed as a percentage compared to the control.

Determination of cellular melanin content

The cells (5×10^4 cells/well) were seeded in a 96-well plate and incubated at 37^{o}C with 5% CO_2 for 24h. Then the cells were treated with 100µL of various concentrations (0.01, 0.05, 0.10, 1.00mg/mL) of cumin extract for 24, 48 and 72h. After that, the cells were rinsed twice with PBS and 200µL of 1M NaOH that containing 1% DMSO was added. Incubating at 80^{o}C for 1h, the absorbance was detected at 492nm by the micro plate reader. Arbutin was used as a positive control. The relative melanin content of the cells was estimated as a percentage compared to the control.

Determination of cellular tyrosinase activity

B16F10 cells (5×10^4 cells/well) were cultured in 96-well plates and incubated at 37^{o}C with 5% CO_2 for 24h. The cells were then treated with 100µL of different concentrations (0.01, 0.05, 0.10, 1.00mg/mL) of cumin extract for 24, 48 and 72h. After that, the cells were collected into trypsin solution and immediately centrifuged at 1200 rpm for 10min. Later, adding 50µL of

1% Triton X-100 to lyse the cells after washing twice with cold PBS. Rapidly freezing at -80°C for 1h and then centrifuged at 1200 rpm for 10min. 20μL of 5mM L-DOPA was added to 80μL of supernatant and incubated at 37°C for 30min, measurement of the absorbance at 492nm using a micro plate reader. Arbutin was acted as a reference. Relative tyrosinase activity was calculated as a percentage of the control (Ko *et al*., 2014).

DPPH scavenging assay

The DPPH scavenging activity was performed according to a previous study (Xu *et al*., 2015) with slight modification. A stock solution of DPPH (0.10mM) was freshly prepared with 80% ethanol. 2mL of cumin extract at various concentrations were mixed with 2mL of DPPH solution and maintained in dark at room temperature for 30min. The absorbance was measured at 517nm. Ascorbic acid was used as a positive control. The DPPH scavenging activity (SA%) of the test sample was calculated as formula (1) below:

$$SA(\%) = [1 - (A_{sample} - A_{background}) / A_{blank}] \times 100 \quad (1)$$

Where, $A_{background}$ is the absorbance of cumin extract under identical conditions as A_{sample} with 80% ethanol substitute for DPPH solution and A_{blank} is the absorbance of the control solution (80% ethanol take the place of cumin extract). The DPPH scavenging activity of cumin extract in concentration providing 50% inhibition, was considered as IC_{50} (μg/mL) and lower IC_{50} value corresponds to stronger antioxidant capacity of cumin extract.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging ability of cumin extract was determined according to a previously reported method (Chen *et al*., 2016) with slight modification. 1mL of various concentrations of cumin extract mixed with 1mL of 9mM $FeSO_4$, 1mL of 9mM salicylic acid-ethanol and 1mL of 8.8mM H_2O_2 were incubated at 37°C for 30min, the absorbance was determinated at 510nm. Ascorbic acid was served as a positive control. The scavenging activity (SA%) was calculated aaccording to the formula (2) below:

$$SA(\%) = [1 - (A_{sample} - A_{background}) / A_{blank}] \times 100 \quad (2)$$

Where, $A_{background}$ is the absorbance of cumin extract under identical conditions as A_{sample} with distilled water instead of H_2O_2 solution, and A_{blank} is the absorbance of the control solution (80% ethanol substitute for cumin extract). The result was expressed as IC_{50} value (μg/mL).

Superoxide anion radical scavenging assay

Superoxide anion radical scavenging activity of cumin extract was tested by a previously described method (Marklund, 1976) with sightly modified. 0.5mL of cumin extract at various concentrations mixed with 4.5mL of 50mM Tris-HCl buffer (pH 8.2) were incubated at 25°C for 20min, then adding 0.5mL of 2.5mM pyrogallol that pre-incubated at 25°C. After the mixtures were incubated at 25°C for 4min, immediately adding two drops of HCl (8M) and measuring absorbance at 420nm. Ascorbic acid was acted as a positive control. Radical scavenging ability (SA%) was calculated using the formula (3) below:

$$SA(\%) = [1 - (A_{sample} - A_{background}) / A_{blank}] \times 100 \quad (3)$$

Where, $A_{background}$ is the absorbance of cumin extract under identical conditions as A_{sample} with distilled water instead of pyrogallol solution, and A_{blank} is the absorbance of the control solution (80% ethanol take the place of cumin extract). The result was expressed as IC_{50} value (μg/mL).

Ethical approval

The experiment research content of the study was supervised by the Experimental Ethics Committee of the Lanzhou University of Technology.

STATISTICAL ANALYSIS

All data were analyzed using the SPSS 17.0 software. The results were expressed as means ± standard deviation (n=3) and statistically analyzed by one way analysis of variance (ANOVA). Values of $*P<0.05$ and $** P<0.01$ were considered statistically significant.

RESULTS

Inhibitory effect of cumin extract on monophenolase activity

The inhibitory effect of cumin extract on monophenolase activity was investigated. There was a obvious lag phase in the process of enzymatic oxidation. It was discovered that the lag time increased with a concentration-dependent manner, however, the steady-state rate decreased with the increase of sample concentration, as shown in fig. 1A. The IC_{50} value of cumin extract was determined to be 1.027±0.061mg/mL, which showed weaker inhibitory effect on monophenolase activity than that of kojic acid (IC_{50} = 3.825±0.028μg/mL), a good tyrosinase inhibitor.

Inhibitory effect of cumin extract on diphenolase activity

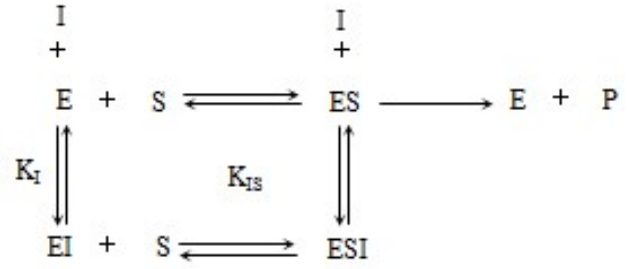
The effect of cumin extract on diphenolase activity was studied. There was no lag time for cumin extract at concentration range between 0.1 and 1mg/mL. When tyrosinase was added, the reaction immediately reached a steady-state, and concentration-dependent inhibition of the tyrosinase was observed as shown in fig. 1B. The IC_{50} value of cumin extract was calculated to be 0.977±0.010mg/mL, which was significantly greater than that of kojic acid (positive control), 2.927±0.032μg/mL.

Inhibition type and inhibition constant

To obtain the inhibition mechanism of cumin extract against tyrosinase, initial velocity versus tyrosinase concentrations at different concentrations of cumin extract were plotted, and a set of straight lines was obtained. As

depicted in fig. 1C, all the straight lines passed through the origin, and the slopes of the lines reduced with the increase of sample concentrations. The presence of cumin extract did not reduce the amount of tyrosinase, but just resulted in the inhibition of tyrosinase activity. The results indicated that cumin extract belongs to reversible inhibitor.

As shown in fig. 1D, the Line weaver-Burk plots indicated that all straight lines intersected at second quadrant. As the concentration of cumin extract increased, V_m value decreased, while K_m value remains increase. This behavior indicated that cumin extract inhibits tyrosinase through two different ways, formation of competitive enzyme-inhibitor (EI) complex and non-competitive enzyme-substrate-inhibitor (ESI) complex to interdict enzyme-substrate (ES) intermediate (Wikul *et al*., 2012). It was strongly indicated that cumin extract was a mixed-type inhibitor of tyrosinase. From the Dixon plots of 1/V versus the inhibitor concentrations, we found that a set of straight lines intersected at the second quadrant and the value of inhibition constant (K_I) was determined to be 0.62mg/mL (fig. 1 E). The speculated binding mode of cumin extract (Scheme 1) was appropriate, which indicated that a kind of inhibitor can combine with the active site of free tyrosinase or tyrosinase-dopa intermediate.



Scheme 1: Putative mechanism of enzymatic hydrolysis of L-DOPA inhibited by cumin extract (I), where E, S and P are tyrosinase, L-DOPA and product, respectively.

Effect of cumin extract on cell viability

The cell viability was measured at different points in time after the cells were treated with various concentrations of cumin extract. As illustrated in table 1, concentrations of cumin extract ranging from 0.01 to 1.00mg/mL had no noticeable effect on cell growth after incubating for 24h ,48h and 72h. When the cumin extract at concentration of 1.00mg/mL for 72h, more than 85.32% of cells were alive. However, the viability of the cells which were treated with arbutin (positive control) at 0.10mg/mL was lower than that treated with cumin extract at 1.00mg/mL (P <0.01), which showed that cumin extract is much safer than arbutin under these experimental conditions.

Effect of cumin extract on cellular melanin content

As illustrated in table 2, the melanin content was distinctly decreased in a concentration-dependent manner after the cells were treated with cumin extract at concentrations between 0.01 to 1.00mg/mL. In addition, as the extension of incubation time, the melanin content gradually decreased. Approximately 52.93% of the melanin content was reduced in comparison with untreated cells at the concentration of 1mg/ml for 72h. At concentration of 0.10mg/mL for 48 to 72h, the inhibitory effect of cumin extract on melanogenesis was greater than that of arbutin (positive control). The results showed that cumin extract is a good melanogenesis inhibitor.

Effect of cumin extract on cellular tyrosinase activity

As presented in table 3, cellular tyrosinase activity decreased in a time and concentration dependant manner. The inhibition rate of cellular tyrosinase activity was around 3.35-44.25% within the concentration and time range of the present test. At the concentration of 1.00mg/mL for 48 to 72h, the inhibitory effect on tyrosinase activity by cumin extract was greater than that of arbutin at 0.10mg/mL (positive control).

Free radicals scavenging activities

As shown in table 4, it has been observed that cumin extract scavenged DPPH, hydroxyl and super oxide anion radicals by the concentration-dependent fashion. When the cumin extract at 500µg/mL, the DPPH radical scavenging activity was up to 74.22±0.38%, and the hydroxyl and super oxide anion radicals scavenging activity were 62.02±0.76% and 55.95±0.50%, respectively. The IC_{50} values of scavenging DPPH, hydroxyl and super oxide anion radicals were 101.94±0.84, 323.74±1.03 and 200.60±0.60µg/mL, which indicated that cumin extract had great ability of scavenging free radicals and exerted efficient antioxidant ability. From the values of IC_{50}, we found that the capacity of scavenging free radicals by cumin extract was weaker than that of standard antioxidant, ascorbic acid (P <0.01).

DISCUSSION

There are three main enzymes in the process of melanin biosynthesis, containing tyrosinase, tyrosinase-related protein-1 (TRP-1) and tyrosinase-related protein-2 (TRP-2) (Lee *et al*., 2017). Tyrosinase is a key enzyme, which has both monophenolase and diphenolase activity. The results of this paper showed that cumin extract had potent inhibitory effect on tyrosinase activity. The reason of inhibiting monophenolase activity may delay the lag time of enzyme catalyzed reaction (Xing *et al*., 2016). It can be suggested that cumin extract inhibits diphenolase activity by preventing the oxygen binding to the enzyme active site, which may be attributed to the special structure of tyrosinase (Ferro *et al*., 2017). Kinetic analysis on diphenolase showed that cumin extract was a reversible mixed-type inhibitor and it may affects the inner bridge of the enzyme activity center. Moreover, the affinity between the enzyme and substrate could decreased due to the

combination of enzyme and cumin extract. The value of K_I was low, which may be demonstrated that the affinity of cumin extract and free enzyme was stronger than that of enzyme-substrate complexes.

The biosynthesis of melanin is a complex process, and the inhibitory mechanism of melanogenesis may be related to the cellular tyrosinase activity, melanin production and regulation of many cytokines (Xing *et al*., 2016). In this paper, we found that cumin extract had no significant

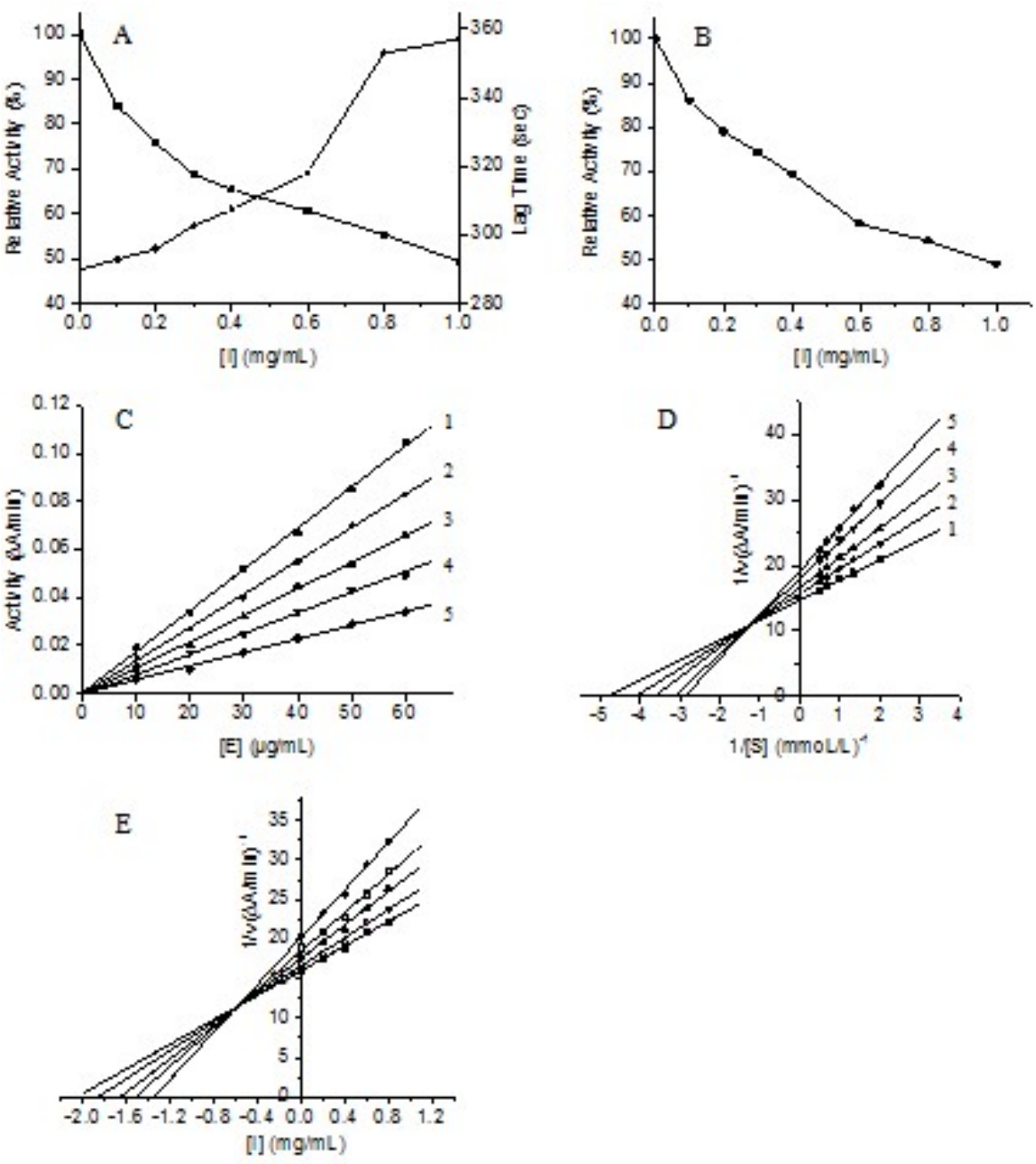


Fig. 1: The effect of cumin extract on mushroom tyrosinase activity. (A) Effect of cumin extract on the lag time and steady-state rates of monophenolase activity. (B) Effect of cumin extract on diphenolase activity. (C) The plots of the initial velocity versus tyrosinase concentrations at different concentrations of cumin extract. (D) Line weaver-Burk plots of mushroom tyrosinase with L-DOPA as a substrate in the presence of different concentration of cumin extract. (E) The Dixon plots of the apparent 1/V versus the inhibitor concentrations with varying concentrations of substrate. The concentrations of cumin extract for curves 1–5 of (C) and (D) were 0, 0.2, 0.4, 0.6, 0.8mg/mL, respectively. Concentrations of L-DOPA for (E) were (●) 0.5mmol/L, (ϒ) 0.75mmol/L, (▲) 1.0mmol/L, (○) 1.5mmol/L, and (■) 2.0mmol/L, respectively.

Table 1: Effect of cumin extract on cell viability

Test Samples	Concentration (mg/mL)	Cell viability (%)		
		24 h	48 h	72 h
Cumin extract	control	100 **	100**	100**
	0.01	95.04±0.92**	94.16±0.28**	92.83±1.25**
	0.05	90.32±0.23	87.47±1.04**	88.38±0.47**
	0.10	90.13±0.10	88.94±0.67**	87.78±0.83**
	1.00	88.20±0.76	86.98±0.30**	85.32±1.42**
Arbutin	0.10	89.75±0.51	82.40±0.29	81.66±0.59

Notes: The results were expressed as means ± standard error (n=5). ** in the same column indicate statistically significant in comparison with arbutin at P <0.01.

Table 2: Effect of cumin extract on cellular melanin content

Test Samples	Concentration (mg/mL)	Melanin content (%)		
		24 h	48 h	72 h
Cumin extract	control	100^{a}	100^{a}	100^{a}
	0.01	95.5 ± 0.82^{b}	92.48 ± 1.34^{b}	91.41 ± 1.50^{b}
	0.05	85.09 ± 0.40^{c}	85.63 ± 0.37^{c}	77.52 ± 0.35^{c}
	0.10	74.48 ± 1.27^{d}	64.72 ± 0.53^{e}	59.17 ± 0.71^{e}
	1.00	73.61 ± 0.56^{e}	65.40 ± 1.40^{d}	47.07 ± 0.36^{f}
Arbutin	0.10	71.50 ± 0.74^{f}	65.67 ± 0.68^{d}	61.42 ± 0.46^{d}

Notes: The results were expressed as means ± standard error (n=5). Different small letters in the same column indicate significant difference values at P <0.05.

Table 3: Effect of cumin extract on cellular tyrosinase activity

Test Samples	Concentration (mg/mL)	Tyrosinase activity (%)		
		24 h	48 h	72 h
Cumin extract	control	100^{a}	100^{a}	100^{a}
	0.01	96.65 ± 0.73^{b}	95.22 ± 0.48^{b}	95.15 ± 0.13^{b}
	0.05	93.14 ± 0.22^{c}	88.34 ± 0.44^{c}	81.62 ± 0.86^{c}
	0.10	92.11 ± 0.28^{d}	76.91 ± 1.26^{d}	74.27 ± 0.87^{d}
	1.00	90.32 ± 0.89^{e}	58.28 ± 0.72^{f}	55.75 ± 0.50^{f}
Arbutin	0.10	68.28 ± 0.40^{f}	62.76 ± 0.35^{e}	58.48 ± 0.60^{e}

Notes: Values are means (±standard deviation) (n=5). Different small letters in the same column indicate significant difference values at P <0.05.

effect on cell growth in B16F10 melanoma cells, but significantly reduced melanin content and cellular tyrosinase activity, indicating that cumin extract was safe to become a cosmetic agent and cumin extract may suppressed cellular melanin synthesis through down-regulation of tyrosinase activity in melanocyte. Previous studies have demonstrated that cumin contains abundant phenolics and flavonoids (Rebey *et al*., 2012; Alinian *et al*., 2016). In particular, phenolics and flavonoids have similar structures with tyrosine or L-DOPA and may be served as substrate analog inhibitors against melanogenesis (Roh *et al*., 2015; Bouzaiene *et al*., 2016; Boissy and Manga, 2017). Based on these results, we reasoned that the inhibition of cell-free mushroom tyrosinase activity was related to the cellular tyrosinase and melanin synthesis in melanocytes.

Oxidative stress is associated with the patho-physiology of inflammation, degenerative disease, skin ageing, pigmentation and so on (Pandey *et al.*, 2017). The antioxidant activity is related to different mechanisms, such as decomposition of free radicals scavenging, reducing capacity and peroxides (Mao et al., 2006). Removing excess free radicals can slow down the process of skin ageing and prevent melanogenesis (Villareal *et al.*, 2010). Spices are one of the effective sources of antioxidants, some of them may be more efficient and safer than the synthetic antioxidants (Alinian *et al.*, 2016). In this paper, we found that cumin extract exhibited strong radicals scavenging activities, which was aligned with the result of a previous study that illustrated cumin was a antioxidant capable of scavenging hydroxy and DPPH free radicals (Thippeswamy and Akhilender Naidu, 2005). It was suggest that radicals scavenging activity may be

Table 4: DPPH, hydroxyl and superoxide anion radicals scavenging activities

Samples	DPPH scavenging activity (%)					IC_{50} (μg/mL)
	10μg/mL	50μg/mL	100μg/mL	200μg/mL	500μg/mL	
Cumin extract	15.49±0.14	31.57±0.44	52.71±0.26	66.59±0.43	74.22±0.38	101.94±0.84
Ascorbic acid	32.02±0.83	56.44±0.50	71.88±0.42	80.02±0.98	91.37±1.20	30.08±0.45
	Superoxide anion radical scavenging activity (%)					IC_{50} (μg/mL)
	10μg/mL	50μg/mL	100μg/mL	200μg/mL	500μg/mL	
Cumin extract	14.38±0.16	26.37±0.16	42.47±0.55	51.72±0.30	62.02±0.76	200.60±0.60
Ascorbic acid	21.29±0.92	42.02±0.96	57.69±0.48	74.91±1.03	89.03±0.25	59.62±0.61
	Hydroxyl radical scavenging activity (%)					IC_{50} (μg/mL)
	10μg/mL	50μg/mL	100μg/mL	200μg/mL	500μg/mL	
Cumin extract	11.29±0.38	21.41±0.64	33.55±0.48	43.77±0.51	55.95±0.50	323.74±1.03
Ascorbic acid	15.99±0.83	30.32±0.64	49.52±0.29	65.02±0.71	81.87±0.25	101.65±1.25

Notes: The results were expressed as means ± standard error (n=3), IC50 represents a 50% inhibitory concentration.

one of the inhibition mechanisms of cumin extract as a tyrosinase inhibitor, and may be related to the melanin synthesis (Panich *et al.*, 2010).

It is possible that the skin-whitening effect of cumin extract is achieved by inhibiting tyrosinase activity and melanin production in melanocytes, along with scavenging free radicals. Therefore, natural plant extracts that have inhibitory effect on melanin synthesis without cytotoxicity, could be a promising source for skin-whitening agents.

CONCLUSION

In conclusion, this study indicated that cumin extract had strong anti-tyrosinase and antioxidant activities. The inhibitory kinetic study revealed that cumin extract inhibited diphenolase by acting as a reversible mixed-type inhibitor. Additionally, cumin extract decreased the cellular melanin content and cellular tyrosinase activity without affecting cell viability in B16F10 melanoma cells. So, cumin extract is a good melanogenesis inhibitor. These results indicate that cumin extract can become potential skin-whitening material for the cosmetics.

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